European Society for Developmental, Perinatal and Paediatric Pharmacology, 9th Congress

The 9th Congress was held in Marburg, Germany on 16–19 June 2004. The President for the meeting was Hannsjoerg Seyberth. The Congress discussed forthcoming legislation in the European Community, pharmacovigilance, pharmacokinetics, pharmacgenetics and prostanoids.

There were 20 oral free communications (O) and 38 poster presentations (P). The abstracts for 25 of the invited lectures are also shown below (L).

L1
Pharmacogenetics of drug metabolising enzymes – Impact of development

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From the 1970s through the mid 1990s, a considerable amount of investigation in the field of developmental pharmacology was focused upon application of the tools of pharmacokinetics to specifically characterise the association between development (i.e. age) and its impact upon the absorption, distribution, metabolism and excretion of drugs. Without question, the insights provided from the resultant large body of research not only re-defined "developmental pharmacology" but most importantly, have provided the knowledge required to understand, predict and control drug exposure-response relationships in pediatric patients. The unraveling of the human genome during the past decade has provided for the development and integration of two new "tools" in clinical pharmacology; namely, pharmacogenetics and pharmaco-genomics. When applied to investigations in developmental pharmacology, these facilitating technologies enable exploration of the role of genetic factors as determinants of drug disposition and/or action in a given individual (ie., pharmacogenetics) or evaluating the genome-wide response of a cell or tissue to drug exposure (i.e. pharmacogenomics)[1].

It is now widely appreciated that the combination of human ontogeny and genetic constitution exerts profound effects upon both pharmacokinetics and pharmacodynamics. As has been previously demonstrated, pharmacokinetic data can provide a "road map" for development by revealing an age-specific "pattern" reflective of change and/or reveal those periods of life where the most dramatic differences occur[2]. While the arguments and indications from such data are often quite compelling with respect to their potential scientific and/or clinical significance, there are still critical "missing links". Namely, what are the independent and/or collective factors that produce developmental differences in drug disposition (e.g., drug metabolism) and when developmental differences in pharmacokinetics are observed, how much of the difference is attributed to development *per se vs* normal interindividual variability in the processes that collectively, are the determinants of drug disposition? Answering this question requires the integration of translational science (e.g. developmental pharmacogenetics/ pharmacogenomics) into the design and conduct of investigations in Pediatric Clinical Pharmacology. The power afforded by this approach will provide new discoveries about normal human development and its true contribution to variability in pharmacokinetics, pharmacodynamics and pharmacotherapeutics.

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L2
Pharmacogenetics of transporters

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During the last 5 to 10 years our understanding of the processes which are involved in the absorption and distribution of drugs within the body has changed fundamentally. Until recently drug absorption and transfer from blood into brain was considered to be a passive process depending on the physicochemical properties and the protein binding of a drug. However, it is becoming apparent that transport proteins are of major importance for these processes. Of particular interest is the *MDR1* gene (*ABCB1*) product P-glycoprotein (Pgp), which translocates a large number of substrates with a diverse chemical structure, such as physiological compounds and important drugs such as cardiac glycosides, anticancer agents, immunsuppressants, HIV-protease inhibitors, statins, certain antiepileptics, and opioids. So far, several SNPs have been identified in the *MDR1* gene. Among these mutations the variants at the position 2677 and 3435 are of relevance since they are associated with alteration of Pgp expression and function for the respective geno-/haplotypes. However, in contrast to drug metabolising enzymes (e.g. CYP2D6) for which loss of function mutations or gene amplification manifest as distinct phenotypes in the population, the impact of MDR1 polymorphisms on pharmacokinetics and pharmacodynamics of Pgp substrates is moderate. On average, there is only a 2 up to 3 fold difference in the level of Pgp expression in various tissues (e.g. intestine, renal tubular cells, human placenta). In addition to alteration of drug disposition by MDR1 polymorphisms interesting observations have been reported regarding the impact of *MDR1* genetics on treatment outcome and host susceptibility to diseases such as inflammatory bowel diseases, HIV, renal cell carcinoma, Parkinson's disease and refractory seizures.

In addition to Pgp various other uptake (e.g. OATP-C) and efflux transporter (e.g. MRP family) exist and their relevance as well as the impact of genetic polymorphisms for drug therapy and treatment outcome have to be further investigated. Thus, a better knowledge on the role of genetic variants on transporter expression and function will contribute to a better understanding of interindividual and interethnic differences in drug disposition and effects in children, adolescents and adults.

L3
Impact on drug efficacy and adverse reactions in children

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Variations in genes for drug-metabolising enzymes, drug receptors and transporters have been associated with inter-individual variations in efficacy of drugs and occurrence of adverse drug reactions. Pharmacogenetics could therefore be used

- to improve therapeutic efficacy : many drugs show limited efficacy and even the most effective therapies do not work in 20% or more of treated patients
- to minimise adverse effects. Adverse reactions are responsible for more than 10% hospitalisations in some European countries. A recent review has shown that "59% of drugs in the ADR studies were metabolised by at least one pharmacogenetic enzyme, in comparison to 22% of randomly selected drugs in the United States

The best-known example is variation in the drug metabolising enzymes of the cytochrome P450 family. In the CYP2D6 gene alone, thee are more than 70 allelic variants, responsible for variations in enzyme activity resulting in low, high or even very high activity and rapid metabolisation of CYP2D6 substrates. Probably, TPMT pharmacogenetic polymorphism is the perfect example of the impact of pharmacogenetics on drug efficacy and adverse reactions Individuals with an impaired ability to metabolise thiopurines (0.3–0.5% of Caucasian individuals) are at risk of life-threatening adverse reactions, while patients with an high activity (90%) require higher dosage than patients with an intermediate activity 10%) to reduce adverse reactions and increase efficacy.

Although they are numerous candidate genes for pharmacogenetics (metabolism and transport, targets, disease related genes), those significantly associated with a given drug response are limited. The reasons are the following

- genetic control is normally complex, and even when a variant has an important effect, clinical exploitation may be difficult
- current pharmacogenetic research has limited reliability (small sample size, genetic information limited, genes studied independently, very few studies check for interactions)

As a consequence, the number of pharmacogenetic tests that are performed clinically is limited and with the exception of TPMT, none is used routinely in the clinic. Pharmacogenetics in principle, have the potential to increase health care disparities, and this requires explicit consideration. However, more experience with functional variants is required before attempting to use genome wide profiles diagnostically. The only option is to carry prospective studies to evaluate whether a genetic diagnostic improves outcome.

L4
The future of pharmacogenetics/ pharmacogenomics

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The human genome has now been mapped by virtue of enormous academic and financial investments. A new era of functional genomics including pharmacogenomics begins. The society cherishes great expectations of the impact of the new knowledge on the possibilities to find new ways of identification of drug targets and pharmacological treatment strategies, e.g. in children. Proteomics will be important in this context but the function of proteins must be tested *in vivo*, in intact cells as well as in the intact organism. Obviously there are several congential inherited diseases that lend themselves to combined genetic and proteomic studies. Currently, only about 500 proteins are targets for pharmacological treatment. Considering the existence of at least 30 000 genes any scenario to investigate the majority of these products is fictive today. The long way from *in silico* prediction of protein structure from the genome, via *in vitro* HTS assays, preclinical *in vivo* studies and finally *clinical* studies will require massive financial investments.

Identification of outliers in pharmacological response or adverse drug reaction (ADR) studies will remain a useful approach to discover genes of important drug metabolising enzymes, receptor systems, etc. With access to large cohorts of patients it is possible to find such outliers and to look for associations between these phenotypes and specific SNPs or constellations of SNPs. Networks of pediatric investigators are required for large-scale data collection. Functional pharmacogenomics at the clinical level will be important to identify new genetic variants of safety and response rate. Identification of non-responders *prior* to clinical trials would rationalise and economise drug development. This is particularly important in paediatrics.

Successful research in this field needs a number of prerequisites. First, robust clinical endpoints for clear identification of "response", "non-response", ADRs, etc. and interaction with national or international morbidity registries will be extremely important. Second, DNA/tissue banking needs to be developed and subjected to quality assurance. The ethical issues of biobanking are important to consider. Third, networking and interdisciplinary collaboration, preferably over the internet, will facilitate clinical data capture.

L5
Computer guided pharmacovigilance?

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Adverse effects related to drugs are common, particularly in children, since in this age group drugs are not routinely tested. Pharmocovigilance is needed to monitor adverse drug reactions (ADRs) to reduce their frequency, severity and consequences. Within the last years computerised methods for detecting ADRs have been established in adult medicine. We have recently shown that implementing laboratory signals helps to detect ADRs in children (Weiss *et al.*, 2002). To implement and measure the effect of automated computerised laboratory signals (ALS) as a detection support tool for ADRs in hospitalised children an observational prospective study was conducted.

A prospective, 6 month survey was performed on a 22 bed general paediatric ward, where ADRs were identified by weekly visits of a pharmaco-epidemiological team (paediatrician, pharmacologist, pharmacist). Beside intensive chart review automatic laboratory signals (ALS) were generated by a computer based monitor system. ADRs were classified by type of affected target organ according to WHO-ART system organ classes. Absolute changes over the defined limits (newALS) as well as changes between two measurements (deltaALS) were chosen.

A total of 396 patients (439 admissions) received 1999 drugs. Out of the cohort 73 ADRs were detected in 52 patients (13.1 % based on the admissions). According to WHO-ART system organ classes gastrointestinal system disorders are the most common ADRs (n=18), followed by blood disorders (n=10). Out of 27434 laboratory tests 1569 abnormal laboratory signals were presented as alert (1269 as newALS and 300 as deltaALS). The computer monitoring system finally reported 37 ADRs in 27 patients (50.7%) and 33 ADRs were found by treating physicians (45.2%).

The findings demonstrate that a computer monitoring system adapted from adult medicine is feasible and effective in the paediatric age group. Increasing specificity in adult as well as in pediatric is unavoidable for creating a system which will be accepted by physicians in daily practice.

L6
Targeted pharmacovigilance for children in hospitals

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Adverse drug reactions (ADRs) are a significant problem in children in hospital. A systematic review of prospective studies of pharmacovigilance showed that 9.5% of children in hospital experience an ADR[1]. At least 12% of these ADRs are severe. The types of ADRs experienced by children are varied and may be associated with significant mortality[2]. Certain types of medicines are more likely to be associated with significant ADRs, for example anticonvulsants, cytotoxics and anaesthetic agents[2].

The aim of pharmacovigilance is twofold. Firstly, it is a means of detecting new, previously unsuspected ADRs. Secondly, it needs to be more specific and look at ways of preventing the occurrence of ADRs. The latter is best achieved by targeted pharmacovigilance focusing on either an individual drug[3] or a group of drugs[4]. Targeted prospective pharmacovigilance has been useful in drawing up guidelines to minimise ADRs in the future.

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L7
Pharmacovigilance in paediatric primary health care

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Introduction: About 2% of paediatric hospital admissions are supposed to be caused by an ADR. Moreover, an incidence of about 15 ADRs per 1000 outpatient children receiving pharmacological treatment has been reported. Though these figures are somewhat lower than corresponding data for adults, it is conceivable that children are actually at a higher risk of suffering from an ADR than adults due to a different body composition, immature hepatic and renal function, as well as exposure to off-label prescribed drugs that lack paediatric labelling. However, knowledge about the frequency and characteristics of ADRs occuring in paediatric primary health care remains very limited.

Methods: We performed a retrospective, cross-sectional, observational analysis of spontaneous adverse drug reaction (ADR) reports in Sweden in the year 2000 and included all reports concerning drugs prescribed for outpatients younger than 16 years. Each ADR was classified with respect to its causality, seriousness, and type of reaction. Data analysis comprised to certain, probable, or possible ADRs. The extent of off-label prescribing was also evaluated among reported drugs with respect to age, dose, indication, formulation, and route and frequency of administration. Moreover, the number of ADRs related to licensed and off-label prescribing was calculated for different therapeutic groups. The data will be discussed in perspective of other studies addressing the current status of pharmacovigilance in paediatric primary health care in Europe.

Results: We identified 112 outpatient-linked reports corresponding 158 ADRs of which almost one third were classified as serious reactions. Antiasthmatic drugs were most frequently suspected as a cause of an ADR. The average proportion of off-label drug prescribing amounted to 42.4%. It was more frequently associated with serious (51.0%) than non-serious ADRs (38.5%) and mostly due to a non-approved age or dose. The most common clinical manifestations were psychiatric disorders (25.9%) and mucocutaneous inflammatory reactions (18.4%).

Conclusions: Drugs reported to have caused an ADR are also frequently prescribed in an off-label manner and the clinical manisfestations appear quite different from the inpatient setting. It is suggested to further identify unlabelled drugs frequently contributing to in particular serious ADRs in a paediatric population by a most careful postmarketing surveillance.

L8
PGE_2-mediated relaxation of the ductus arteriosus: effects of gestational age on EP receptor expression, signaling and vasomotor control

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Background: In the preterm newborn, a patent ductus arteriosus is due in large part to the increased sensitivity of the immature ductus to prostaglandin E_2 (PGE_2). PGE_2 acts through three G-protein coupled receptors (EP2, EP3, and EP4) which activate both adenyl cyclase and K_{ATP} channels. We explored these pathways to identify the mechanisms responsible for the increased sensitivity of the immature ductus to PGE_2.

Methods and results: We measured EP receptor content (mRNA and protein), receptor binding, cAMP production, and isometric tension in rings of ductus taken from immature (65% gestation) and mature (95% gestation) sheep and baboon fetuses. Ductus relaxation and cAMP generation were augmented in response to selective EP receptor agonists in the immature ductus. Glybenclamide (a K_{ATP} channel antagonist) decreased the sensitivity of the ductus to PGE_2 at both gestational ages. However, it did not eliminate the significant difference in PGE_2 sensitivity between the two age groups. In contrast, the presence of a selective Protein Kinase A inhibitor, Rp-8-CPT cAMPS, eliminated the developmental difference in sensitivity to PGE_2, suggesting that the increased sensitivity of the immature ductus to PGE_2 may be due to increased signaling through the Protein Kinase A pathway. Both the selective and nonselective EP receptor agonists produced a greater increase in cAMP production in the immature ductus than in the mature ductus. In addition, the immature ductus was more sensitive to the relaxing effects of cAMP itself. Increased cAMP production in the immature ductus appeared to be due to increased EP2, EP3 and EP4 receptor binding. However, the increased binding was not due to increased receptor mRNA or protein content. Nor was there a difference between the immature and mature ductus in the maximal adenyl cyclase activity.

Conclusion: Two mechanisms explain the increased sensitivity of the immature ductus to PGE_2: (1) increased cAMP production due to increased binding of PGE_2 to the individual EP receptors, and (2) increased potency of cAMP on Protein Kinase A-regulated pathways.

L9
Cyclooxygenases and prostaglandin receptors in the mouse ductus arteriosus

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Prostaglandins play a major role in the prenatal patency and postnatal closure of the ductus arteriosus (DA). In our studies, we have used COX-1 and COX-2 deficient mice, as well as COX-isoform specific inhibitors, to study the roles of the COXs in the physiology of the ductus. Our studies indicate that COX-2 deficiency or inhibition, but not COX-1, contributes to DA pathology in the mouse. Studies by others with prostaglandin receptor deficient mice have indicated that only EP4 deficiency caused a patent DA. Thus, COX-2 deficiency and EP4 deficiency appear to have similar DA effects. To further investigate the roles of the receptors in ductus closure, receptor ligands/agonist were studied for their ability to induce DA closure. Of the ligands studied, only a PGH_2 analogue and aTxA_2 analogue caused DA constriction. Thus, our data suggest that COX-2 appears to have an active role in normal ductus physiology and that COX-1, and not COX-2, inhibition may delay premature labor without adverse DA effects.

L10
Cyclooxygenase-2 in human and experimental ischemic proliferative retinopathy of the newborn: implications in retinopathy of prematurity

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The pathogenesis of retinopathy of prematurity (ROP) involves a number of inflammatory mediators, the sequence of which remains largely unknown. Inflammatory mechanisms influence vitreal neovascularization. Cyclooxygenase-2 (COX-2), a gene product induced in inflammation, promotes tumor angiogenesis. We investigated the role COX-2, its major product PGE_2 and its receptors in intravitreal neovascularization associated with ROP. Experiments were conducted on the mouse and rat pup models of O_2-induced retinopathy. In addition, COX-2 expression was also studied in humans with a comparable ischemic retinopathy, namely of diabetes. We observed increased expression of COX-2 in retinal astrocytes of the nerve fiber layer in the murine and rat models of ischemic proliferative retinopathy *in vivo*, in human diabetic retinopathy, and in hypoxic astrocytes *in vitro*. The profile of EP_3 expression coincided with that of COX-2. Specific COX-2 but not COX-1 inhibitors prevented intravitreal neovascularization, without affecting the degree of avascular area. Effects of COX-2 inhibition were reversed by PGE_2 and the selective EP_3 agonist M&B28767, but not by modulation of EP_2 and EP_4 activity. COX-2 inhibition induced a downregulation of eNOS and an upregulation of thrombospondin-1 (TSP-1) and its CD36 receptor consistent with the observed antiangiogenic effects of COX-2 inhibition; EP_3 stimulation reversed effects of COX-2 inhibitors on eNOS, TSP-1 and CD36. Elucidation of the mode of action of EP_3 on gene expression revealed an intracellular site specifically on the nuclear membrane (by confocal and electron microscopy), which upon stimulation of isolated nuclei induced calcium transients and eNOS expression. Findings disclose 1) an important role for COX-2 in ischemic proliferative retinopathy, such as ROP; 2) a previously undescribed regulation of TSP-1 and CD36; and 3) a totally novel mechanism of action of EP_3 in gene induction, notably directly on nuclear membrane.

L11
From oxygen sensing mechanisms to modern treatment of pulmonary hypertension.

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In the last decade our knowledge of the O_2 sensing mechanism and the signaling pathways that mediate the cellular response to hypoxia in the pulmonary circulation has considerably improved. Several studies have provided direct evidence that hypoxic vasoconstriction of pulmonary arterial smooth muscle cells (PASMCs) is mediated, at least in part, by the inhibition of one or several K^+ channels leading to cell depolarization, opening of voltage-gated Ca^{2+} channels, and myocyte contraction. In the ovine model of persistent pulmonary hypertension of the newborn (PPHN) we have shown that the contribution of the calcium-sensitive K^+ channels (Kca) to membrane potential and oxygen sensitivity is decreased compared with control animals and that this may contribute to the abnormal perinatal pulmonary vasoreactivity associated with PPHN.

Iloprost, a stable prostacyclin analog, under hypoxic conditions increases K^+ current, hyperpolarizes resting membrane potential, and decreases cytosolic Ca^{2+} in PASMCs, effects that can be inhibited by iberiotoxin. Consequently, we conclude that the ability of iloprost to cause vasodilatation in pulmonary hypertension may be mediated by activation of KCa channels.

Pulmonary hypertension has become a treatable disease. The most effective substances have vasodilating properties, but is not clear if this is their most important effect or if antiproliferative effects are more important. For smooth muscle cells, there is a coupling between vasoconstriction and proliferation and between vasodilatation and apoptosis. Therefore, vasodilatation may be an index of antiproliferative efficacy.

