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Comparative in vitro studies on different 6-mercaptopurine formulations for use in children

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6-Mercaptopurine (6MP) is used in the therapy of various diseases in childhood. However, there is no formulation available worldwide that is appropriate for children. In hospital pharmacies different approaches have been used to prepare formulations suitable for children. Due to the lack of experimental data, most pharmacists can only hope that their individual formulations are stable and suitable for paediatric use. We compared the drug dissolution of 6MP from seven different drug formulations. These included commercially distributed tablets that were divided, three different capsules, two different suspensions and a newly developed tablet. In our investigations on extemporaneously prepared formulations, the suspension had the best dissolution profile releasing equivalent amounts of the drug. The newly developed tablet containing 10 mg 6MP shows the most accurate dosing and a fast drug dissolution. It will allow the safe administration of 6MP to children.

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Introduction

6-Mercaptopurine (6MP) has been used for many years throughout the world as an antineoplastic agent in various life-threatening diseases such as acute lymphoblastic leukaemia (ALL), Non-Hodgkin-Lymphoma, and Crohn's disease. In the paediatric population, ALL is the commonest malignancy (30% of all cancer diseases) with an incidence of 1 in 30,000 children[1]. 6MP is used in almost all therapy protocols for ALL in childhood. In the German ALL-BFM 2000 study, for the induction phase the 6MP dose is 60 mg/m^2 body surface per day; for the consolidation phase 25 mg/m^2/day and for the maintenance therapy over 24 months 50 mg/m^2/day. In other therapy regimens, the MP dose is calculated by the body weight of the children, e.g. 2.5 mg/kg daily. The desired daily doses for the treatment of ALL in childhood may range from 7.5 mg to 125 mg according to the varying body surfaces or body weights (Figure 1) (personal communication, Schrappe M).

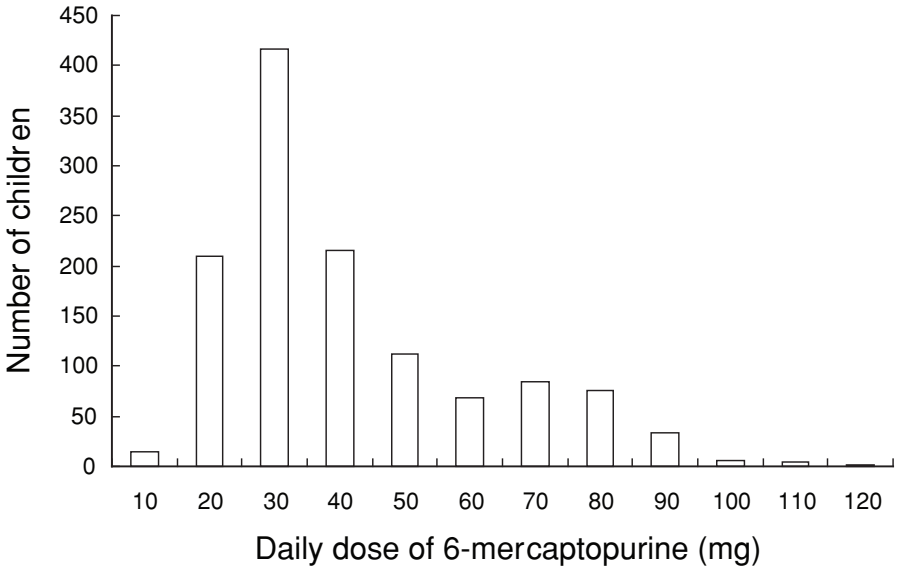

Figure 1 Daily doses of 6MP in the standard maintenance therapy of acute lymphoblastic leukaemia in childhood and the number of paediatric patients in the ALL-BFM 2000 protocol, Germany, 2003.

Today, the only 6MP product with a marketing authorisation is a 50 mg tablet (6MP monohydrate), distributed under the trademark Puri-Nethol® by GSK or Teva. It is clear from the prescribed doses in the ALL–BFM study, that for standard maintenance therapy, most children need doses other than the 50 mg tablet. Less than 10% of the treated children received either 50 mg or 100 mg as a daily dose. The majority of the children needed different doses which were dispensed by tablet splitting or by extemporaneous drug formulations prepared by hospital or community pharmacies.

In the UK, a solid formulation containing 10 mg 6MP monohydrate per tablet had been made available by GSK using an exceptional permission granted by the British regulatory office based on the "Medicines Act 1968". In 2000, GSK announced that they were stopping production of their irregularly licensed products in UK, including the 10 mg MP tablets. In August 2004, the last available batch ran out of the specified life-time. The discontinuation raised significant concern[2-4].

In recent years, the medical and pharmaceutical community have become aware that the off-label and unlicensed use of drugs in the paediatric population is common practice[5]. In the treatment of malignancies in children, 19% of all prescribed drugs are unlicensed and 26% are used in an off-label manner[6]. Unlicensed and off-label use is often caused by the lack of an appropriate drug formulation.

There are exceptional safety issues concerning the preparation of drugs for paediatric oncology[7]. It has previously been demonstrated that splitting 50 mg Puri-Nethol® tablets into pieces results in poor accuracy of dosing, ranging from 49 to 157% of the desired tablet mass[8]. Mass uniformity was not achieved, even if commercially available tablet splitters were applied by a pharmacist well experienced in the preparation of extemporaneous formulations. It was concluded from the study that there is a possible danger of contamination with cytotoxic and mutagenic dust for each individual who splits Puri-Nethol® tablets without protective measurements.

The aim of our study was to compare the various methods used for the preparation of extemporaneous formulations of 6MP for use in children. Tablet pieces, capsules and suspensions from two hospital pharmacies in Germany and a manufacturer of "specials" in the UK, Nova Laboratories, were tested and evaluated for dosage uniformity, handling, drug dissolution and stability during the storage and in-use periods. Two 6MP tablet formulations, the discontinued GSK tablet and a 10 mg tablet newly developed by our working group in order to replace the formerly "special" medication in UK, were included in the investigations.

Methods

Materials

Milled 6MP monohydrate was obtained by A.E. Tiefenbacher, Hamburg, Germany. It meets the requirements of both Ph. Eur and USP. 6MP USP (lot I-1, Phast, Homburg, Germany) was used as the reference standard. The impurity MP disulfide (97.3%) was purchased as certified in-house standard from Fermion Oy, Oulu, Finland.

Hypoxanthine was purchased from Sigma Aldrich Chemie GmbH, Steinheim, Germany, and used as laboratory standard for impurity testing as delivered.

Puri-Nethol® 50 mg tablets, lots 4C073 and 4C052 for the German market and lot 411497 for the British market, were purchased from GSK. Puri-Nethol® 10 mg tablets, lot A45741A, were supplied by the Paediatric Oncology Pharmacists Group, UK. 6MP 10 mg capsules (lot 0458n018) were obtained from Nova Laboratories (Wigston, UK) and capsules with 12.5 mg 6MP (lot 030904) by the hospital pharmacy of the University hospital in Münster, Germany. Capsules with splitted tablet pieces (lot 408E09) containing about 12.5 