Continuous intravenous epoprostenol has been used since 20 years for treatment of primary pulmonary hypertension (PPH) and has been approved in the US and many European countries for treatment of pulmonary arterial hypertension (PAH) NYHA class III and IV. Iloprost, as continuous intravenous infusion, has been approved for PPH and secondary PH in New Zealand and inhaled iloprost has been approved by the EMEA for PPH, NYHA III. Treprostinil has been approved in the US for PAH, NYHA II-IV. Beraprost, as oral medication was effective in a European 3-month trial in PPH but not in a 1-year trial in the US. New application forms of prostanoids are being developed. The endothelin receptor antagonist bosentan has been approved by the FDA and the EMEA for PAH, NYHA class III (class IV, US only). Phosphodiesterase 5 inhibitors play an important role in the treatment of patients worldwide but have not been approved for pulmonary hypertension. Calcium channel blockers have been shown to be beneficial to a small subset of PPH patients, those who show a strong acute vasodilatory response.

As a consequence, a treatment algorithm of the "Arbeitsgemeinschaft Pulmonale Hypertonie" of the German Societies of Pulmonology, Cardiology and Pediatric Cardiology has been developed. The treatment options depend on NYHA class and the response criteria. Once a therapy has been established, the clinical response has to be monitored by suitable parameters like 6-min walk test, peak VO2 and NYHA class. Neurohormonal parameters like BNP and TNT may be helpful. Non-specialized physicians are asked to consult an experienced center for pulmonary hypertension after the diagnosis of increased pulmonary pressures has been made.

L12
Hypertension and renal prostanoids

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Introduction: Prostaglandins comprise a family of five distinct cyclooxygenase (COX) metabolites playing critical roles in regulating blood pressure, and kidney function as is evident in hypertension associated with the use of NSAIDS and COX2 inhibitors (like rofecoxib and celecoxib). By blocking prostaglandin formation, COX2 inhibitors, like non-selective NSAIDs reduce fever and inflammation, but may also cause renal failure, congestive heart failure, hyperkalemia and hypertension. The role of COX1 derived prostanoids remains less certain. We therefore performed studies to determine whether these isozymes exerted different effects on blood pressure, renal function, and synthesized different prostaglandins.

Methods: COX1 and COX2 inhibitors were used to treat mice followed by an infusion of Angiotensin-II (AngII). Mean arterial pressures and urine output was determined. Similar studies were performed in COX1, and COX2 knockout mice and mice genetically lacking the down-stream prostaglandin receptors for PGE2 (EP1, EP2). GC/MS was used to examine the prostaglandin profile in mouse kidney in control (resting) and AngII infused animals.

Results: While COX2 inhibition augmented the pressor effects of AngII, COX1 inhibition reduced the pressor action of AngII, suggesting the prostanoids derived from COX2 exert a depressor effect while those derived from COX1 contribute to the pressor effects of AngII. In normal mice, PGE2 is the major product in renal cortex and medulla followed by PGI2 (6-keto PGF1α), PGF2α, and TxA2 (TxB2). PGD2 was undetectable. Intravenous infusion of Ang II significantly increased PGE2 and PGI2 in both renal cortex and medulla, and this was blocked in renal medulla by COX2 inhibition, and by either COX1 or COX2 inhibition in renal cortex. Ang II dramatically increased PGF2a only in the renal cortex, and this increase in PGF2a was suppressed with either COX1 or COX2 inhibition. PGE2, the major prostaglandin can therefore be produced via either COX1 or COX2.

To test whether PGE2 exerts divergent vasoactive effects through its family of four receptors (EP1, EP2, EP3, EP4) we used receptor selective agonists and receptor knockout mice. EP2 knockout mice lose the normal depressor effect of PGE2. In these mice PGE2 exerts a pressor effect. This pressor effect may be through an EP1 receptor since the EP1 knockout mice exhibit reduced blood pressure and a blunted pressor response to EP1 selective receptor selective PGE2 analogs.

Conclusion: COX2 derived PGE2 may exert a depressor effect through EP2 receptors, while COX1 derived PGE2 may exert a pressor effect via activation of EP1 receptors. These studies further suggest COX1 and COX2 derived PGE2 may have differential access to specific PGE2 receptors.

L13
Adverse effects of COX inhibitors on the newborn kidney

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Nonsteroidal anti-inflammatory agents (NSAIDs) are administered to pregnant mothers to prevent the premature onset of labor or to treat polyhydramnios, and to premature infants to promote the closure of a hemodynamically symptomatic patent ductus arteriosus (PDA). They act by inhibiting prostaglandins synthesis. NSAIDs easily cross the placenta and reach the fetus. Prolonged intrauterine cyclooxygenase inhibition may interfere with renal morphogenesis, with consequent dysgenesis of the renal parenchyma. In both fetuses and neonates, NSAIDs also produce hemodynamic changes that can adversely affect the circulation in many organ systems. Deleterious renal effects of indomethacin have been described shortly after this agent was broadly used for the closure of PDA.

Prostaglandins are derived from the cyclooxygenases (COX1 and COX2) mediated metabolism of arachidonic acid. These prostanoids are actively synthetized within the kidney, where they act locally to modulate vascular tone. In addition to increasing renal blood flow, the vasodilatory PGs increase renin release, sodium excretion and free-water clearance. The presence of active vasodilatory PGs is of special importance for the kidney when perfusion is compromised or pathophysiologically limited. Such is the case in fetuses and neonates, who present with very low systemic and renal perfusion pressures and low GFR.

Because of the well described adverse effects of indomethacin, the search for alternative drugs was mandatory. Ibuprofen, a non-specific inhibitor of the cyclooygenases, has been used in human neonates, with the resultant claim that it was as efficacious as indomethacin on the ductus, but without its renal adverse effects. In experimental studies however, ibuprofen has the same dose-dependent deleterious effect on the immature kidney as indomethacin or aspirin. NSAIDs impair PGs synthesis by inhibiting the cycoloxygenases 1 and 2 (COX1 and COX2). The hope that COX2-selective inhibition could spare the kidney has neither been confirmed in adult patients, nor in newborn animals. In the latter, nimesulide, a COX2-preferential inhibitor induces the same dose-dependent deleterious effects on the kidney. Also the integrity of COX2 appears crucial for fetal nehrogenesis: small kidneys with few glomeruli, cortical microcysts, dysplastic tubules and medullary hypoplasia are seen when COX2 is inhibited in mice and rats during pregnancy.

Conclusion: Synthesis of PGs is crucial for early renal development and for the physiological preservation of GFR and renal perfusion during the fetal and early neonatal periods. All NSAIDs, non-selective and COX2-selective, should be used with great caution during pregnancy and in the perinatal perod.

L14
Function of prostanoids in COX-dependent nephrogenesis

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Impairment of renal development has been observed in newborns of women treated with nonsteroidal anti-inflammator drugs. The renal abnormalities range from oliguria to renal dysgenesis. Nonsteroidal antiinflammatory drugs are known to block cyclooxygenase enzymes and an important role for prostaglandins in renal development of functional maturing is suggested. Two isoforms of cyclooxygenase (COX) are known to exist and both are expressed in the adult and fetal kidney. COX-1 is constitutively expressed, wheras COX-2 expression is under the control of different stimuli, most important cytokines and growth factors and its expression may vary. Gene knockout studies have shown that COX-1 disruption does not interfere with normal renal development. In contrast, COX-2 null mice exhibit renal dysgenesis associated with hypoplastic glomeruli and reduced cortical mass. Adminstration of a COX-2-selective inhibitor is able to mimick the COX-2 knockout defect, which further indicates that COX-dependent mechanisms are invovled in nephrogenesis. In order to investigate the role of prostaglandins for normal renal development we examined the efficacy of various prostglandin receptor ligands for the rescue of renal dysgenesis in COX-2 knockout mice.

L15
New targets in the transepithelial Na^+ transport in the kidney: options for more specific drugs

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The epithelial sodium channel (ENaC) located in the apical membrane of polarized epithelial cells mediates Na^+ transport across tight epithelia. In the kidney ENaC is expressed in the aldosterone-sensitive distal nephron (ASDN), allowing the fine tuning of Na^+ absorption under the control of aldosterone, vasopressin or insulin. In the lung, ENaC is expressed along the airways and regulates the ASL volume.

Constitutive activation of ENaC-mediated Na^+ absorption in the ASDN leads to severe forms of salt-sensitive hypertension, and conversely ENaC loss of function cause salt-loosing nephropathies. In the airways an increase in ENaC-mediated Na^+ absorption produces a cystic fibrosis-like disease in mice, whereas loss of ENaC function leads to a severe defect in the clearance of fetal lung liquid.

The important physiological and pathophysiological roles of ENaC in the maintenance of extracellular salt and volume balance as well as in regulation of ASL volume makes ENaC a key pharmacological target for drugs in the treatment of diseases such as hypertension or cystic fibrosis. Our understanding of ENaC function at the molecular level and the identification of interacting proteins that regulate ENaC activity using genomic ad proteomic tools, allow the development of new strategies for a better pharmacological control of ENaC-mediating Na transport.

L16
The potential role of renal chloride channels in salt-sensitive hypertension

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Background: The chloride channel ClC-Kb is expressed in the basolateral cell membrane of the distal nephron and participates in renal NaCl reabsorption. Loss-of-function mutations of ClC-Kb lead to classic Bartter syndrome, a rare salt-wasting disorder. Recently, we identified the ClC-KbT481S polymorphism, which confers a strong gain-of-function effect on the ClC-Kb chloride channel. The present study has been performed to explore the prevalence of the mutation and its functional significance in renal salt handling and blood pressure regulation.

Methods & Results: As evident from electrophysiological analysis with the 2-electrode voltage-clamp technique, heterologous expression of ClC-KbT481S in Xenopus oocytes gave rise to a current that was 20-fold larger than the current produced by wild-type ClC-Kb. Biophysical properties of ClC-Kb ion channel function like anion selectivity or pH sensitivity were not affected by the T481S mutation. The prevalence of the mutant allele was significantly higher in an African population from Ghana (22%) than in whites (12%). As tested in 1 white population, carriers of ClC-KbT481S were associated with significantly higher systolic (by about 6.0 mm Hg) and diastolic (by about 4.2 mm Hg) blood pressures and significantly higher prevalence (45% versus 25%) of hypertensive (>140/90 mm Hg) blood pressure levels. Individuals carrying ClC-KbT481S had significantly higher plasma Na^+ concentrations and significantly decreased glomerular filtration rate.

Conclusion: The polymorphic variant ClC-KbT481S of the renal epithelial Cl-channel ClC-Kb strongly activates ClC-Kb chloride channel function in vitro and may predispose to the development of essential hypertension *in vivo*. ClC-Kb chloride channels thus provide a new target for antihypertensive drug development.

L17
Pharmacological chaperones as potential treatment for X-linked nephrogenic diabetes insipidus

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Introduction: In many mendelian diseases, some mutations result in the synthesis of misfolded proteins that cannot reach a transport-competent conformation. In X-linked nephrogenic diabetes insipidus, most of the mutant vasopressin V2 receptors (AVPR2) are trapped in the endoplasmic reticulum and degraded. They are unable to reach the plasma membrane and promote water reabsorption through the principal cells of the collecting ducts.

Methods: We are reporting two types of experiments: *in vivo* studies to assess clinically a short-term treatment with a non-peptide AVPR2 antagonist and *in vitro* studies in cultured cell systems.

Results: In patients, SR49059 decreased 24-hour urine volume (11.9±2.3 L to 8.2±2.0 L, $P<0.005$) and water intake (10.7±1.9 L to 7.2 ± 1.6 L, $P<0.05$). Maximal increase in urine osmolality was observed on day 3 (104±8 mOsm/kg to 147±38 mOsm/kg, $P=0.05$). Na, K, creatinine excretions and plasma sodium were constant throughout the study. *In vitro* studies indicate that two non-peptide vasopressin receptors antagonists SR49059 and YM087 rescued cell surface expression and function of mutant V2 receptors. Nonsense mutations were not affected by the treatment.

Conclusions: Misfolded vasopressin V2 receptor mutants were rescued *in vitro* and also *in vivo* by non-peptide antagonists. This therapeutic approach could be applied to the treatment of several hereditary diseases resulting from errors in proteins folding and kinesis.

L18
Classification and rescue of ROMK mutations underlying hyperprostaglandin E syndrome/antenatal Bartter syndrome

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Mutations in the renal K^+ channel ROMK (Kir 1.1) cause hyperprostaglandin E syndrome/ antenatal Bartter syndrome (HPS/aBS), a severe tubular disorder leading to renal salt- and water wasting. Several studies confirmed the predominance of alterations of current properties in ROMK mutants. However, in most of these studies analysis was restricted to non-mammalian cells and electrophysiological

methods. Therefore, for the majority of ROMK mutations, disturbances in protein trafficking remained unclear. Aim of the present study was the evaluation of different pathogenic mechanisms of 20 naturally occurring ROMK mutations with consecutive classification into mutational classes and identification of distinct rescue mechanisms according to the underlying defect.

Therefore, mutated ROMK potassium channels were expressed in *Xenopus* oocytes and a human kidney cell line and analyzed by two electrode voltage clamp analysis, immunofluorescence, and Western Blot analysis. We identified 14 out of 20 ROMK mutations that did not reach the cell surface indicating defective membrane trafficking. High expression levels rescued 6 out of 14 ROMK mutants leading to significant K^+ currents. Two early inframe stop mutations could be rescued by aminoglycosides, resulting in full-length ROMK and correct trafficking to the plasma membrane in a subset of transfected cells. In addition, 3 trafficking mutations could be rescued by the addition of butyrate to the culture medium.

In conclusion, most of the investigated ROMK mutations displayed a trafficking defect that might be rescued by pharmacological agents acting as molecular chaperones. The evaluation of different disease-causing mechanisms will be essential for establishing new and more specific therapeutic strategies for HPS/aBS patients.

L19
The Best Pharmaceuticals for Children's Act, the study of off-patent drugs and the NICHD-FDA Newborn initiative

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The BPCA enacted in 2002 has established a process to study off-patent and on patent drugs in children. While the BPCA has extended the economic incentives for the study of on-patent drugs in children, it contains significant new provisions.

The distinguishing features include the development of a prioritised list of off-patent drugs, the establishment of a formal NIH (NICHD)-FDA interaction and the recognition on the need to study drugs in newborns.

The organisational changes at FDA with the creation of the Office of Pediatric Therapeutics will be briefly outlined. Provisions of BPCA concerning pediatric oncology will be mentioned. The role of the Institute of Medicine in reviewing ethical issues on pediatric trials will be briefly outlined.

The evolving process of list development will be discussed from the initial opinion based system to an evidence based process. The drugs in lists including recently published 2004 prioritized list will be reviewed.

The nature of the FDA/NIH partnership will be reviewed including the process of developing Written Requests by the FDA and their translation into NIH Contracts will be detailed.

Some of the problems involved in fitting regulatory requirements with scientific needs will be reviewed.

The implementation of the BPCA with the establishment of a hybrid model of trials with a permanent coordinating center and the establishment of PODS (Pediatric Off-Patent Drug Study) Centers Networks for Drug/Indication will be detailed. The study of lorazepam for sedation of pediatric ICU patients will be used as an example.

Finally the NICHD-FDA Newborn Drug Development Initiative will be described.

The overall objective of this 5 year initiative includes the 1) the development of a process that involves academia, clinicians, and the FDA to develop an approach for:

- the design and conduct of clinical trials for to- be-marketed drugs
- the conduct of clinical trials of drugs used off label with inadequate data to support such use
- the harmonisation of trial design and conduct of academic drug studies.

The formation of work groups for the initial phase for the development of templates for studies of drugs use for (1) pain control; (2) neonatal seizures and hypoxic ischemic encephalopathy; (3) apnea of prematurity and broncho-pulmonary dysplasia; and (4) cardiac failure will be detailed. The outline of future plans will be detailed.

L20
Development of drugs for orphan groups of patients

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Different groups of patients are left alone with their disease. The reasons are manifold: the prevalence of the disease can be low, dealing with a very rare condition. In such situations, drug development is often neglected by industry since the financial return is too limited when considering the potential market. This attitude is at variance with the Academic research, investing much and investigating a lot in rare (metabolic) conditions likely to contribute substantially to the understanding of science and progress in medical care. Such analysis applies also to somewhat different situations: the pathological condition might not be rare in an age group (prematurity in neonates), but the group by itself (birth rate) represents a low absolute number of potential patients. Further on, a medical condition might sometimes prevail in several locations in the world (malaria, TBC, HIV…) but these populations might not be easily accessible for appropriate investigations living in remote areas or lacking a sufficient health care system; whatsoever, the poor financial backing remains the main cause of carelessness. Finally a frequent situation leading to similar difficulties relates to the complexity of a clinical condition (severe infection or simply old age) inducing multiple organ failure and necessitating polymedication (heart + renal failure, infection, pain…). Here again, chronic clinical conditions can happen to turn out as nearly unique situations.

The problem is not new but its awareness has increased in the society and several initiatives were undertaken at different levels. First patients have organized themselves in associations, creating lobby groups. Health authorities have offered incentives for drug developers (industry, academia) in setting a new legal frame (Orphan Regulation in US/EU). Regulators have adapted their requirements to the condition (alternative methodologies) and even contribute to facilitate the development of new medicines (scientific advice, protocol assistance). This approach revealed to be effective for rare conditions, especially in the field of paediatric (metabolic) diseases and in oncology.

On the European level a reflection has started aiming at issuing a regulation facilitating the development of better medicines for children in the same way as the Orphan regulation achieved it for very rare conditions. Currently some initiatives were undertaken at the level of the European Medicine Evaluation Agency (EMEA): a paediatric group was called together in order to organise a survey about availability of medicines, labelling… Guidance for the formulation of choice, good practices for extemporaneous formulations,… were also discussed. An inventory of the paediatric needs is also currently being set up.

This example indicates that the development of drugs for "Orphan" groups of patients remains a difficult problem. Not to quote elderly people, pregnant and/or lactating women… an effort should be made to target the medicines more precisely. The task will not be made easier when the pharmaco-genetic background of the population will intervene as an additional parameter.

L21
Non-interventional studies as a basis for approval of drugs for children

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For any type of study to be used as a basis of approval of medicinal products random error and bias of the study has to be sufficiently small for the relevant question posed to be answered reliably. Observational studies can often reduce random error substantially by involving very large numbers of individuals with a specific outcome, thereby providing useful evidence about any large effects of treatment on relatively uncommon outcomes (for example rare but serious adverse-effects). However, very large numbers of individuals are difficult to obtain into paediatric studies. Due to their inherent potential for moderate or large biases, observational studies have little role in the direct assessment of any more moderate effects of treatment on major outcomes, which generally are all that can realistically be expected from most treatments for most common serious conditions[1]. It has been suggested that observational studies can provide useful information when there are substantial barriers to the conduct of randomised trials, as with children. Difficulties in obtaining reliable evidence in randomized trials as a consequence of such obstacles are not, however, sufficient to justify the use of unreliable evidence from observational studies that may, due to potential biases, be importantly misleading.

With these very important limitations there may remain places for use of observational studies in approval of drugs for children. Possible examples could be efficacy studies of a medicinal product approved under exceptional circumstance for a serious rare disease with no previous satisfactory treatment, provided that a valid control can be found. Observational studies may also be the only feasible way to detect unexpected rare major adverse effects, especially delayed effects and effects of long-term treatment, however, use of more rapid methods of detection if available is preferable.

When considering the use of any non-interventional studies as a basis for approval of drugs for children we have to keep in mind the general principle that paediatric patients should be given medicines that have been appropriately evaluated for their use. Key concepts of the evaluation process are a favourable risk/benefit ratio and management of the uncertainties related to clinical research and paucity of data. Increases of our knowledge and understanding as well as development of new methods will probably expand the possibilities to use non-interventional studies for approval of drugs for children. However, their contribution may remain as supportive and in reduction of uncertainties in the decision making process and not as hard evidence for efficacy and safety.

Reference

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L22
PK-PD modelling in paediatrics: a general overview

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In general the amount of information available about pharmacokinetics (PK) and pharmacodynamics (PD) properties of drugs used in adults is limited, due mainly to difficulties such as the recruitment of patients, and the number of samples taken with invasive methods needed to characterise the PK and PD relationships.

This situation will change in the future as a consequence of the improvement of the analytical methods that will require samples with less volume, the increase in the application of the population approach methods that allow to describe sparse data, imbalance studies, etc, and the request of drug regulatory agencies that companies study drug to be given to children prospectively.

In the mean time paediatricians are forced to find the appropriate dosing regimen on the basis on the information available from another population. Pharmacokinetic/pharmacodynamic (PK/PD) models are useful in this case because they allow one to explore the time course of drug response under different dosing, demographic and/or disease scenarios. However, the question about the type of relationship between the PK and PD properties between children and adults (in other words, how to scale the PK and PD information across populations?) is crucial. There is a considerable experience in this area with regard to PK, but not with regard PD.

Several examples will be presented covering a wide range of therapeutic areas (aids, cancer, analgesia, etc) and continuous and non-continuous responses. In particular, the examples dealing with non-continuous responses will help to expand the use of this type of analysis in paediatrics since they provide a framework to incorporate important clinical responses such as survival times, probability of an event to occur into a PK/PD model.

Comparison of PK and PD parameter estimates between children and adults will be presented when possible, and the critical aspects in the design of PK/PD studies in children will be discussed.

L23
Prediction of drug clearance and its variability in neonates, infants and children using SIMCYP

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Background: Physiologically based pharmacokinetic models to assess the exposure of neonates, infants and children to given doses of xenobiotics are likely to be more successful than simplistic allometric scaling, particularly in young children. However, such models require comprehensive information on the ontogeny of physiological and biochemical processes. The Simcyp software incorporates genetic, physiological, demographic and clinical attributes of patient populations pertinent to *in vitro-in vivo* extrapolation of xenobiotic metabolism into libraries that can be used for automated prediction of drug clearance (CL). Previous applications of Simcyp to predict CL has been successful in infants and children from 2 years of age[1]. Recently, information on the ontogeny of specific cytochrome P450 (CYP) enzymes, both in the liver and small bowel, has been incorporated into the algorithms such that predictions of CL from birth can also be made.

Methods: The drugs studied were midazolam (oral and IV), caffeine, warfarin, cisapride and omeprazole. *In vitro* Vmax and Km values and *in vivo* clearance data for each drug were obtained from the literature. Two thousand virtual subjects (birth to 18 years) were simulated for each drug. For each specific age band, Simcyp predicted values of weight normalised CL were compared with observed values.

Results: There was close agreement between the observed and predicted CL values across the age bands such that 58 out of 69 predictions (84%) were within 2-fold of the observed value.

Discussion: The *in silico* prediction of pharmacokinetic parameters in paediatric populations will not replace clinical studies. However, it provides a valuable aid to decision making with regard to first-time dosage in children and study design. The clinical study then becomes a "confirmatory" rather than an "exploratory" step in paediatric drug development.

Reference

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L24
How to use PK-PD modelling in paediatrics

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A pediatric decision tree has been developed by CDER to guide the recommendation of pediatric bridging studies that link adult efficacy/safety data to those in children. The decision tree recommends different types of pediatric studies based on the existing knowledge of the specific disease and the pharmacokinetics/pharmacodynamics (PK/PD) of the drug. If the PK/PD relationship of a drug is similar between adults and children or the PK/PD relationship can be determined in the pediatric population, only the pharmacokinetic studies are recommended for the bridging and dose determination. A bridging study is designed to generate the necessary evidence to permit extrapolation of the efficacy and safety database from adults to pediatrics. Bridging studies can be used to minimize the cost and time required for developing a drug in the sub-population. Full-scaled efficacy/safety studies can be avoided if appropriate PK/PD are available to establish the links between the adult and pediatric populations. Examples will be given to illustrate how the PK/PD studies can be used for pediatric bridging.

L25
Clinical trial optimisation in paediatrics using prior knowledge and sparse sample

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Introduction: In essence the design of trials for the paediatric population are more complex than those performed in adults since it is generally desired to learn more from less intensive studies. To learn more from less requires that the design be optimised, in some way, to provide the best scenario from which useful information can be gathered. This problem has been addressed in adult populations using clinical trial simulation as the tool. Clinical trial simulation is being used increasingly in the drug development process in an effort to reduce the number of studies performed by maximising the chance of a successful outcome[1].

Difficulties in the use of clinical trial simulation as a tool for designing paediatric trials lies in the development of methods for scaling issues for PK/PD models used in adults and/or animals/in-vitro to pediatrics and determination/definition of the desired covariate distribution models.

Methods: Any modelling and simulation (M&S) project first begins by defining the objective(s) of the project, this should certainly also be the case for in the paediatric field. Then, the following steps are generally performed:

- Modelling and simulation plan is written.
- All data needed to meet the objectives are defined and sourced.
- Exploratory analyses are performed (initial model development).
- Full model development takes place.
- Model evaluation is carried out (testing to define if the model is a good description of the data).
- Simulations are run to optimise subsequent trial design.
- Presentation of results and appropriate decision making.

With respect to sourcing the data needed to perform the M&S project, this may come from recent investigator's brochures, clinical protocols and reports, submission documents, summary basis of approvals on the FDA web site, the internet, etc. With respect to the paediatric field there will probably be less data available than is usual, thus the data analyst must be prepared to scale, extrapolate and/or interpolate from information from other drugs in the same chemical class that may already be available, whether in adults or children.

Further, it is generally desirable to include as few children as possible in any study, and thus characterisation of covariate relationships may be a problem (e.g. the influence of age and weight on pharmacokinetic parameters). Models do exist in the literature and these can be employed.

Conclusions: Clinical trial simulation is a powerful tool that offers much to developers of drugs for the paediatric population and is being increasingly recognised by the regulatory bodies.

Reference

1. Bonate PL. Clinical trial simulation in drug development. Pharm Res 2000; 17: 252-256.

O1
A major influence of cytochrome 2C19 genotype on the steady-state concentration of N-desmethylclobazam

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Introduction: N-desmethylclobazam (NCLB), the major metabolite of the anti-epileptic drug clobazam (CLB), exerts a large influence on the therapeutic and adverse effects of CLB. A substantial inter-individual variability has been observed in the ratios of NCLB concentration/CLB dose and of the NCLB/CLB concentration. In the present study, we tested whether CYP2C19 genotype was associated with an altered metabolism of CLB and NCLB among 16 patients receiving CLB, giving careful consideration to a unique Japanese population structure in which the *2 and *3 alleles are the prevalent (35% altogether) and the exclusive mutant alleles.

Methods: A group of 16 patients (1.5 to 33 years, media*n*= 7 years) who had received oral administrations of tablets or granules of CLB with the dose unchanged at least for 4 weeks at the time of study were studied after obtaining written informed consent. We assumed that the spot concentrations represented the steady-state concentrations because the steady-state is reached within about 4 days of repeated doses for CLB and within 10 days for NCLB. Genomic DNA was isolated from whole blood, and CYP2C19*2 and CYP2C19*3 mutations were detected by direct sequencing.

Results: The NCLB/CLB concentration ratio in three patients with two non-functional alleles (two homozygotes for *2 or one compound heterozygotes for *2 and *3) were six-fold higher (P<0.0001) than those of seven patients with normal alleles (*1 homozygotes). Patients with one non-functional allele (six heterozygotes for *2 or *3) exhibited intermediate trait. Similarly, NCLB concentration/CLB dose ratio elevated as the allele count of the non-functional allele increased (P<0.0001).

Conclusion: We document here a genotype-phenotype correlation between CYP2C19 polymorphisms and those ratios. Patients with two mutated CYP2C19 alleles show significantly higher ratios than those with the wild type genotype: patients with one mutated allele exhibited intermediate an intermediate trait. That is, the degree of elevation in the ratios was dependent on the number of mutated alleles of CYP2C19 (gene-dose effect).

O2
Ontogeny of cytochrome 3A isoenzymes and P-glycoprotein in the human intestine

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Introduction: Cytochromes P4503A (the predominant CYP450 subfamily) and P-glycoprotein (P-gp, an efflux plasma membrane protein) are mainly located in enterocytes and hepatocytes. The CYP3A/P-gp system contributes to the first pass metabolism of many drugs, resulting in a limited

bioavailability. During the neonatal period, a shift between CYP3A7, the fetal form, and CYP3A4 occurs in the liver but data on expression of the CYP3A/P-gp complex in the intestine are very limited.

Methods: localisation and expression of CYP3A and P-gp were studied in 59 normal duodenal biopsies from Caucasian children aged 1 month to 17 years using an immunohistochemistry and a real time quantitative RT-PCR technique.

Results: The immunoblot analysis using a polyclonal antibody ("Nuage") recognizing CYP3A4, CYP3A5 and CYP3A7 showed that CYP3A protein could not be detected in all the enterocytes in the samples till 6 months of age while it was present in all of the older samples. P-gp protein (monoclonal antibody "C494") was expressed at the apical surface of all enterocytes. mRNA quantification of the CYP3A4, 3A5, 3A7 and P-gp was performed using highly specific real time RT-PCR. Villin mRNA expression was used for normalization and the results were expressed as the expression ratio of the number of mRNA copies of the gene of interest over villin. All CYP3A mRNA levels were highly variable. CYP3A4 and CYP3A5 mRNA levels were high during the first year of life (gene/villin ratio 0.165 ± 0.103 and 0.164 ± 0.161 respectively) and decreased significantly with age (P<0.0001 and P=0.005 respectively) while CYP3A7 was only detected at a low level in 64% of samples, whatever the age. P-gp mRNA level was variable with an expression ratio ranging from 0.08 to 1 and a significant increase in expression between 6 and 12 months of age (P=0.0471).

Conclusion: Our results showed that neonates and infants had a significant expression of CYP3A and P-gp mRNA in the intestine, with a maturation profile which appeared different in the intestine and liver. Additional studies are required to quantify protein activity and determine the clinical impact of these results. However, this could already explain why drugs substrates of the CYP3A/Pgp system, such as nelfinavir, exhibit limited bioavailability in young HIV1 infected patients.

O3
Association of genetic polymorphisms of VEGF and risk of proliferative retinopathy of prematurity

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Introduction: The intention of our retrospective study was to determine whether VEGF genetic polymorphisms were associated with risk of proliferative retinopathy of prematurity (ROP), a condition characterised by abnormal retinal neovascularization which can lead to retinal detachment and result in blindness.

Methods: We enrolled 86 very low birth weight infants (birth weight <1500 grams) who had been treated with cryo/lasertherapy because of the risk of proliferative ROP (treated group). Their VEGF T-460C and G+405C genotypes were determined from dried blood samples and were compared with VEGF genotypes of 115 VLBW infants who were not treated with cryo/lasertherapy (untreated group).

Results: We found that the allele frequency of VEGF +405C was higher in the treated group than in the untreated group (0.30 vs. 0.41, P<0.05). The likelihood of being treated for ROP was higher in heterozygous and homozygous carriers of VEGF +405C alleles (odds ratios adjusted for risk factors of ROP [95% CI]: 2.00 [1.02–3.92], P=0.04 and 3.37 ([1.17–9.65], P=0.007, respectively). VEGF -460TT/+405CC haplotype was more prevalent in the treated patients than in the untreated patients (13/86 *vs* 1/115, P<0.001), and the association remained significant (P<0.01) even after the adjustment for risk factors of ROP (gestational age, supplemental oxygen therapy and gender).

Conclusion: These findings suggest that the VEGF genotype may be associated with risk of proliferative ROP in VLBW infants.

O4
Topiramate pharmacokinetics in children

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Purpose. To study the pharmacokinetic parameters of topiramate (TPM) in children under 4 years of age.

Methods. Twenty two children aged from 6 months to 4 years of age with a pharmacoresistant epilepsy of any seizure type were recruited. This was an open label prospective study. Children were grouped by enzyme-inducing (group IND, n=8), enzyme-inhibiting (group INH, n=6), other antiepileptic drugs (group OTHER, n=7), and one patient had both enzyme-inducing and inhibiting drugs. After a progressive titration of dose, blood samples were collected just before and 0.5, 1, 1.5, 2, 4, 6, 8 and 12 hour after the TPM administration. TPM plasma concentration were measured using a GC-MS validated method. Pharmacokinetic parameters were studied in all patients with a non-compartmental method.

Results. Ten patients (45%) were responders. TPM was well tolerated. Patients in group IND had a significantly higher drug clearance compared to other groups (CL/F, 170 ± 68 ml/h/kg in group IND, 99 ± 27 ml/h/kg in group INH, 93 ± 28 ml/h/kg in group OTHER) and therefore lower TPM plasma concentrations. The mean steady-state area under the curve (0-12 hour) was significantly lower in group IND compared to group OTHER.

Conclusions. Children aged <4 years who receive concomitant enzyme-inducing drugs need higher mg/kg doses to achieve the TPM plasma concentrations of those who do not. Furthemore whatever the co-medication they require much greater body-adjusted dosage to achieve plasma concentration comparable to those observed in adults.

O5
Sex- and age-related differences in doxapram pharmacokinetics in neonates: a population analysis

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Introduction. Doxapram, a respiratory stimulant, is now widely used in France to treat apnoea in neonates badly responsive to methylxanthines. Its therapeutic index is narrow with a wide interindividual variability in kinetics. It may be responsible for severe side effects. We have previously shown that monitoring of plasma concentrations of doxapram (dox) and its active metabolite ketodoxapram (ketodox) leads to a much better tolerance in premature infants. The aim of this study was to determine which covariates could contribute to differences in pharmacokinetics in neonates.

Methods. Plasma dox and ketodox concentrations were measured (2.1 samples/patient) by HPLC in 191 neonates (110 boys, 81 girls, BW=1.14±0.29 kg, GA=28.6±2.0 weeks, post-conceptional age (PCA)=30.8±2.1 weeks) given doxapram per os or by continuous infusion. Data were analysed using a NONMEM (non linear mixed-effects modeling) program according to a three-compartment model. Effects of developmental, demographic and clinical factors were tested on volume of distribution (Vd), clearance (Cl) and elimination constant rate (Ke) for a concentration target of 1mg/L for dox and 2 mg/L for dox+ketodox.

Results. The inclusion of the covariates sex and PCA into a multivariate intermediate model and in a backward stepwise approach was statistically significant ($P<0.001$). The very wide interindividual variability in dox Ke decreased from 90% to 71% when taking into account these covariates. Cl was higher in girls than in boys (0.51±0.04 vs. 0.32±0.02 L/kg/h; $P<0.01$).

Conclusion. These data demonstrate the influence of development and sex in dox elimination. This could be the initial step for developing a Bayesian dosing strategy in order to optimize individual dosage regimen.

O6
Pharmacokinetics of propacetamol in preterm and term neonates

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Introduction: The aim of this study was to describe propacetamol pharmacokinetics in term and preterm neonates in order to suggest dosing regimens in line with an earlier report on enteral and rectal paracetamol in neonates.

Methods: A population pharmacokinetic analysis of paracetamol time-concentration profiles in 48 neonates was undertaken using non-linear mixed effects models (NONMEM). Neonates received either a single (n=30) or multiple doses (n=18) of intravenous propacetamol infusion(s). Neonates had a median postnatal age of 1 day (range, 1-76 days). Median post-conceptual age (PCA) was 35 weeks (range, 27-42 weeks) and median weight was 2.4 kg (range, 0.51-4 kg).

Results: The population volume of distribution estimate and between subject variability (%) for a one compartment model with zero order input and first order elimination was 70.4 (30.7%) L/70kg. Clearance increased from 2.85, CV 40.7% at 27 weeks PCA to reach 7.05 L/h/70kg by 42 weeks PCA (standardised to a 70 kg person using allometric "1/4 power" models). Between occasion variability for volume of distribution and clearance were 17.4% and 26% respectively.

Conclusions: A mean paracetamol steady state target concentration above 10 mg/L at trough can be achieved by loading dose of 40 mg/kg and maintenance doses of 20 mg/kg/6h in 28-week PCA neonates, 25 mg/kg/6h at 32 weeks, 30 mg/kg/6h at 36 weeks and 20 mg/kg/4h at term. The role of the oxidative enzyme CYP2E1 and production of the hepatotoxic metabolite NAPQI is unknown in premature neonates. Therefore, lower doses scaled by age related clearance and centred on a daily dose of 60 mg/kg/day in a child of 6-8 y with a clearance of 0.25 l/h/kg (12.5 l/h/70kg) may be more appropriate, resulting in maintenance doses of 15 mg/kg every 6 hr at 28 PCA weeks, 10 mg/kg every 4 hours at 32 weeks, 12.5 mg/kg every 4 hours at 36 weeks and 15 mg/kg every 4 hours at 40 weeks.

Warning: all doses are reported as prodrug. One gram of propacetamol equals 0.5 gr of paracetamol.

O7
Age and weight related differences in the pharmacokinetics of stavudine

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Objective(s): To study the influence of age, bodyweight, gender and renal function on the pharmacokinetics of stavudine and to examine the relevance of the recommended stavudine dose for human immunodeficiency virus-infected in a heterogeneous population according to these covariables.

Design: Individual stavudine time-concentration data was obtained from therapeutic drug monitoring. The following covariates: were recorded for each patient: age, bodyweight, gender, urea, creatininemia

Methods: Pharmacokinetic data were analyzed by a nonparametric population approach. The relationships (if any) between pharmacokinetic parameters and covariates were then assessed. Individual estimates of an exposure factor – AUC(0-24 h) - were computed for recommended drug dosing schedules based on age and body weight. Influence of these factors on AUC(0-24 h) were investigated by nonparametric statistical tests.

Results: Estimated mean apparent clearance (CL/F) and distribution volume (V/F) were 5.90×10^{-1} (L/kg /h) and 9.73×10^{-1} (L/kg) respectively. Age and body weight were found to play a role in explaining the large interindividual variability of CL/F. A major evidence influences of body weight and age groups on means AUC(0-24 h) were also evidenced. The medians of estimated AUC(0-24 h) for adults less than 60 kg , between 60 kg and 80 kg, and more than 80 kg were 3.29, 4.03 and 3.13 µg ml-1 h respectively. By a Kruskal-Wallistest the hypothesis of no influence of body weight on AUC(0-24) was rejected ($P=5.3 \times 10^{-3}$). The medians of AUC(0–24 h) for neonates, infants, , children between 2 and 6 years, children more than 6 years and adults were 2.51, 2.55, 3.30, 3.70, and 3.75 µg ml-1 h respectively. By a Kruskal-Wallis test the hypothesis of lack of age influence on AUC (0-24) was rejected ($P < 10^{-4}$).

Conclusion: This study identified weight and age as major determinant of stavudine clearance and AUC of stavudine. Neonates, infants and adults above 80 kg appeared to be underexposed to stavudine compared to a standard adult population

O8
Population pharmacokinetics of indomethacin in children with salt-losing tubulopathies

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Objective: To determine the pharmacokinetics of indomethacin in children with hyperprostaglandin E syndrome/ antenatal Bartter-Syndrome.

Method: 211 serum concentrations were obtained from 38 patients during 93 hospital stays. Median (range) weight, length and age were 16.6 kg (3.0–70.3 kg), 108 cm (51–176 cm) and 5.4 years (0.24–21.1 years), respectively. The data were analysed using NONMEM V 1.1.

Results: A one compartment model was used to fit the data. Since not enough data were available to estimate the absorption constant ka properly, it was fixed to previously reported values.

Body surface area was an important determinant of both clearance (CL) and volume of distribution (V). Inclusion of other covariates (age, creatinine) did not significantly improve the fit of the data.

The final models were CL/F (l/h) = 2.48 × BSA and V/F (l) = 23.8 × BSA, where BSA = body surface area (m^2).

Conclusion: The pharmacokinetics of indomethacin in children with HPS does not differ from previously reported data in other populations.

The variability of the pharmacokinetics could not be explained completely by the model. Therapeutic drug monitoring still remains important in order to adjust dosing.

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O9
Abbreviated cyclosporin A pharmacokinetic profiles in paediatric renal transplant recipients as predictors for the risk of acute rejection episodes

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Cyclosporin A (CsA) is a cornerstone of immunosuppressive therapy in pediatric renal trans-plant recipients, but its therapeutic index is narrow. Serious clinical consequences may occur because of underdosing or overdosing. Hence, individualization of CsA dosage by therapeutic drug monitoring is indisputable. The current focus of cyclosporin A monitoring in adult trans-plantation is on the early portion of the CsA area under the concentration-time curve (AUC), particularly in the first four hours postdose designated as AUC0-4 and on the blood concentra-tion 2 hours postdose (C2) as a highly predictive marker for AUC0-4. Unfortunately, results obtained in adults may not simply be transferred to the pediatric population due to different pharmacokinetic characteristics in children.

Full-time (12 h) and absorption profiles (AUC0-4) of CsA were obtained in 61 pediatric renal transplant recipients aged 3.2 to 17.4 years on an immunosuppressive triple regimen with CsA, mycophenolate mofetil and methylprednisolone. CsA dosing was based on body surface area and adjusted to CsA trough levels. Pharmacokinetic (PK) profiles were obtained 1 and 3 weeks and 3 and 6 months posttransplant. Patients with an AUC0-4 < 4400 µg × h/L at both PK sampling periods in the first 3 weeks posttransplant had an adjusted relative risk of 48.4% to suffer an acute rejection episode (ARE), whereas in patients with at least one AUC0-4 above this threshold the adjusted relative risk for an ARE was only 13.1% (P<0.02). The single PK parameters C0 or C2 did not discriminate between patients with and without acute rejection. The PK parameters C1.25 (r^2 = 0.64) or C2 (r^2=0.60) showed a stronger relationship to the ab-sorption profile than C0 (r^2=0.15). An abbreviated profile consisting of the PK parameters C0.5;2 or C0;0.5;2 showed the closest correlation to the absorption profile (r^2 = 0.89) and the lowest percentage prediction error. These data indicate that absorption profiling in pediatric renal transplant recipients has the potential to optimise immunosuppressive therapy with CsA in order to reduce the incidence of AREs.

Acknowledgements: On behalf of the members of the German Study Group on Pediatric Renal Transplantation

O10
Dose-finding study of ibuprofen in patent ductus arteriosus

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Objective: Intravenous ibuprofen has been found as effective as indomethacin and associated with less side effects for treating patent ductus arteriosus (PDA). The dose regimen used (10-5-5 mg/kg/day) was based on few pharmacokinetic data and the efficacy in extremely immature infants has not yet been adequately addressed. This study was designed to determine the minimal efficient dose regimen (MEDR) of ibuprofen (1 course) required to close PDA. The success was defined according to a target closure rate and gestational age (GA): 80% closure in 27 to 296/7 weeks GA neonates and 50% closure in < 27 weeks GA neonates.

Methods: A dose-finding double-blind study using the continual reassessment method was performed in 40 neonates (20 in each GA group) with PDA (echocardiography), between day 3 and 5 of life. Four different dose regimens were tested: a loading dose (5, 10, 15, 20 mg/kg) followed by 2 doses ($^1/_2$ loading dose) at 24 hour intervals. The efficacy was evaluated on echocardiography 24 hours after the third infusion. The rate of ductal closure, the need for additional treatment and surgical ligation and the side effects were recorded.

Results: In 27–296/7 weeks GA neonates, the MEDR was 10–5–5 mg/kg with a 77% (95%CI: 56–92) probability of success. The 15–7.5–7.5 mg/kg dose regimen had a best probability of success (88%); but minor renal effects appeared to be slightly more frequent. In < 27 weeks GA neonates, the MEDR was 20–10–10 mg/kg with a 54.8% (95%CI: 22.3–83.9) probability of success. Slight renal effects were more frequently observed than in  27 WGA group for all the tested doses. The conventional dose regimen gave very low a-posteriori probabilities of success (30.6%).

Conclusion: This study confirmed that the currently recommended dose regimen (10-5-5 mg/kg) of ibuprofen was associated with a high PDA closure rate (80%) and with little side effects in premature infants of 27 to 296/7 weeks GA. The failure rate was much higher below 27 weeks GA and higher dose regimen (20-10-10 mg/kg) could be envisaged to achieve a higher closure rate than with conventional dose regimen. However, larger phase 2 studies comparing the 2 dose regimens are warranted before recommending these doses for PDA closure.

O11
Development of a paediatric dosing regimen for sotalol

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Introduction: Antiarrhythmic pharmacotherapy with sotalol is often the only treatment alternative in neonates and infants with supraventricular tachycardia (SVT). A rapid onset of an effective and safe sotalol therapy is frequently prevented because of age-dependent interpatient variability in pharmacokinetics and pharmacodynamics and uncertainty regarding adequate dosing.

Methods and Results: We developed age-specific dosage guidelines for sotalol based on a population pharmacokinetic covariate analysis. Eighty-one sotalol plasma concentration profiles of pediatric patients with SVT (median age 0.76 yrs [range 0.03-41]) were analyzed after oral doses of 1.0–9.9 mg/kg. Interindividual differences in oral clearance and volume of distribution could largely be attributed to size differences, with an additional age-effect on clearance in children <1 year. Integrated pharmacokinetic/pharmacodynamic analysis indicated a higher sensitivity of neonates towards QTc interval prolongation compared to older patients. In a subgroup of 15 patients, half of the patients converted into sinus rhythm at sotalol trough levels of 0.4 µg/mL and 90% at 0.6 µg/mL. Using these concentrations as start and target exposure, dosing recommendations (starting dose/target dose in mg/kg per day) were derived for five age groups: Neonates 2/4; infants <6 months 3/5; infants <2 years 3/6; children <6 years 3/5; children >6 years 2/4.

Conclusion: The development of a dosage guideline for sotalol treatment of pediatric SVT provides an example for rational drug dosage coping with interpatient variability in pediatrics. The approach provides a dosing framework that can be easily switched to an individually guided therapy based on effective sotalol trough levels.

O12
Ketoprofen excretion in human milk after intravenous administration after elective caesarean section

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Introduction: Ketoprofen is a NSAID administered in women after caesarean section to treat postpartum pain. Breast feeding is usually contraindicated, as data regarding milk transfer are not available. The present study was undertaken to quantify ketoprofen milk transfer after maternal intravenous administration of ketoprofen during post-partum.

Methods: After giving informed consent, 26 women receiving repeated intravenous bolus doses of ketoprofen (100 mg every 12 hours) were included. Repeated blood and milk samples were collected at steady-state over 12 hours and after extraction, plasma and milk concentrations of ketoprofen measured by high-performance liquid chromatography – UV detection using naproxen as internal standard. The lower limit of quantification for ketoprofen in plasma and milk was 20 ng/ml. Data analysis was performed as follows: the maximum dose that the infant would ingest was calculated assuming a rate of ingestion of 150 ml/kg/day. The result is given in absolute amount per kilogram and also expressed as a percentage of weight adjusted maternal daily dosing.

Results: Ketoprofen was detected in all plasma samples (n = 24) and in a limited number milk samples (n = 57/75). The peak concentration was 1214 ± 100 ng/ml [42 – 3367] at 2.5 hours in maternal plasma and 108 ± 100 ng/ml [20 – 177] at 4 hours in milk. Using the maximum peak concentration detected, the amount of ketoprofen that a sucking infant would ingest in a day is at the maximum 26 µg/kg/day (0.7% of the weight-adjusted maternal daily dose).

Conclusion: Small amounts of ketoprofen were found in the milk after maternal intravenous administration and we conclude that breast-feeding under ketoprofen at usual doses is permissible.

O13
Safety of psychotropic drug use during breast feeding: lack of evidence despite a lot of doubts

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Introduction: Mother's milk represents the most complete form of nutrition for the first six months of life. Psychiatric disorders have a higher prevalence among women than men and psychotropic drug prescription is greater in this population.

Evidence shows that the postpartum period is characterised by a high risk of emotional change: depression occurs in over 10% of women and puerperal psychosis in 0.1-0.2%, almost always requiring drug treatment.

Psychotropic use during breastfeeding is thus a significant issue and clinicians must consider the risks to the infant.

The problem is evident in the Regional Centre for Drug Information's (CRIF-Mario Negri Istitute for Pharmacological Research) experience where, between 2000-2003, psychotropic use during lactation was one of the most common queries (14%). In contrast with such a need, adequate evidence is lacking, and a systematic review of the literature was therefore performed.

Methods: A search was conducted in the MEDLINE (1967-present) and EMBASE (1975-present) databases. The *keywords* used included all categories of psychotropic agents, all specific substances, and breast feeding, lactation, drug milk level and breast milk.

Results: Of the 1040 articles retrieved, 495 focused on psychotropic drug use by nursing mothers. Reviews made up the majority of the collected references, while primary studies represented only one third of the total.

The most frequently investigated agents were anticonvulsants (43%) and antidepressants (36%).

Considering the 106 psychotropic drugs marketed in Italy, 44% had no data on breast milk excretion and, for 19%, available studies were dated more than 10 years ago. In all, no data was found for 60% of antipsychotics, while, on the contrary, studies were found for 65% of anticonvulsants. In the last decade, studies on 14/22 antidepressants were performed, while only scant data on anxiolytic agents (6/32) were collected.

Conclusion: It is not possible to evaluate the safety during lactation of nearly half of all psychotropic agents marketed in Italy. Furthermore, for a surprising 80% of anxiolytics, widely used drugs that have been available on the market since the '70s, no studies have been performed in the last ten years. The fact that more review articles than primary studies were retrieved also raises questions.

Further studies are needed to guarantee mothers who breast feed and their lactating infants safe psychopharmacological treatment.

O14
Antipyretic misuse in children: Determinants of excessive acetaminophen doses

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Purpose: Investigate factors affecting administration of supratherapeutic acetaminophen doses to children of two diverse cultural- ethnic backgrounds.

Methods: A structured questionnaire was administered to 101 Jewish and 100 Bedouin Moslem parents or usual caregivers attending a Pediatric Emergency Department with their children 0-5 years of age because of fever.

Results: Children's age was 20±17 months (mean ± SD); caregivers interviewed were the mother and/ or the father (97%) or the grandmother (3%). Most caregivers (65%) gave antipyretics for no or minimal elevations of body temperature, only 53% gave individual acetaminophen doses within 10% of the recommended dose (9–16.5 mg/kg/dose), 35% gave >16.5 mg/kg/dose, and 21% repeated the dose at intervals equal or less than 3 hours. Differences existed among the two cultural–ethnic groups in the source of knowledge regarding antipyretic use (P<0.001). More Bedouin than Jewish caregivers exposed their children to inappropriate antipyretic doses (P<0.001) and for temperatures equal or less than 38 degrees C (P<0.001). However, after adjusting for the cultural- ethnic background, administration of supratherapeutic doses of acetaminophen was influenced by use of rectal suppositories (OR 4.9, 95% CI 2.3-10.2, P<0.001) vs oral liquid preparations, and inversely by child's weight (OR 0.72 for each additional kg, 95% CI 0.6-0.9, P=0.002).

Conclusions: Lighter body weight and use of acetaminophen suppositories were risk factors for administration of supratherapeutic acetaminophen doses. Socioeconomic variables rather than the family's cultural – ethnic background contributed to acetaminophen misuse. Successful antipyretic educational programs should be targeted to the socioeconomic characteristics of the population, which may prevail in specific cultural- ethnic groups.

O15
The successful implementation of an ADR program at a children's hospital

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Introduction: The significant risk of adverse drug reactions (ADRs) in children has been highlighted in recent years. Examples include chloramphenicol induced grey baby syndrome[1] and fulminant hepatitis associated with sodium valproate[2]. We describe the successful implementation of a comprehensive ADR program in a paediatric setting.

Methods: In December 1998, an ADR committee was formed at the Royal Children's Hospital in Melbourne, Australia, to develop comprehensive ADR reporting and feedback. The committee, which included a paediatric clinical pharmacologist, pharmacists and nurses, met monthly and discussed all reported ADRs. The service covered three city hospitals (total ~1000 beds) which cared for both women and children. A computerised reporting format was introduced and reporting incentives included an acknowledgment letter and several small rewards such as pens and chocolates. Follow-up was comprehensive. Clinicians received a full report and patients were sent alert cards (laminated, credit card size). All reports were forwarded to the national reporting agency. Potential allergic reactions were issued an interim alert card and followed by appropriate testing. The system was promoted within the hospital and feedback was obtained from both clinicians and patients.

Results: Over 5 years, this system resulted in tripling of ADR reports to 15 per month. While 50% of reports were thought beta-lactam allergic, only 5 actual penicillin allergies have been identified with the majority testing as non-allergic. Several protocols have been produced to reduce commonly reported ADRs such as vancomycin related red-man syndrome and diclofenac induced diarrhoea.

Conclusion: The establishment of a comprehensive ADR program has identified many avoidable ADRs and allowed implementation of management guidelines for both individuals and the institution. In Australia, hospitals are ranked on their reporting of ADRs (considered a key performance indicator). Over this period of time, the Royal Children's Hospital had moved from 11th to 4th in the national ranking. Other Australian hospitals are now modelling their ADR programs upon our successful one.

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O16
The evolution of the European paediatric clinical trial register: DEC-net

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Introduction: A review of the better known clinical trial registers showed that none focuses on paediatric medicines and that few paediatric studies are included in the databases. Furthermore, the registers were often not equipped with suitable search options for retrieval of paediatric studies. This situation is mostly due to the fact that paediatric studies are few, but also to the fact that not enough importance is given to making paediatric drug data available.

DEC-net* is a European paediatric clinical trial register that is being set up by members from four countries, Italy, France, Spain, and UK. The project began in 2003 and is the first register dedicated to paediatric trials. Its main objective is to aid the dissemination of clinical trial results to ensure that drugs used in children are evaluated properly with respect to safety and efficacy. The register aims to become a stepping-stone for planning new studies, promoting collaboration among researchers, preventing trial duplication and inappropriate funding, and facilitating patient recruitment to trials by providing easy access to information.

Methods: The four participating groups have collaborated closely in carrying out the project. The programmers, who are part of the Italian group, designed the online input form taking into consideration all cross-checks, coded answers, and multilingual database options agreed on by the members in order to reduce errors and decrease input time and are currently perfecting the register's search and retrieval modules. The members have been contacting ethical committees, research associations, etc., to ask for collaboration in sharing trial data to make the register as representative as possible of their national paediatric situation and have been inputting data.

Results: To date, the group has created the register and DEC-net website, www.dec-net.org, which acts as the foundation for the project's different aspects, hosting the register and providing useful, updated information.

Conclusion: The first paediatric clinical trial register is well underway and the participants hope that other countries will join in the future and that the register will represent the first step in moving Europe (at least) towards greater awareness of the need to prioritise paediatric drug information.

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O17
Sevelamer (SV) vs calciumacetate (CA) in children with chronic renal failure (CRF)

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Objectives of Study: SV, a calcium-free phosphate (PO4) binder, has been widely used for the treatment of hyperphosphatemia in adult hemodialysis (HD) patients. Here we present results from the first European multicenter, open-label, randomized, cross-over study comparing the efficacy and tolerability of SV versus CA in children with CRF.

Methods: Children (aged 0 to 18 years) undergoing HD, peritoneal dialysis or suffering from CRF with a GFR > 20 < 60 ml/min × 1.73 m^2 were randomized to the following treatment scheme:2 weeks washout, 8 weeks of either SV or CA treatment. Serum PO4, Ca and iPTH values were determined every 2 weeks and the dosage of the PO4 binder was adjustet if needed. Serum lipid and vitamin concentrations were determined at the beginning and end of each treatment period.

Results: In total, 43 patients (15 female) 12.7 ± 4.4 years of age were included. 20 discontinued the study (non-qualifying PO4 value after first wash-out: 4; kidney transplantation: 8; other: 8) and 23 completed the study. 3 more patients were not analysed because of non-compliance. Mean serum PO4 and Ca levels were similar in both groups at baseline and equivalence of SV and CA in lowering the serum PO4 levels was demonstrated. The increase of Ca under CA was statistically not significant. Total cholesterol and LDL-cholesterol (-32%) decreased substantially under SV but not under CA treatment. Effects of both treatments on fat- as well as water-soluble vitamins were comparable. The number of side effects did not significantly differ. CA had a significantly increased incidence of hypercalcemia (15,4% vs 3,3%), whereas acidosis was more frequent under Sv (36,7% vs. 3,8%).

n=18	SV		CA	
	Baseline	Final	Baseline	Final
PO4 (mmol/l)	2.62 ± 0.49	2.12 ± 0.36	2.53 ± 0.47	1.99 ± 0.36
Ca (mmol/l)	2.45 ± 0.23	2.40 ± 0.19	2.42 ± 0.21	2.53 ± 0.28
Cholesterol (mmol/l)	5,99 ± 4,34	4,48 ± 1,94	5,98 ± 4,24	5,38 ± 2,26
LDL (mmol/l)	2,63 ± 1,30	1,78 ± 1,1	2,88 ± 1,38	2,76 ± 1,40

Conclusion: Treatment of children with CRF with SV provides adequate PO4-control without increased risk of hypercalcemia. The marked reduction of lipid levels and the lower rate of hypercalcemic episodes may augment the long-term beneficial effect of this treatment.

O18
Concerted NO-EDHF action: A new potential mechanism for ductus arteriosus patency

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There is evidence that nitric oxide (NO), formed spontaneously or in response to appropriate stimuli, may act in concert with prostaglandin E2 (PGE2) to maintain patency of the ductus arteriosus. To better define this proposed function for NO, experiments were carried out in the isolated ductus from wild-type and NO synthase (eNOS and iNOS)-deleted mice[1]. L-NAME (100 µM) contracted the wild-type preparation (0.36±0.03 mN mm-1) and its effect remained virtually unchanged following deletion of iNOS (0.50±0.13 mN mm-1). Conversely, a smaller L-NAME contraction was seen with the eNOS (-/-) ductus (0.17±0.04 mN mm-1). Bradykinin (Brady, 10 pM–10 µM) relaxed dose-dependently (max 39 ± 4 %) the ductus precontracted with indomethacin (2.8 µM), and this response required an intact endothelium to occur. No obvious change was noted in the Brady relaxation between wild-type and NOS-deleted preparations. Furthermore, Brady action was not affected by L-NAME, alone or in combination with zinc protoporphyrin (ZnPP, 10 µM). However, when excess potassium (K+) was added to L-NAME and ZnPP, there was a partial (K+, 20 mM) or complete (K+, 55 mM) inhibition of Brady relaxation in the wild-type preparation. Similarly absent, or greatly reduced, was the Brady relaxation in preparations pretreated with TRAM-34 (1 µM) plus apamin (0.2 µM). We conclude that the mouse ductus generates NO at rest, partly via eNOS. Brady relaxation, however, is not sustained by either NO or carbon monoxide (CO), but rather by endothelium-derived hyperpolarizing factor (EDHF). NO and EDHF qualify as effectors for prenatal patency of the vessel. (supported by the Heart and Stroke Foundation of Ontario, Grant T-3329; and MIUR Italy, FIRB no. RBNEO1W9 PM).

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O19
Identification and assessment of new pharmacological targets by human inherited disorders

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Inherited renal tubular disorders associated with hypokalemic alkalosis can be differentiated into two major phenotypes: (i) the classic Bartter syndrome (BS) and its hypocalciuric-hypomagnesemic variant, designated as Gitelman syndrome (GS), and (ii) the hypercalciuric hyperprostaglandin E syndrome/antenatal Barrter syndrome (HPS/aBS). Causative are defects of solute transporters and ion channels which lead to impaired salt reabsorption along the distal nephron. BS is caused by mutations in the chloride channel ClC-Kb. ClC-Kb is widely expressed along the distal nephron and mediates Cl-exit through the basolateral membrane into the blood. GS is caused by mutations in the thiazide-sensitive NaCl cotransporter (NCCT) which is exclusively expressed in the distal convoluted tubule. Mutations in either the furosemide-sensitive NaK2Cl cotransporter (NKCC2) or the renal potassium channel ROMK lead to HPS/aBS. Both, NKCC2 and ROMK reside in Henle`s loop. In addition, ROMK is expressed in the cortical collecting duct, contributing to urinary K+ secretion.

It is notable that two (NCCT and NKCC2) out of four affected proteins are target molecules of widely used diuretics. In order to assess whether ROMK and ClC-Kb are attractive candidates for the development of novel diuretics, we evaluated the clinical and laboratory findings of affected individuals. The study cohort comprises 40 patients, half of them carrying mutations in ROMK and ClC-Kb, respectively.

Individuals with defective ROMK invariably were born prematurely due to polyhydramnios (median 32 gestational weeks, range 28–36). The newborns developed severe salt wasting and volume depletion. Urinary concentration ability was abolished with maximal osmolality between 165 and 424 mosmol/L (median 316). Interestingly, potassium wasting is not typical in the majority of patients. In contrast, in 8/20 patients, transient hyperkalemia was found in the neonatal period. During follow-up the lowest serum potassium level ranged between 2.1 and 3.8 mmol/L (median 3.0). Urinary calcium was high (range 8.2–38 mg/kg/d, median 14) and within the first months of life all patients developed medullary nephrocalcinosis. Prostaglandin E formation was markedly elevated leading to systemic manifestation like fever, vomiting, and diarrhea in two thirds of patients.

The clinical signs of ClC-Kb patients are much more variable. Age of presentation ranged between first week of life and 11 years (median 2 months). In 5/20 patients, pregnancy was complicated by mild polyhydramnios. Isosthenuria was present in 1/20 only, hypercalciuria and nephrocalcinosis in two, hypocalciuria in six, and hypomagnesemia in eight out of 20. A consistent finding was severe hypokalemia (range 1.7–3.3 mmol/L, median 2.5). Because renal potassium losses could not sufficiently reduced by indomethacin high potassium intake was required in all patients.

Taken together, ROMK represents an attractive target molecule for a powerful diuretic with less hypokalemic side effects. Pharmocological targeting of ClC-Kb might result in a mixed furosemide/thiazide effect reflecting the current clinical practice of using the additive effect of furosemide and thiazides.

O20
Characterisation of four new ClC-Kb mutations associated with Bartter syndrome

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The term "Bartter syndrome" is used for a phenotypically heterogenous group of autosomal-recessive salt-losing nephropathies presenting with hypokalaemic alkalosis and low blood pressure. They have in common a disturbed transepithelial sodium chloride reabsorption in the distal nephron. Underlying mutations have been identified in different transporters such as the apical Na-K-2Cl-cotransporter (NKCC2), the potassium channel ROMK, the basolateral chloride channel ClC-Kb and its ß-subunit barttin.

We identified four different point-mutations of ClC-Kb in children suffering from Bartter syndrome (Q79R, G167S, P463delA, Y531X). To analyse the functional effect of these mutations wildtype and mutated ClC-Kb channels together with barttin were heterologously expressed in Xenopus laevis oocytes. We determined ion channel activity by two-electrode voltage-clamp analysis and channel surface expression by a luminescence reaction emanating from an extracellular HA-epitope inserted into the ClC-Kb channel protein.

In presence of barttin expression of wildtype ClC-Kb gave rise to voltage-independent chloride currents without rectification behaviour. As expected, the mutants P463delA and Y531X did not induce chloride currents different from non-injected oocytes. Significant chloride currents, though smaller than wildtype ClC-Kb induced currents, were observed upon expression of mutants Q79R and G167S.

In congruence with these findings the mutants Q79R and G167S reached the cell membrane, whereas P463delA and Y531X were retained intacellularly. In conclusion we have identified four new ClC-Kb mutations associated with Bartter syndrome and showed that different mechanisms impair ClC-Kb ion channel function.

P1
Spectrum and management of peritoneal dialysis associated peritonitis in children: Initial results of a global registry

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Peritoneal dialysis (PD) is the dialysis modality of first choice in pediatric end stage renal disease. Bacterial peritonitis remains the most relevant complication of PD. Despite a gradual decrease in incidence with improved equipment technology, sequelae of peritonitis remain the most common reason for PD failure in children.

Specific recommendations for the antibiotic treatment of peritoneal dialysis associated peritonitis in children have recently been developed by an expert group of the International Society for Peritoneal Dialysis (ISPD). The ISPD guidelines recommend stratification of empiric antibiotic therapy (1st generation cephalosporin vs. glycopeptide, combined with ceftazidime in all cases) according to risk factors for severe infection. Antibiotic therapy is then adapted according to the resistogram after receipt of culture results.

To assess the validity of these clinical practice guidelines, a global consortium of 54 pediatric dialysis centers (Europe including Turkey, 35; North America, 15; Latin America, 3; South Korea, 1) has started to report peritonitis data online in an internet-based registry. Since January 2002, 332 peritonitis episodes have been reported in 271 children.

In 28 % of episodes effluent cultures remained sterile. Of the culture positive cases, 22% were caused by S. aureus (3% MRSA), 21% by coagulase-negative staphylococci, 9% by streptococci, 5% by enterococci, 6% by other grampositive species, 35% by gramnegative bacteria and 2.5 % by fungi. Marked regional variation was observed for the incidence of culture-negative, fungal and coagulase-negative staphy-lococcal infections. In infants less than 2 years of age, initial clinical signs and symptoms tended to be more severe and gramnegative infections more frequent. Peritonitis due to candida, but not any other organisms, occurred more frequently in children carrying gastrostomies. Of those children in whom peritonitis occurred at home (89% of all episodes), 70% were hospitalised for a mean of 8 (± 6, range 1-36) days.

Of all peritonitis episodes, 76% presented with at least one risk factor qualifying for glycopeptide treatment (severe abdominal pain, 46%; history of nasal/exit site colonization with S. aureus, 26%; high fever, 21%; age <2 yrs, 11%; history of MRSA infection, 5%). Analysis of the treatment results awaits the accumulation of 500 cases. It is likely that then an assessment of the validity of the current treatment guidelines will be possible.

P2
Use of psychotropic medications in children and adolescents: a study from Italy

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Introduction: Although the available evidence concerning the safety and the efficacy of psychotropic drug use in the pediatric population, especially antidepressants, is scant, their prescribing to children and adolescents has increased significantly, as several epidemiological studies have documented. To date, data on the extent of psychotropic medication use in the Italian youths is scant. A drug utilisation study in order to evaluate the prescribing of psychotropic drugs to children and adolescents in Italy was therefore performed.

Methods: The prevalence of psychotropic drug prescriptions was estimated in a sample of 568,770 children <18 years old during 2002. Moreover, the trend in prevalence of psychotropic prescriptions between 1997 and 2002 was evaluated. Data were collected from the ARNO database, a system that merges information regarding out-of-hospital prescriptions, the population and the community setting into a single database.

Results: During 2002, 1947 children < 18 years old received psychotropic drugs (3.42‰). Antidepressants were prescribed to 1600 youths (2.81‰) were prescribed antidepressants, antipsychotics to 448 (0.79‰) and lithium to 33 (0,1‰). The prevalence rate of psychotropic drug prescriptions was significantly higher in girls than boys (Chi-square = 9.9; P=0.002) and increased with increasing age, with a statistically significant trend (P<0.0001). Selective serotonin reuptake inhibitors (SSRIs) were prescribed to 1200 antidepressant users (75%). All the SSRIs (the most prescribed was paroxetine) are unlicensed paediatric drugs with the exception of sertraline for obsessive compulsive disorder in children > 6 years of age.

The prevalence of psychotropic prescriptions increased between 1997 and 2002 with a statistically significant trend (P<0.0001). There was a huge increase in antidepressant prescription prevalence due to a 4.5 fold increase in the prevalence of SSRI prescriptions between 2000 and 2002 (from 0.47 to 2.11‰); the prevalence of antipsychotics also increased, but with a lesser extent.

Conclusion: Although the prevalence of psychotropic drug prescriptions in Italian children is lower than that reported in other countries, the increase in SSRI prescriptions raises some concerns. Data on safety and effectiveness of these antidepressants in pediatrics are still limited and further studies are needed to guarantee evidence based therapeutic approaches to children, adolescents and their families.

P3
A three-month prospective study of adverse drug reactions and drug utilisation on paediatric wards

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Introduction: In a previous study we observed ADRs in 13.8% of hospitalised children. Aim of the current study: Monitoring for ADRs using pre-specified definitions of ADRs and standardisation of methods, analysis of the documented medication prescribed for all hospitalised children with the diagnoses "respiratory infections" and "epilepsy" with regard to off-label drug use and ADR incidences.

Methods: Data collection between 01.03.2003 and 25.05.2003 on two general paediatric wards and the paediatric intensive care unit at Helios Klinikum Wuppertal (Germany). ADR collection by daily ward rounds, chart review, interview of nurses and physicians, parents and patients. Documentation of the ADR related medication as well as all medication of children with respiratory diseases and epilepsy in a Microsoft ACCESS database. Causality was evaluated by the WHO- and Naranjo-Score. Off-label use with regard to age was defined according to the German SPCs.

Results: 631 children were admitted during the study period, 199 suffered from an airway disease, 23 had a diagnosis of epilepsy. 65 ADRs occurred in 41 (6,5%) children, 25 of them had a respiratory illness and 6 an epilepsy. 45% of ADRs were mild, none was fatal. 57% of the ADRs consisted of gastro-intestinal, 11% of CNS symptoms. Antibiotics were responsible for 61% of ADRs, followed by antiepileptics (13%). A total of 1062 prescriptions were administered among children with respiratory diseases, 88 (8%) of them were off-label, concerning 44 children (22%): 12 with ADRs (48%) and 32 without ADRs (18%). Regarding frequently prescribed drugs causing ADRs in this collective, cefuroxime was considered to be responsible for ADRs by 14 of 63 treated patients (22%), Amoxicillin by 7 of 35 (20%), and Ampicillin by 6 of 33 (18%).

Conclusion: In contrast to our previous study we observed a lower number of ADRs (however a comparable spectrum of symptoms and causative drugs), possibly due to strict ADR definitions and raised vigilance. The potential relationship between off-label drug use and increased risk of ADRs needs further investigations. Antibiotics are still the major cause for ADRs in paediatrics and the indication for their prescriptions should be carefully monitored.

P4
Unlicensed and off-label drug prescribing in a paediatric tertiary referral centre

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Introduction: The off-label and unlicensed use of medicine in children is considered suboptimal. Recently there have been international efforts to improve the quality of the approved product information (PI) to include indications and dosage for children. A recent comprehensive review of Australian PI's indicated that up to 80% of licensed medicines had inadequate information for their use in children[1]. It is therefore important to continue to monitor the off-label and unlicensed use of medicine in children.

Objectives: To determine the extent, nature and reasons for off-label and unlicensed drug use on a medical ward in a tertiary referral paediatric centre

Method: A prospective survey of the medication charts of consecutive children discharged from a 48-bed paediatric medical ward was conducted over one-month. Prescriptions were evaluated for off-label and unlicensed use of drugs with reference to the approved PI. Reasons for inclusion of a drug in either category were recorded.

Results: One-hundred and sixty-one different medications were prescribed over 1133 prescriptions to 361 children. Forty-nine percent of prescriptions were for off-label or unlicensed use of drugs. Eighty-four percent of children prescribed a medication were prescribed at least one drug in an off-label or unlicensed fashion. The commonest single reasons for the off-label classification of prescriptions were differences in dose and dosing frequency from that suggested in the PI. The principle reason for the unlicensed use of drugs was alteration of formulation to allow administration to younger children.

Conclusion: Off-label and unlicensed use of medications is prevalent in tertiary level paediatric in-patient care in Australia. Variations in dosing or dosing frequency from those recommended in the product information account for a significant proportion of off-label drug use.

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P5
Are off-label drug prescriptions by paediatricians loyal to regulatory or guideline standards?

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Introduction: Although off-label (OL) drug use is common to all ages and healthcare settings, it is recognised that this type of use is not necessarily inappropriate. In the paediatric field, OL drug use is often inevitable because only adult therapeutic data exists. It is therefore evident that the problem is related to an inadequate drug evaluation and registration process.

The authors assessed OL drug use in the Italian paediatric community setting and analysed a sub-sample of the prescriptions, distinguishing between non-compliance with regulatory standards (i.e. OL use) and non-compliance with guidelines, in order to determine approriateness of prescribing.

Methods: Information on all patients visited by 35 general paediatricians in southern Italy in a 13-week period was collected and their prescriptions analysed for OL status. Pharyngotonsillitis was chosen as a sample indication for evaluating the approriateness of off-label prescriptions based on a gold standard (five sets of guidelines).

Results: Information was collected on 9917 patients (8476 prescriptions). In all, 14% of prescriptions were OL. When the 1675 prescriptions for pharyngotonsillitis were analysed, 8% were OL and 63% were not in accordance with the guidelines. On the other hand, 55% of these prescriptions did not adhere to the guidelines, but were not OL either.

Conclusion: OL prescribing exists also in the in the Italian community setting, although it is generally lower than in other European countries.

A few studies comparing OL drug uses with guideline recommendations found that they generally correspond, indicating that the quality of prescribed drug therapies is not necessarily related to the drugs' license status. In this context, the results of this study were surprising because the extent of OL drug use for pharyngotonsillitis, a common paediatric condition, was low, while non-adherence to guidelines was high, highlighting the fact that, although many prescriptions adhere to regulatory standards, they do not adhere to evidence-based guidelines. The reasons could be several, from outdated product licenses to un-preparedness of practitioners.

There is thus a problem in the lack of harmonisation between evidence and official information available to doctors (SPCs). It is essential that evidence available in the literature be made easily accessible to practitioners, especially by improving and up-dating product leaflets.

P6
Systematic review of randomised controlled trials of oral and IV medication in children 1996–2002

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Introduction: A gold standard for evidence based medicine is the double blind placebo controlled randomised controlled trial (RCT). These have been said to be difficult to perform in the paediatric population due to ethical considerations. We aimed to assess in what areas research is taking place in the

paediatric population, the design of these studies, and whether it was changing over the period of 1996-2002 in children (0-16 years).

Methods: RCTs were identified from the MEDLINE database. Details of study design, continent of origin, year of study, type of drug treatment and speciality area were obtained from the abstract. Only trials of oral or intravenous medications were included. The areas of oncology and HIV were excluded as much is known about research in these areas.

Results: 744 trials were identified spread equally over the years studied. 82% of studies were on purely the paediatric population and just over half were in children under the age of 6 years (58%). Abstracts were available for 728 trials (98%). The specialties most studied were general paediatrics 16.3%, infectious diseases 14.9%, neonates 12.9%, developmental paediatrics 11.7% and neurology 8.5%. Most studies originated from North America (41%) and Europe (30%). Over half of the trials were placebo controlled (52%) and a quarter drug versus drug trials (26%). Studies were double blind in nature in just over half of the cases (53%), a quarter were open (24%) and just under a quarter were unable to be determined from the abstract (20%). There was no significant difference by year in the number of double blind or placebo controlled trials. Most involved a single centre (74%) and the mix of single to multi centre studies did not significantly change with time. Only 7 trials (1%) were multinational. A third of studies looked at a known treatment indication (36%) and a third looked at new treatments (35%). Over a quarter of abstracts did not state the dose of the medication tested. Most studies looked at small numbers of patients with 60% involving less than 100 children.

Conclusion: RCTs are taking place within the paediatric population. There has been no change in the type of trials over the seven year period of 1996-2002. Most trials took place in North America and Europe, although this may reflect the database studied. Most trials involved a single centre.

P7
Salivary sampling method for therapeutic drug monitoring in newborns and infants

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Introduction: In order to achieve an efficient therapy combined with limited toxicity, an individual doses regime by using Therapeutic Drug Monitoring (TDM) is needed for newborns and infants. Frequent venepunctures are stressful, especially for newborns. An alternative to venepunctures is TDM via saliva sampling, available for some drugs. Salivettes® are approved for collecting saliva in clinical, but this method is not useful in young infants.

Methods: Pared salivary and serum samples were obtained from 2-days-to-7-weeks newborns (600–5,000 g) receiving digoxin (n=46), phenobarbital (n=23), or theophylline (n=14). Saliva was collected with special cotton wool sticks and was centrifugated in the same way as the Salivettes®. The drug concentrations were determined by using the TDx (Abbott) fluorescence polarisation immunoassay.

Results: The saliva-to-serum ratios for digoxin, phenobarbital and theophylline were determined with 0.92 ± 0.21, 0.37± 0.07 and 0.60 ± 0.10 respectively. A significant correlation (P<0.0005) between concentration of serum and saliva was observed for all drugs with correlation coefficients (r) of 0.887, 0.961 and 0.955 for digoxin, phenobarbital and theophylline respectively. A 30% deviation between the calculated serum concentrations and the measured serum concentrations was found in 8.7% and 7.1% of the phenobarbital and theophylline samples, but in 19.5% of the digoxin samples.

Conclusion: This study indicates that the salivary sampling method is reliable for TDM of phenobarbital and theophylline in newborns and young infants, however, only of limited usefulness for digoxin.

P8
Propofol 6% SAZN is safe as a sedative following major craniofacial surgery in infants less than 2

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Introduction: Infants admitted to an intensive care unit (ICU) often need analgesia and sedation to relieve pain and anxiety. Propofol is a short acting anesthetic often used as a sedative in adult ICUs and for procedures in children. Propofol as a sedative for prolonged infusion in children has become controversial due to the so-called propofol infusion syndrome[1]. Objective of this study was to determine safety of propofol 6% during infusion <24 hours after major craniofacial surgery in non-ventilated infants <2 years of age.

Methods: We conducted a prospective clinical trial including non-ventilated infants <2 years of age after major craniofacial surgery. Following informed consent, infants received either midazolam or propofol 6% for postoperative sedation if COMFORT behavior scores were >17. The COMFORT behavior scale was used as it is validated in the Pediatric ICU environment[2]. Triglyceride (TG) and Creatine phosphokinase (CK) levels were determined before, during and after infusion. High TG levels were defined as above 2 mmol/L and high CK levels at 300 U/L according to guidelines of our laboratory. To detect possible side effects of propofol, blood pressure (BP), saturation (ST), heart frequency (HF) and temperature (T) were determined hourly.

Results: Of 57 infants, 19 received propofol, 23 midazolam, 10 no sedatives at all, and 5 both sedatives. Median age of infants did not differ between the four groups and was 10 months (range 3 to 17). Median duration of infusion was 12 hours (range 6 to 17) for propofol and 14 hours (range 5 to 17) for midazolam. All TG and CK levels were below upper safety limits. During infusion of propofol, BP, ST, HF and T values remained within reference values for the age group.

Conclusion: After more than 12 hours of infusion, no significant increases in the triglyceride (TG) and creatine phospho kinase (CK) levels during propofol infusion were detected, neither adverse events were observed. These results demonstrate the usefulness of propofol 6% SAZN as a short acting sedative for non-ventilated postoperative infants less than one year of age.

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P9
Nephelometry to study compatibility of intravenous drugs in neonates

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Introduction: The need to administer different types of drugs by intravenous route, combined with a limited number of venous accesses in neonates necessitates caregivers to co-administer different drugs trough the same lumen. This practice might be hampered by the limited information on biochemical compatibility between different drugs.

Nephelometry is a technique in which changes in transillumination through a liquid solution and the grade of dispersion of light are used to test biochemical compatibility

since a more inhomogeneous solution will be associated with increased dispersion of light. An increase of the FNU (Formazine Turbidity Unit, water = 0.02 FNU) > 0.5 NFU is considered to be a marker of biochemical incompatibility. By using different drugs of various concentrations and various contact times, this in vitro setting can be used to simulate the in vivo situation. We here report on the use of nephelometry to study biochemical compatibility of tramadol (Contramal®) with other frequently prescribed drugs.

Methods: The initial concentration (50 mg/ml) of the commercially available vial had a FNU of 0.089 at time 0 and 0.052 at time 4-5 hours. Since this is the highest concentration used in the unit, this concentration was used to test compatibility of tramadol in combination (1/1) with either heparine (5000 IE/ml), doxapram (20 mg/ml), insuline (0.7E/ml), ranitidine (25 mg/ml), furosemide (10 mg/ml), T3 (0.02 mg/ml), hydrocortisone Na-succinate (50 mg/ml), isosorbidedinitraat (1 mg/ml), milrinonelactate (1 mg/ml), noradrenalinebitartraat (1 mg/ml), Adrenaline HCl 0.1 mg/ml, dobutamine HCl 12.5 mg/ml, dopamine HCl (40 mg/ml), midazolam HCl (5 mg/ml) or fentanylcitrate (0.05 mg/ml).

Results: Based on increased turbidity (> 0.5 FNU), incompatibility of tramadol with furosemide (462 FNU) and with midazolam HCl (9.33 FNU) were documented immediately following mixing of both drugs. All other combinations did not result in increased turbidity even if the contact time was increased up to 5 hours.

Conclusions: Nephelometry might be a useful tool to study biochemical compatibility of simultaneous administration of different drugs taking various concentrations and different contact times into account.

P10
Pharmacodynamics of methohexital in neonates

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Introduction: Methohexital is an ultra-short acting barbiturate that can be used for short procedural anaesthesia. In contrast to reports on its use in adults and children, there are still few data on pharmacodynamics (PD) of this drug in neonates.

Methods: Prospective observational evaluation of sedation (grade 1–4) and relaxation (grade 1–4) in two cohorts of neonates in whom methohexital was administered to enable either endotracheal intubation or chest tube removal (intravenous bolus administration of 2.6 mg/kg of methohexital). In addition, Near Infra Red Spectroscopy (NIRS) was used to document the effect on cerebral blood flow (CBF) and cerebral tissue oxygenation index, expressed by the Tissue Oxygenation Index (TOI) during and following methohexital administration.

Results: Sixteen infants were evaluated during endotracheal intubation (EI), 16 during chest tube removal (CTR) and NIRS was performed during 8 additional CTR procedures. In EI procedures, maximal sedation (grade 4) was documented for at least 2 minutes in all infants and all infants returned to baseline sedation (grade 1) within 10 minutes after administration. Maximal relaxation (grade 4) was achieved in all EI procedures for at least 2 minutes, with normalisation within 8 minutes after IV bolus. In CTR procedures, median sedation grade was 1, 3, 2, 2 and 1 before, 1, 3, 5 and 10 minutes after administration. Median relaxation was 1, 3, 3, 2 and 1 before,1,3,5 and 10 minutes after administration. Muscular twitching (n=6) or hiccups (n=6) were noticed as side effects. In 8 neonates, TOI remained stable during the first 4 minutes following administration but CBF decreased significantly at in the second minute following administration with subsequent return to baseline values.

Conclusions: 2.6 mg/kg seems to be an effective dose in neonates for short acting sedation and relaxation. This dose is higher compared to PD studies in older children and adults. These PD effects are short lasting (2-5 minutes) and the clinical evaluation of duration is much in line physiological (NIRS) measurements. Methohexital has a marked effect on cerebral blood flow but not on cerebral oxygen content (tissue oxygenation index), reflecting decreased oxygen consumption. Although not observed during both prospective studies, bronchospasms are a potential side effect, already noticed during routine use of this drug in the unit.

P11
Implementation of paediatric aspects into the global drug development process

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Introduction: Most modern drugs have not been adequately tested in children. The smaller children are, the less extrapolation of adult ADME data is feasible. In the 1960ies children were described as 'therapeutic orphans', but without consequence until the 1990ies. Today pharmaceutical industry is increasingly expected to consider the potential use of new drugs in children.

Methods: In 2002, implementation of pediatric aspects into drug development was assessed in Novartis by a cross-functional team representing clinical pharmacology, clinical development, technical development, preclinical safety and toxicology, registration, safety and epidemiology, marketing, project management and government liaison. A pediatric strategy was decided and an internal pediatric advisory group (PAG) was established.

Results: Principal guideline is ICH E 11 'Principles for Clinical Evaluation of Medicinal Products in the Pediatric Population'. Its main scenarios are (1) serious and life threatening diseases that affect primarily children, (2) serious and life threatening diseases that affect both adults and children, and (3) all other diseases. Scenarios 1 & 2 could justify early development in children. This would result in shifting several development steps to earlier stages, mainly preclinical safety & toxicology and a pediatric formulation. Each project team has to consider the potential use of the new compound in children. This requires careful balance of the potential therapeutic value, ethical concerns, inherent risks and business considerations. An internal training program on pediatric drug development has been initiated.

Conclusions: The decision to include children early into drug development requires a careful case-by-case analysis. In the complex and multidisciplinary modern drug development process the introduction of paediatric thinking is a major challenge even if no decision to initiate early pediatric development is made. It requires extensive internal training and organized exchange of internal knowledge & experience. Although the decision for early paediatric development will probably be the exception reserved for therapeutic brake-throughs, a careful analysis of paediatric aspects at several development decisions points is becoming integral part of drug development.

P12
Terlipressin as rescue therapy for intractable hypotension in children following septic shock

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Background: Intractable hypotension due to septic shock is associated with high mortality rates (40-60%) in critically ill children worldwide. Recently, vasopressin and terlipressin have been used as treatments for hypotension not responsive

to vasopressors and inotropes. We describe our experience with terlipressin as a rescue therapy in children with intractable hypotension.

Methods: Records of fourteen patients in sixteen occurrences of septic shock that were treated between January 2003 and February 2004 with terlipressin as a rescue therapy were reviewed. Data such as mean arterial blood pressure (MAP), heart rate, oxygenation index were retrieved.

Results: Shortly after beginning treatment, significant improvements in hemodynamic and respiratory indexes were noted. Blood pressure increased significantly (mean arterial blood pressure increased from 56±13.8 to 73±21.2 mmHG 1 hour after terlipressin administration, $P<0.05$). Oxygenation index decreased from 10.3±7.8 to 7.3±7.4 ($P<0.05$), 6 hours after beginning treatment and PaO2 increased from 95.4± 45.3 mmHg before terlipressin administration to 106.4± 77.9 mmHg ($P<0.05$) 6 hours after beginning of treatment. The dose of adrenaline was decreased or stopped in 8 patients as a result of terlipressin administration. Five out of 14 patients survived.

Conclusions: Terlipressin is associated with a significant improvement in hemodynamic and respiratory indexes in children with intractable hypotension due to septic shock. Further clinical, pharmacokinetic and pharmacodynamic studies are needed to prove terlipressin's effectiveness and safety in infants and children in shock.

P13
Pharmacogenetics of CYP3A5: effect of corticosteroids in children with acute lymphoblastic leukemia

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Introduction: The treatment of children with acute lymphoblastic leukaemias (ALL) according to EORTC protocol 58951 is divided into three phases (induction, consolidation, maintenance). During the induction phase, patients are randomly assigned to receive either prednisone or dexamethasone (associated with anticancer drugs). The response to corticotherapy is evaluated by the quantification of the residual disease at day 35 of treatment and has a major impact on prognosis and treatment: patients with an RD lower than <10-2/100 000 cells are classified as responders. Corticosteroids are both substrats and inducers of cytochrome P4503A isozymes (CYP3A). Among the three isoforms, CYP3A5 is under genetic control with 70% of Caucasians not expressing CYP3A5 and carrying 3 of the 7 mutations described (*2, *3, *6). In the present work, the impact of CYP3A5 genetic polymorphism on the initial response to corticosteroids was studied.

Methods: Children were included at the time of diagnosis. According to the EORTC protocol 58951, they received corticosteroids in a randomized manner. They were genotyped genotyped for CYP3A5 using a PCR-RFLP method and the residual disease was measured at day 35.

Results: 225 children were studied. Among them, 27 had a residual disease higher than 10-2/100 000 cells. The distribution of CYP3A genotypes was as follows: *1/*1: 2,7%, *1/*3: 18,8% and *3/*3: 78,5%, in agreement with already published data. CYP3A5*2 and *6 mutations were not found There was no difference in the response to corticosteroids according to individual CYP3A5 genotypes or to the corticosteroid administered.

Conclusion: In this preliminary study, which included 225 children, our results did not evidence an impact of CYP3A5 pharmacogenetics polymorphism on the initial response to corticosteroids, measured by the residual disease. Additional polymorphisms (drug metabolizing enzymes and transporters) will be tested, while increasing the number of patients studied.

P14
Effect of age on 6-MP metabolic profile during the maintenance phase in children with acute leukaemia

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Introduction: 6-mercaptopurine (6-MP) is a thiopurine analogue administered for the treatment of acute lymphoblastic leukemia. It is an inactive pro-drug that undergoes extensive metabolism resulting in the formation of active metabolites 6-thioguanine nucleotides (6-TGN) and inactive 6-mercaptopurine methylated metabolites (6-MMP) under the genetic control of the enzyme thiopurine methytransferase (TPMT). 6-MP metabolic profile (6-MMP/ 6-TGN) was proposed as a tool to optimize therapeutic response while monitoring the risk for drug induced toxicity.

Methods: 52 children treated according to the EORTC protocol 58951, aged 6.7; 4.1 years at the time of ALL diagnosis, were included at the time of diagnosis. TPMT genotype was determined before the initiation of treatment. During maintenance therapy, the individual daily dose of 6-MP (50 mg/m2/day) was adapted to maintain white blood cell count within the therapeutic range of 2000-3000 cells/mm3 and 6-MMP/6-TGN ratio was measured every month.

Results: 47 children were homozygous wild type for TMPT activity while 5 children were heterozygous. Due to TPMT pharmacogenetics, impact of long-term 6-MP metabolism on 6-MP metabolic profile during maintenance therapy was only analyzed in homozygous patients. As prognosis is dependant on age, patients were divided into two groups: younger than 6 years ($n=30$) and 6 years and older ($n=17$). A repeated measures analysis of variance showed that when the whole group of children was considered, 6-MMP/6-TGN ratio did not vary according to age ($P=0.14$) while a significant difference in the evolution of 6-MMP/6-TGN ratio was observed between the two groups ($P=0.02$) with repeated 6-MP administrations.

Conclusion: In the patients homozygous wild type for TPMT activity, and using a repeated measure model, changes in the 6-MMP/6-TGN ratio was significantly different between patients younger and older than 6 years. This could suggest a possible saturation of the 6-TGN pathway with repeated 6-MP administrations in the younger patients. The impact on 6-MP efficacy and toxicity remains to be evaluated.

P15
Severe salt wasting and deafness in a child with mutations in two chloride channels

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Salt wasting kidney disorders with hypokalemia and metabolic alkalosis ("Bartter syndrome") are clinically and genetically classified into several entities. The classic Bartter syndrome (cBS), the Gitelman variant of Bartter syndrome (GS) with underlying defects in the sodium-chloride cotransporter, NCCT, and the chloride channel, ClC-Kb, respectively, typically manifest during early infancy or adolescence. In contrast, the antenatal Bartter or Hyperprostaglandin E syndrome (aBS/HPS), caused by mutations in the sodium-potassium-chloride cotransporter, NKCC2, and the potassium channel, ROMK, is characterised by maternal polyhydramnios resulting in premature birth, and by severe volume depletion in the early neonatal period.

Recently, a new phenotype was identified within the aBS/HPS subgroup, in which severe renal salt wasting and sensorineural deafness are both present (also called BSND; antenatal Bartter syndrome with sensorineural deafness). As the underlying genetic defect, mutations in the BSND gene, coding for a beta-subunit (Barttin) of the chloride channels ClC-Ka and ClC-Kb, could be identified. This beta-subunit invariably associates with both chloride channels to allow for their correct membrane targeting in epithelial cells of both the kidney and the inner ear.

Here, we present a child with renal salt wasting and deafness, in which mutations in the *BSND* gene could be excluded. Rather, we were able to identify a digenic defect of the closely adjacent genes encoding for ClC-Ka (*CLCNKA*) and ClC-Kb (*CLCNKB*) consisting of a deletion of the complete *CLCNKB* gene in combination with a point mutation W80C in *CLCNKA*. For the newly identified ClC-Ka (W80C) mutant, severely reduced current amplitudes were observed in the Xenopus oocyte expression system.

In conclusion, this case provides strong evidence of genetic heterogeneity among patients exhibiting severe renal salt wasting and deafness and convincingly proves the regulation of ClC-Ka and ClC-Kb by their beta-subunit barttin. By providing deeper insight into the mutually compensating role of both chloride channels along the nephron, this observation may contribute to the development of a pharmacological inhibition of renal chloride reabsorption via ClC-K channels.

P16
Expression of potassium channel ROMK in fetal and adult human kidney

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The renal outer medullary potassium channel ROMK (Kir 1.1) is a crucial element of K+ recycling and secretion in the distal tubule. Mutations in the ROMK gene (KCNJ1) lead to hyperprostaglandin E syndrome/antenatal Bartter syndrome (HPS/aBS), a life-threatening hypokalemic disorder of the newborn. The localisation of ROMK channel protein, however, remains unknown in man. We generated an affinity-purified specific polyclonal anti-ROMK antibody raised against a C-terminal peptide of human ROMK. The antibody recognized a 45 kD protein band in human kidney tissue by immunoblotting. In human kidney sections, the antibody showed intense staining of epithelial cells in cortical and medullary thick ascending limb, connecting tubule, and collecting duct. Moreover a strong expression of ROMK protein was detected in cells of the macula densa. In epithelial cells of thick ascending limb expression of ROMK protein was mainly restricted to the apical membrane. In human fetal kidney expression of ROMK protein was detected only in distal tubules of mature nephrons. No expression was found in comma or S shapes. This observation indicates a differentiation-dependent expression of ROMK. In biopsy specimens of patients suffering from Bartter syndrome we observed ROMK expression dependent on the genetic defect. In summary these findings support the proposed role of ROMK channels in potassium cycling and in the regulation of K+ secretion and present a rationale for the phenotype observed in HPS/aBS patients with ROMK deficiency.

P17
Role of prostaglandin E2 receptor EP4 in furosemide induced salt losing tubulopathy

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Exaggered formation of PGE2 is a key symptome of hyperprostaglandin E syndrome/antenatal Bartter syndrome (HPS/aBS), an idiopathic renal disease characterized by NaCl wasting, water loss and hyper-reninism. Inhibition of PGE2 formation by cyclooxygenase inhibitors significantly improves patient mortality and morbidity, which gives evidence to a link between rise in PGE2 and manifestation of clinical symptoms in HPS/aBS. However, the pathogenetic role of PGE2 in HPS/aBS awaits clarification.

Chronic blockade of the Na-K-2Cl cotransporter NKCC2 by diuretics such as furosemide causes symptoms similar to HPS/aBS and provides a useful animal model. In control mice and in EP1-/-, EP2-/-, EP3-/-, and EP4-/- we determined effect of chronic furosemide application (7 days) on urine output, sodium and potassium excretion and renin secretion. Suppression in diuresis and electrolyte excretion was detected in EP1-/-, EP3-/- and EP4-/-, whereas the largest decrease in salt and water loss was observed in EP4-/- mice. No difference compared to wild type (wt) was observed in EP2-/- mice. Regarding renin activation following furosemide application a significant decrease was observed only in EP4-/-. Inhibition of EP4 receptors by the specific antagonist ONO-AE3-208, attenuated renin mRNA expression as well as plasma renin concentration. Further, application of the EP4 antagonist diminished diuresis and sodium excretion signifcantly. The glomerular filtration rate (GFR) by EP4-/- mice is not different to wt, indicating that the reduction in diuresis and salt loss is not a consequence of lower rate of filtration. In summary our data show that EP4 receptor is mediating PGE2 induced renin secretion and that EP1, EP3 and EP4 receptor are mediating PGE2 forced salt and water excretion. We suggest a significant role of the EP4 receptor in mediating the pathogenic effects of PGE2 in HPS/aBS.

P18
Paediatric reference values of eicosanoids in spontaneous urine samples

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Introduction: Eicosanoids (prostaglandines and leukotrienes) are important mediators involved in many disease entities (e.g. inflammatory bowel disease, asthma, rheumatoid arthritis, renal tubular disorders). The effect and metabolism of eicosanoids is affected by several drug, such as NSAIDs, diuretics and receptor-antagonists (e.g. montelukast). Therefore, eicosanoids can be used as biomarkers reflecting both - the activity of a disorder as well as the effect of the drug. Actually, the only published paediatric reference values are measured in 24 hour urine samples[1, 2] correlated to time and body surface area (BSA) (ng/hr/1.73m^2). This method is not helpful for the use in PK/PD-studies where rapid changes should be detected. The aim of the study was to establish reference values for the urinary excretion of eicosanoids in relation to the urinary creatinine-concentration (ng/mg Crea) in spontaneous urine samples.

Method: We measured eight eicosanoids (PGE2, PGE-M, 6-k-PGF1a, 2,3-dn-6-k-PGF1a, TxB2, 2,3-dn-TxB2, 11-dh-TxB2, and LTE4) and the creatinine excretion in every naturally urinary fraction given during the collection interval of 24 hours, and in the cumulated 24 hour urine sample. Included were 60 healthy children (28 females) between 1.1 and 16.5 years (median age: 11.4 y.). For the statistical evaluation, median, 10th, 25th, 50th, 75th, and 90th percentile of the eicosanoid concentration of overlapping fractions was calculated for every hour. The eicosanoids were analysed using gas chromatography tandem mass sprectrometry.

Results: 227 samples were collected (median of 3.5 samples per person). We found no circadian rhythm for all eicosanoids, except for LTE4 when the concentration was correlated to the creatinine concentration. There was no difference for age or sex for eicosanoids in the examined age-group.

Conclusion: Our results suggest, that eicosanoid concentrations in spontaneous urine samples in relation to urinary creatinine concentration are reflecting the excretion rate of eicosanoids adequate as compared with the excretion

rate in 24 hour urine collections correlated to time and BSA. Therefore measurement of eicosanoids in spontaneous urine samples may be helpful when used as biomarkers in PK/PD-studies where either the disease or the drug affect eicosanoid metabolism.

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P19
UDP-glucuronosyltransferase 1A6 activity in neonates

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Introduction: Data on maturational aspects of UDP-glucuronosyltransferase (UGT) activity in neonates are still limited and are mainly based on either continuous infusion of morphine or single dose paracetamol (acetaminophen, APAP). We therefore measured urinary metabolites of APAP in neonates during repeated admininistration of propacetamol to study the effect of

(i) postconceptional age (PCA), (ii) postnatal age (PNA) and (iii) repeated exposure to APAP using intravenous propacetamol as a probe drug to study UGT-1A6 activity.

Methods: APAP-glucuronide (APAP-G), APAP-sulfate (APAP-S) and free APAP were determined using HPLC in urine samples collected during repeated administration of propacetamol. Spearman rank correlations were used to study the effect of PNA, PCA and repeated administration on the the relative contribution of APAP-G to overall APAP elimination (APAP-T = G+S+free)

Results: 147 urine samples were collected in 23 neonates. Mean APAP-G/APAP-T ratio was 0.17 (SD 0.11). A significant correlation of APAP-G with PNA (r=0.5, P<0.0001), with PCA (r=0.23, P=0.0055) and with repeated administration (r=0.29, P=0.0005) was documented. Even after correction of PCA and PNA in a multiple regression model, correlation of repeated administration with G/T ratio still remained significant (P=0.001).

Conclusions: Besides PNA and PCA, repeated exposure to APAP also had a significant effect on UGT-activity, reflected by an increasing APAP-G/APAP-T ratio during repeated administration of propacetamol. These findings in neonates are in line with earlier reports on UGT-activity in adults during repeated administration. It might also explain the relative higher APAP-G/APAP-S (0.7) ratio in the study of Van der Marel et al since these authors also reported on APAP metabolism during repeated administration in infants (median age 11 months).

This observation further underscores the need to study different aspects of drug metabolism, including repeated administration, in this specific population.

P20
Intrathecal administration of GABA, glycine and their antagonist in neonatal rat pups

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Introduction: Noxious stimulation in infants causes 'whole body movements' which mature into individual responses localised to an arm or a leg .While there are a number of possible mechanisms underlying this lack of inhibitory control, our study focuses on the responses of the immature spinal cord to GABA and glycine. In mature neurones, the effect of GABA and glycine is hyperpolarizing (inhibitory) because the receptors are coupled to Cl- channels, and the Cl- equilibrium potential is more negative than the cell's resting potential, allowing Cl- to flow into the cell. However, in young neurones, intracellular Cl- is elevated, such that opening of the Cl- channels leads to outward Cl- flux and depolarisation (excitation).

Methods: Experiments were carried out on rats aged P3, P10, P21 (where P refers to 'postnatal day'). Intrathecal administration of GABA, glycine and their antagonists was performed under light halothane anaesthesia. The investigator administering the drug was blinded as to whether the solution was active drug or saline control. Following recovery from anaesthesia mechanical withdrawal thresholds were tested using Von Frey hairs, applied to the dorsal surface of the hind paw. After the testing period of one hour, the animals were sacrificed and dissected to assess the injection site.

Results: Intrathecal strychnine and bicuculline produced a reversible reduction in mechanical withdrawal thresholds in P21 rats, similar to that reported for adults. In rat pups of P10 and younger, strychnine and bicuculline raised mechanical thresholds. In contrast, intrathecal GABA produced a reversible sensitisation in the P10 and P3 rats, causing a lowering of their withdrawal threshold. This was also the case with glycine, although the effect was of a smaller magnitude.

Conclusion: These results show that the inhibitory neurotransmitters GABA and glycine have the reverse effect on spinal sensory processing in young pups compared to older rats. The implications of these findings for the care of human neonates are potentially far reaching. Many neonates on intensive care units receive infusions of benzodiazepines while on a ventilator, and it is possible that their actions in the youngest neonates differ markedly from those in older patients.

P21
P-Glycoprotein reduces the materno-fetal transfer of talinolol in the human placenta

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Objective: Placental syncytiotrophoblasts are known to express the efflux pump P-glycoprotein (P-gp) which is supposed to be an integrated part of the human "placental barrier". But only limited information on its pharmacokinetic significance is available, we studied the function of placental P-gp with the probe drug talinolol before and after administration of the P-gp inhibitor verapamil.

Methods: Dual closed loop in vitro perfusion of isolated cotyledons (n=13): A) Perfusion experiments in 4 phases (n=8): (1) stabilisation and control phase (1.5h); (2) addition of talinolol (0.8 µM) into first circuit (either maternal or fetal) and measurement of talinolol transfer (2h); (3) washout phase (0.5h) followed by; (4) addition of talinolol into the second circuit. The order of talinolol administration (first maternal or first fetal) was arbitrarily chosen. B) Materno->fetal transfer of talinolol was also studied with and without blocking of P-gp (verapamil 30 µM, n=5). Antipyrine and creatinine were used as reference substrates for intact perfusion conditions, without active transport properties. P-gp mRNA content of perfused cotyledons were measured using Taq-man real time PCR. P-gp protein were determined by immunoblot.

Results: The talinolol/creatinine permeability ratio was higher for the feto->maternal compared to the materno->fetal direction (0.66±0.19 vs 0.39±0.07; n=8; P<0.005). After inhibition of P-gp with verapamil talinolol/creatinine permeability was increased from 0.48±0.05 to 0.55±0.05 (n=5; P<0.05). There was no significant correlation between talinolol kinetics and P-gp or P-gp mRNA tissue content.

Conclusion: P-gp is a component of the human placental barrier which may protect the fetus from exposure to xenobiotics. Further transporters seem to be involved in the transfer of talinolol.

P22
Identification of apoptosis-modifying proteins in KELLY neuroblastoma cells

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Neuroblastoma is a neuroblastic tumor of the primordial neural crest and is the most common extracranial solid tumor of childhood. Tumors may regress spontaneously, reflecting induction of apoptosis or differentiation, or they may exhibit extremely malignant behavior with very low cure rates. The induction of cell death or apoptosis in tumor cells with malignant growth rates may be a valuable approach to treat neuroblastoma. We studied the induction of apoptosis in the human neuroblastoma cell line KELLY. Nonsteroidal antiinflammatory drugs (NSAID) are known to affect neoplastic growth in humans. In our model the addition of different NSAID caused a time and concentration dependent apoptotic death of neuroblastoma cells. The strongest effect was observed using flufenamic acid. Already at 50 microM flufenamic acid induced apoptosis determined by FACS-assisted DNA determination. To identify the molecular mechanisms responsible for the flufenamic acid induced cell death we studied protein expression in KELLY neuroblastoma cells. Using two dimensional gel electrophoresis combined with MALDI-TOF differentially expressed proteins were identified. We observed the induction of two proteins, HSP75 and p47 to be induced, and two proteins, HSC 54 and enolase 1alpha, to be suppressed within 3-6 h following addition of 500 microM flufenamic acid. HSP75, heat shock protein 75, is suggested to affect apoptosis. HSC54, heat shock cognate protein 54, belonging to the HSP70 family, is known to be expressed under conditions of cellular stress. p47 has recently been identified as a cofactor of p97-mediated membrane fusion. Whether p47 may play a role in cell survival or cell death is unknown. Enolase-1alpha is known to be induced in different types of solid tumors, however, the role of enolase-1alpha in tumor cells remains to be clarified. By means of RT-PCR and one-dimensional and two-dimensional immunoblotting we verified the differential expression of these four proteins. We suggest that the development of specific ligands modifying the activity of these four candidates may help to suppress neoplastic behavior of neuroblastoma cells.

P23
Role of keratinocytes in cutaneous drug reactions with sulfamethoxazole: Toxicity and metabolism

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Introduction: Cutaneous drug reactions (CDRs) are the commonest adverse drug reactions (ADRs). The anti-microbial agents with the highest reported CDRs are trimethoprim-sulfamethoxazole (TMP-SMX). 2% to 4% of immuno-competent patients receiving TMP-SMX have CDRs, a number which rises in the HIV positive patient population (40% to 60%). SMX is responsible for most of the CDRs. The role of keratinocytes (Kcs) in CDRs is poorly understood.

Methods: Kcs from the HaCaT line were incubated with increased concentration of SMX (0-3200 µM) and SMX-HA (0-1600 µM) and with different incubation times (2, 4, 6, 12 and 24 hours). After incubation, cellular viability was determined using an MTT cytotoxicity assay. Haptenation assay was performed by incubating 100 µM of SMX, SMX-HA and SMX-NO and 1% DMSO during 1 hour in HaCaT. A second haptenation assay was performed by incubating SMX 800 µM during 24 hours in HaCaT. Cells were harvested and lysed and SMX-protein haptenation was determined using Western blot analysis.

Results: There is a concentration- and time-dependent decline in cell viability of HaCaT with SMX-HA. At 800 µM at all incubation times maximum cell viability is 30.0% ± 2.3%. With the parent drug (SMX), declining cell viability begins at 12 hours and 200 µM. There is a more significant cell death after 24 hours incubation (at 800 µM, viability of 55.8% ± 7.7%, $P<0.05$.) For the haptenation assay with SMX and its metabolites, there is significant haptenation of proteins with SMX-HA and SMX-NO (bands between 30 to 80 KDa). In the SMX haptenation assay, selective haptenation of proteins between 30 to 80 KDa is seen after 24 hours which are comparable with SMX-HA and SMX-NO bands.

Conclusion: The time-course MTT assay in HaCaT cell line with SMX and SMX-HA demonstrates that Kcs are sensitive to sulphonamide reactive metabolites. With SMX, association of long incubation times and high concentrations with a decline in cell viabilitysuggests that the drug is metabolized to reactive metabolites by Kcs. SMX-HA and SMX-NO protein haptenation provide a potential mechanism for CDRs by presenting drug through MHC groove. The SMX 24 hour incubation suggests that the drug is metabolized in Kcs and then binds to protein which correspond to those found with SMX-HA and SMX-NO.

P24
Expression of cyclooxygenases and prostaglandin E2 receptors EP1 and EP2 in growth plate chondrocytes in vitro and in situ – PGE2 dependent proliferation of chondrocytes

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Prostaglandin E_2 (PGE_2) is important in bone development and metabolism. Although cultured chondrocytes were reported to produce PGE_2, both the identity of the cyclooxygenase form involved and the expression of PGE_2 receptors has not been clearly defined. Therefore, we examined the spatial distribution of COX-1 and COX-2 as well as EP1 and EP2 receptors in the growth plate in situ and in vitro.

PGE_2 synthesis was determined by mass spectrometry, cell proliferation by DNA [^{3}H]-thymidine incorporation, mRNA expression of cyclooxygenases and EP receptors by RT-PCR on cultured cells and in homogenized growth plates. To determine cellular expression frozen sections of the rat tibial growth plate or primary chondrocyte cultures were stained by means of immunohistochemistry using polyclonal antibodies directed towards COX-1, COX-2, EP-1 and EP-2.

Cultured growth plate chondrocytes transiently secreted PGE_2 into culture medium. It appears that both COX-1 and COX-2 contributed to PGE_2 formation, as both enzymes were expressed in chondrocytes in vitro and in vivo. Exogenously added PGE_2 stimulated DNA synthesis in a dose dependent fashion and gave a bell-shaped curve with a maximum at 10^{-8} M. The EP-1/EP-3 specific agonist, sulprostone, and the EP1-selective agonist, ONO-D1-004, increased DNA synthesis, whereas the effect of PGE_2 was suppressed by ONO-8711, an EP1-selective antagonist. The expression of EP1 and EP2 receptors in situ and in vitro was demonstrated by means of RT-PCR and immunohistochemistry. Although the EP2 antibody stained all chondrocytes in situ, the EP1 antibody spared the reserve zone cells. In cultured cells, EP1 was expressed in a subset of cells only. The most intense staining for the EP1 receptor was found in polygonal cells surrounded by matrix.

Based on these data we suggest that growth plate chondrocytes express COX-1 and COX-2 and are responsible for PGE_2 release, which stimulates cell proliferation via the EP1 receptor.

P25
Increased oxidative stress in children with dilated cardiomyopathy

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Introduction: This study evaluated oxidative stress in children with dilated cardiomyopathy. Oxidative stress appears

to increase in the failing myocardium and may contribute to ventricular dysfunction in patients with dilated cardiomyopaty

Methods: Twenty-nine children (17 male, 12 female, age range 7 months to 18 years) with dilated cardiomyopathy were included in the study. All patients included in this study had advanced heart failure (NYHA modified classification II to VI) and ejection fraction < 40%. A control group included twenty-nine healthy children subjects (17 male, 12 female, age range from 1 to 18 years). Blood sample was taken for glutathion reductase (GR), glutathion peroxidase (GSH-Px), catalase (CAT), superoxid dismutase (SOD), vitamin E and vitamin C.

Results: SOD (P<0.001) and GR (P<0.001), activity was significantly higher in children with dilated cardiomyopathy compared with healthy controls. CAT (P<0.001) and Vitamin C(P<0.001) activity was significantly lower, while GSH-Px (P<0.05) and vitamin E was unchanged.

Conclusions: We conclude that children with dilated cardiomyopathy exhibit abnormalities of a range of markers of increased oxidative stress. These data demonstrate a progressive increase in free radical injury and encroachment on antioxidant reserves with the evolution of dilated cardiomyopathy .They also suggest that oxidative stress may be an important determinant of prognosis.

P26
Single nucleotide polymorphisms of VEGF gene are associated with risk of congenital valvuloseptal heart defects

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Introduction: Altered expression of vascular endothelial growth factor (VEGF) has been suggested as a link between embryonic hypoxia and the development of congenital heart defects (CHDs). We examined the possible association of a functional genetic variance of the VEGF gene and the risk of CHD by investigating the prevalence of VEGF gene haplotypes (T-460C and G+405C) in children with CHD.

Methods: Dried blood samples were collected from 102 CHD children and from 112 healthy control neonates. Genotyping was done with appropriate PCR-RFLP (VEGF G+405C) and real-time PCR methods (VEGF T-460C).

Results: The prevalence of -460C did not differ between the CHD group and the control group. However, we found that the VEGF +405C allele was more prevalent in the CHD patients than in the control group (0.42 *vs* 0.21, P<0.001). VEGF +405GC and +405CC genotype presented independent risk of CHD (odds ratios [95% CI]: 1.45 [1.10-1.92], P=0.007 and 2.94 [1.43-6.04], P<0.00001, respectively). We found –460CT/+405CC haplotypes occurred exclusively among CHD patients (13/102 vs. 0/112, odds ratio [95% CI]: 2.26 [1.93–2.64], P<0.0001), while –460TC/ +405GG haplotype was associated with lower risk of CHD (16/102 *vs* 36/112, odds ratio [95% CI]: 0.58 [0.37–0.89], P=0.0064). The severity, location or complexity of CHD did not appear to be related to the VEGF genotype in our patient population.

Conclusion: Genetic polymorphisms and haplotypes of the VEGF gene, specifically those which alter the gene's production, may be associated with increased risk of congenital heart defects.

P27
Polymorphism of the oestrogen receptor-1 gene exerts gender-dependent effect on perinatal morbidity

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Introduction: Infants experience a sudden cessation of ooestrogen supply after birth. Assuming that premature cessation of high ooestrogen levels may have a role in the development of perinatal complications. We tested the association of a genetic polymorphism (SNP) of the oestrogen receptor (ER)-1 gene in immature infants with perinatal morbidity. This SNP may have an effect on the individual sensitivity to oestrogen.

Methods: 153 preterm infants (79 boys and 74 girls) were enrolled (gestational age 30+/-3 week, birth weight 1221+/-359 g). The allele prevalence was related to the healthy reference values obtained in 138 healthy term infants (86 boys, 52 girls). The ER-1 PvuII polymorphism was determined with PCR-RFLP methods. The association of genotype with perinatal complications was tested by chi-square test and stepwise logistic regression analysis.

Results: ER-1 PvuII prevalence was similar in preterm infants and in controls. It was not associated with intrauterine growth retardation. In preterm girls the ER-1 PvuII polymorphism was not associated with acute renal failure (ARF), necrotizing enterocolitis (NEC), retinopathy (ROP), cardiac failure, persistent ductus arteriosus (PDA) and sepsis. In boys, homozygote carriers of ER-1 PvuII SNP are at lower risk for NEC (odds ratio [95% CI]: 0.76 [0.58-0.98], P=0.045), PDA (OR: 0.36 [0.11–1.13], P=0.052) and cardiac failure (0.76[0.59–0.97], P=0.06). The associations between the PvuII polymorphism and PDA, NEC remained significant after the adjustment for risk factors.

Conclusion: We concluded that carrier state of ER-1 gene PvuII SNP is not associated with preterm birth, but it may have an impact on some perinatal complications in boys.

P28
Oestrogen substitution with evorel plaster during midpuberty

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Introduction: We have earlier shown that it is possible to mimic the spontaneous levels as well as the diurnal pattern of serum 17-beta-oestradiol in early puberty, by cutting a transdermal 17-beta-oestradiol matrix plaster and attaching a part of it, corresponding to 0.08-0.12 microg oestradiol/kg body weight, to the buttock nocturnally[1]. However, nocturnally application of higher doses mimicked the spontaneous nighttime concentrations of 17-beta-oestradiol seen in midpuberty. But, the levels during daytime remained low.

Objective: To see if it is possible to mimic the 17-beta-oestradiol concentrations seen in spontaneous midpuberty, by keeping half of the night dose during day.

Study patients: Two hypogonadal girls with induced puberty. Their breast stages were 3 and 2; weight 73 and 52 kg and ages were14.7 and 14.8 years.

Methods: A transdermal matrix plaster of 17-beta-oestradiol (Evorel), Janssen Pharmaceuticals-Cilag, Beerse, Belgium; 25 microg/24 hours) was cut into quarters (6.25 microg/24 hours). Two quarters were attached to the buttock overnight. In the morning, one of the quarters was removed and the other remained attached during day. Serum 17-beta-oestradiol was measured every 2 hours during 24 hours by radioimmunoassay with detection limit: 6.0 pmol/L[2]. The results were compared to the 17-beta-oestradiol concentrations seen in spontaneous midpuberty.

Results: The serum 17-beta-oestradiol concentrations mimicked well both the day and night time levels as well as the diurnal variation seen in spontaneous mid puberty.

Conclusion: By keeping half of the night-dose during day, it is possible to mimic the levels as well as the diurnal rhythm seen in spontaneous midpuberty.

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P29
Time-dependent cytotoxicity of reactive sulfonamide metabolites

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Introduction: ADRs are common and important adverse consequences of therapy that complicate the course of 5% of courses of therapy. Hypersensitivity ADRs are serious ADRs the mechanism of which is poorly understood. It has been demonstrated that reactive metabolites of the sulfonamides are important determinants of sulfonamide hypersensitivity. The mechanism(s) for this remain unclear.

Methods: Jurkat E6.1 cells were incubated with increasing concentrations (0–800 µM) of sulfamethoxazole hydroxylamine (SMX-HA) and 200µM parent compound sulfamethoxazole (SMX) over increasing incubation times (0– 24 hours). After incubation, cell viability was determined using a tetrazolium-based assay (MTT).

Results: Concentration-dependent toxicity was demonstrated at all time points (P<-0.05). There was an increase in toxicity from 0 to 4 hours, but there was no increase in toxicity associated with increasing incubation times (12.5 ± 3.5% viability at 800 µM SMX H-A at 4 hrs and 20 ± 4.5% at 12 hrs).

Conclusions: The demonstration of concentration-dependent toxicity is consistent with the potential role of reactive drug metabolites in hypersensitivity ADRs. The lack of increased toxicity with time suggests that reactive drug metabolites may have a threshold effect. The molecular determinant(s) of this effect are under study in our laboratory.

P30
Paediatric prescribing competency – can we assess it?

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Background: Children are more vulnerable to medication errors than adults due to doses being calculated on an individual patient basis, altered pharmacokinetic and pharmacodynamic abilities to handle and tolerate drugs and the lack of availability of suitable licensed paediatric drug formulations. Prescribing errors comprise a large proportion of these errors. Most studies looking at prescribing ability have focussed purely on basic calculation skills and failed to mirror the specialist clinical environment. This study aimed to develop a tool to assess practitioner's abilities to prescribe safely and accurately for children.

Methods: A tool was developed from a literature search, standard paediatric textbooks and drug formularies. It aimed to test prescribers' potential to commit errors commonly associated with paediatric prescribing. To assess the capacity of the tool to identify quantifiable differences in prescribing ability, it was distributed to multi-disciplinary paediatric health professionals who were asked to complete it within a time limit of 30minutes to simulate the pressures of the clinical environment. The tool comprised 6 multi-part questions and addressed a number of different areas of prescribing skills including calculation of doses, intravenous infusion volumes and rates, specialist drug knowledge including side effects, drug administration issues and prescription chart writing and review.

Results: Total scores ranged from 20 to 56 (maximum possible score 73) from 24 health professionals who completed the questionnaire. The tool demonstrated respondent's differing abilities in the specific areas of competencies it was designed to explore and identified a number of weaknesses in individual's prescribing ability.

Conclusions: A number of limitations of the tool were identified which will be used to inform further work in the development of this assessment aid e.g. re-design to allow electronic return of responses. The eventual aim is to develop a tool to evaluate the impact of prescribing training for medical students and junior medical staff on their ultimate ability to prescribe safely and effectively for children.

P31
Calcineurin inhibitor reduction after paediatric renal transplantation

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Introduction Nephrotoxicity of calcineurin inhibitors is an important factor in the development of chronic allograft nephropathy. We tested the hypothesis that reduction of calcineurin inhibitors in longterm immunosuppression is effective and safe in preserving allograft function after paediatric renal transplantation.

Methods

(1) We retrospectively analyzed the medical reports of 17 patients (median 14.3 yr after renal transplantation (range 8.3–17.6)). All patients analyzed were on ciclosporin A (CSA) monotherapy, before Mycophenolatmofetil (MMF) was started. After three months CSA was gradually tapered over a median of 12 months (4–37) reaching constant trough levels (C0) = 60 ng/ml.

(2) In a prospective pilot study 19 patients (median 4.5 yr after renal transplantation (1.0–11.0)) were treated with CSA or Tacrolimus (TAC), both in combination with MMF. Until now 10/12 patients have completed CSA reduction according to C0 levels from 100–150 to 50-70 ng/ml over 9 months. Until now 6/7 patients on TAC have decreased their C0 levels from 4–8 to 2–3 ng/ml. MMF was given in constant doses.

Results

(1) In the retrospective analysis median GFR (Schwartz) was 103.1 (37.3–125) ml/1.73 m2/min 12 months before MMF start, 66.1 (45.1–144.0) at MMF start, and 71.42 (51.1–150.7) after CSA reduction. One patient showed a borderline rejection.

(2) In the prospective study, median GFR for CSA patients was 101.8 (63.0-205.0) twelve months before enrolment, 108.7 (80.1–167.0) at the start of the study, and 95.0 (64.5–121.5) three months after the end of CSA reduction. In TAC patients median GFR was 73.53 (58.3–88.1) 12 months before enrolment, 69.1 (41.6–104.7) at the enrolment, and 55.9 (39.9–106.9) three months after the end of TAC reduction. In CSA patients the mean number of infections could be reduced from 2.6 ±0.5 during the first three months to 1.5 ± 0.3 during month 9 to 12 (P < 0.05). One CSA patient and one TAC patient showed signs of discrete interstitial rejection combined with signs of drug toxicity.

Conclusion In the retrospective analysis introduction of MMF and CSA reduction acutely improved transplant function. In the prospective study transplant function was stabilized with lower doses and C0 levels of CSA. TAC reduction could not stop the slow loss of renal function present in these patients anyhow. A low risk of rejection was seen. Long term observation and further randomized controlled trials are needed.

P32
Evaluation of the cardiac biomarker Nt-pro BNP in children to guide pharmacotherapy

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Introduction: More than 200.000 pediatric patients with congenital heart disease (CHD) in Germany from birth to adulthood need adequate diagnostic classification and medical treatment regarding their cardiovascular status. The natriuretic peptide BNP is synthezised in the ventricular myocardium. Elevation of BNP and of its inactive by-prduct Nt-pro BNP (N-BNP) in plasma indicates dysfunction of the cardiovascular system and serves as a biomarker to guide decisions concerning drug treatment. Reference values in a sufficient number of people are only established for adults with age dependent increasing values and about 84% higher values in women compared to men. To avoid misclassification concerning the cardiovascular status of pediatric patients, we investigated N-BNP in a large group of healthy children in relation to gender and age. These values should serve as reference values.

Methods and Results: Plasma concentrations of N-BNP were measured in 439 healthy subjects of both sexes (250 men and 189 women) with age ranging from 0 to 42 years without any cardiovascular disease, renal or hepatic impairment. Measurements were performed with competitive Enzyme Immuno Assay.

The mean N-BNP values in healthy children, adolescents and adults decreased from 12.6 (0-9 yrs; n=79) to 9.4 (10-14 yrs; n=154) to 6.1 (15-19 yrs; n=99) and to 4.8 fmol/ml (>19 yrs; n=102; each P<0.05). Mean N-BNP values concerning gender did not differ in any age group below 19 yrs, P>0.05). In contrast, in adults women had 78% higher N-BNP values compared to men (P<0.05).

Conclusion: Reference values for N-BNP differed profoundly in children compared to adults. In young children they were about 260% higher than in adults. In contrast to adults, gender plays no role in children for the N-BNP plasma concentrations. As a first step, these reference values will help to avoid isclassification of the pediatric population for N-BNP. As a next step, for a convenient and widespread use, e.g. in multicenter trials, inter-laboratory variability will be established for N-BNP.

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P33
Safety and efficacy of a hepatitis B immunoglobulin preparation combined with hepatitis B vaccination in neonates

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The risk of neonates born to HBsAg-positive mothers to acquire perinatal hepatitis B (hepB) infection ranges from 10 to 20%; viral persistence occurs in 80 to 90% of the infected children of whom 15 to 40% develop serious hepatic diseases like hepatocellular carcinoma, liver failure or liver cirrhosis. A combination of hepB vaccine and specific immunoglobulin has proven to effectively prevent HepB infection of the neonate in 85 to 90%. The hepB immunoglobulin preparation *Hepatect CP* has been used in adults and in children for years but is not licensed in neonates so far.

The aim of this study was to demonstrate, in newborn infants, safety of intravenous *Hepatect CP* and to give indication for clinical efficacy. In a prospective, open, phase III study performed in four centres in Bremen, 22 neonates born to HBsAg-positive mothers received intravenously a single dose of 100 IU (2 mI) *Hepatect CP* within 12 hours after birth. Simultaneously, active immunisation with recombinant hepatitis B vaccine was carried out. The small number of subjects was considered sufficient due to the existing clinical experience with this preparation in adults and older children.

No serious adverse reactions were reported. Vital signs and safety laboratory variables, such as alanin and aspartate aminotransferase (ALT, AST, respectively) and bilirubin were all within normal range of this age group. Primary efficacy, as defined by serum anti-HBs concentration $\geq$ 100 IU/L 3 to 5 days after administration was demonstrated in 21 (95,5%) neonates with a mean of 267$\pm$72,9 IU/L (95% CI 78.2–99.2%). The one neonate who did not reach the predefined anti-HBs level, though, showed a clinically relevant increase from not detectable to 79 IU/L. Follow-up investigation after 4 weeks confirmed the absence of hepB infection.

The results of this study support the concept of combined passive-active immunisation against hepB infection in neonates born to HBsAg-positive mothers. The specific immunoglobulin preparation *Hepatect CP* was well tolerated and appears to effectively prevent viral infection after single administration in this age group.

P34
Pharmacoanthropology – an interdisciplinary challenge for adequate drug therapy in paediatrics

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Introduction: Pharmacoanthropology integrates medical, biological and social fields of research regarding drug use, drug response and toxicity in different populations. In the present review we analysed, if pharmacoanthropological approach could give an innovative input to the optimation of drug therapy in paediatrics.

Material and Methods: We reviewed the problem of drug use in paediatrics regarding the subject's maturation of biological and cognitive parameters and the cultural reflection of childhood.

Results: Medical research produces evidence for the maturation of physiological parameters during infancy and childhood. In this context for example, drug metabolism in the liver is well analysed. In addition, transport mechanisms of drugs in the intestine are the subject of intensive research. Even less information exists about the maturation, function and drug interaction concerning other barrier, such as the blood brain barrier, the endothelial-tissue barrier and the peritoneum, which could be simulated by in vitro models.

In the field of cognitive science have been considerable efforts to analyse cognitive and emotional development, along with the consequences for the health beliefs model in the pediatric population.

From an epistemological point of view it could be demonstrated that understanding of childhood is a parameter which depends on cultural and social history.

Discussion: The analysis of the functional postnatal maturation of organs and tissue should be integrated into auxological research which until now has focussed on traditional anthropometric parameters. The specific problems of drug interactions in children open up new interesting aspects for the use of in vitro models of the blood-brain barrier, peritoneum and blood vessels. The understanding of cognitive and emotional development should influence medical practice and research, especially regarding the development of decision making and ethical judgement in children.

Conclusion: Pharmacoanthropology represents a field of integrated and interdisciplinary research, which allows a comprehensive understanding of the paediatric patient, with consequences for an adequate drug therapy in this population.

P35
Born small for gestational age: A new indication for growth hormone therapy?

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Introduction: Population studies indicate that 5% of newborns are born small for gestational age (SGA) due to intrauterine growth restriction (IUGR). Most of the SGA children (birth weight and/or length below -2SD scores of the population mean or below the 3rd percentile) fail to exhibit catch-up growth in association with various alteration of the action of the GH-insulin-like growth factor I (IGF-I) axis. The availability of human recombinant growth hormone (h-GH) offers a new opportunity to improve height and/weight velocity in short children born SGA with persistent growth failure. This study was initiated to analyse spontaneous growth pattern of children born SGA, however we did not aim to assess efficacy of h-GH therapy.

Methods: Questionnaires were sent to caretakers of children born between January 1st, 1998 and December 31st, 2001 at our department with birth weight below the 10th percentile. Growth charts of 63 children (mean age: 3.2 years, SD: ± 1.1; 30 girls and 33 boys) have been analyzed. Proportion of the children with slow height and/or weight velocity (below 10th and 3rd percentile) was determined.

Results: Of the 5849 infants born during the observation period 308 (5.3%) had birth weight below the 10th percentile, while general incidence of SGA was 4.1%. Vast majority of these children (85.7%) was proportionally small size in weight and length; no gender difference was observed. Growth charts revealed that 27% of the children failed to cross the 10th height percentile curve and 15.9% of them remained below the 3rd percentile. Interestingly, boys born SGA were less likely to exhibit catch-up growth in comparison with girls; 33.3% of the boys remained below the 10th height percentile, 18.2% of them below the 3rd percentile contrasted with the 20% and 13.3% values of the girls. Weight-charts showed that 38% of children born IUGR failed to exceed the 10th, 35.4% the 3rd weight-percentile. Weight and height charts reached their plateau over the age of 3.5 years.

Conclusion: Our study proved enduring height deficit of children born SGA. These observations stress that upon h-GH therapy characteristics of the growth-charts, gender differences should be carefully considered.

P36
Enteral administration of methylene blue in a preterm infant

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Introduction: Methemoglobinaemia is an uncommon cause of non-cardiac cyanosis. Knowledge of potential etiologies and rapid recognition of this condition might be life saving. Nitrate-contaminated drinking water, inhaled nitric oxide, metocopramide, anilines, EMLA or benzocaine oinment are well-known inducers of methemoglobinaemia. We here report on a preterm infant in whom methemoglobinaemia and hemolysis developed after enteral administration of methylene blue.

Case report: A preterm infant (GA 31 weeks, 1135g) received 1 ml of methylene blue (10 mg/ml) diluted in 2 ml of normal saline by naso-oesophageal tube in an attempt to document a broncho-oesophageal fistula. Desaturation without clinical signs of respiratory distress was documented 25 hours after birth (18 hours after administration). Oxygen fraction increased up to 0.5 without effect on transcutaneous saturation monitoring while arterial oxygen tension raised up to 140-170 mmHg. In addition to the discrepancy between arterial oxygen tension and oxygen saturation, the infant also had signs of active hemolysis (Htc drop, hyperbilirubinaemia, increased LDH) and urine became green coloured.

Discussion: The use of methylene blue for diagnostic procedures is a often reported technique in pediatric surgery. In addition to these diagnostic procedures, it is also used as add-on medical therapy in severe sepsis or to treat acquired methemoglobinaemia (recommended dose: 1–2 mg/kg). At these low concentrations, methylene blue is reduced to leukomethylene blue in the erythrocyte. Leukomethylene blue is capable of nonenzymatic reduction of methaemoglobinaemia. At higher dose, methylene blue itself becomes an oxydant, resulting in methaemoglobinaemia, hemolysis and hyperbilirubinaemia. The natural transient reduction in NADH-dependent methaemoglobin reductase puts neonates and especially preterm infants at an increased risk for this side effect.

Conclusions: Methylene blue is a potential dangerous and noxious product, and its use to detect surgical conditions in neonates should be avoided. Methylene blue should be considered a potential cause of acquired methemoglobinaemia, even after enteral administration.

P37
Relationship between hyperglycaemia and retinopathy of prematurity in very low birth weight infants

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Introduction: Retinopathy of prematurity (ROP) is a multifactorial vasoproliferative retinal disorder that increases in incidence with decreasing gestational age. Approximately 80% of extremely low birth weight infants (ELBW) will develop some degree of the disease. At present hyperglycaemia is not listed among risk factors of ROP, however, recent studies revealed a possible causal association between hyperglycaemia and ROP. The purpose of this study was to evaluate the possible relation between glucose levels and development of ROP in very low birth weight (VLBW) infants.

Methods: A retrospective – case control study of all infants born at our department between January 1st, 2000 and December 31st, 2002 with birth weight of less than 1500 g and ROP developed was conducted. We analysed the data of 202 VLBW infants who survived the neonatal period (survival rate: 76.4%, mean gest. age: 29.1 ± 2.6 week, birth weight: 1142 ± 240 g). The incidence of ROP and hyperglycemia among VLBW infants was detected and odds ratio (OR) was calculated to investigate association between the 2 variables.

Results: The incidence of ROP and hyperglycaemia in VLBW infants was 34.5% and 17.2%, respectively. ROP developed in 80% of hyperglycemic infants, OR: 11.9 (CI: 4.5-32.5; $P<0.0001$). When ELBW infants were analysed separately ($n=64$, mean gest. age: 26.8 ± 2.0 week, birth weight: 846 ± 119 g, survival rate: 53.6%) we demonstrated a higher incidence of hyperglycemia among infants with ROP, OR: 3.8 (CI: 1.1-15.3; $P<0.05$). Similar association was observed in the subgroup of 1000-1499 g infants suggesting that hyperglycaemia is a risk factor or a cofounder in the development of ROP.

Conclusions: In this retrospective study we could demonstrate that glucose levels in the first month of life are associated with the development of ROP. Further studies are needed to answer the question: is hyperglycaemia pathophysiologically linked to ROP or just another marker of severity of illness in VLBW infants?

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Urinary tract diseases revealed after DTP vaccination. Key role of cytokine irregularities

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Introduction: Prophylactic vaccinations may sometimes shorten incubation period of some illnesses and/or convert a latent infection/inflammation into a clinically apparent disease. Cytokines play a major role in mediating inflammatory process in various clinical entities and also represent a potential source of tissue damage if their production is not well controlled.

Methods: Literature data cited in this work were selected to illustrate the aforementioned role of cytokines. Medical records of 13 infants and young children aged 3 to 65 months hospitalised over the last 24 years with full blown clinical symptoms of various urinary tract diseases following DTP vaccination were analysed.

Results: It was reported that *in vitro* the whole-cell vaccine induced significantly more pro-inflammatory cytokines production than did the acellular pertussis or diphtheria-tetanus-only vaccine. Because many of these cytokines down-regulate gene expression of major CYP450 and/or other enzymes with the specific effects on mRNA levels, protein expression, and enzyme activity, thus affecting the metabolism of several lipophilic substances, such as steroids, lipid-soluble vitamins, prostaglandins, and exogenous substances, their irregularities may eventually lead to the flare of latent diseases in some predisposed subjects. This is consistent with the dose-, and time-dependent marked increases in the hepatic mRNA expression for IL-1, IL-6, and TNF, as well as the significant depression in expression of mRNA and activities for liver isoenzymes of CYP450 reported after administration of the whole-cell DTP vaccine to mice. DTP vaccine also caused marked induction of INF-g coincident with the maximal inhibition of CYP450 levels, and increased inducible NO synthase mRNA expression, claimed by some authors as being responsible for down-regulation of CYP450 enzymes. Finally, genetic polymorphism of different interleukins (e.g. the constellation of TNF-a and IL-6 genetic variants, and/or the IL-10-592*A allele) may predispose some infants with subclinical infection/inflammation to a more than usually intense inflammatory response in urinary tract after DTP vaccination.

Conclusion: In some genetically predisposed subjects, DTP vaccination acting as an excessive stimulus may produce cytokine irregularities which cause CYP450 and/or other enzymes down-regulation and finally uncover latent urinary tract diseases.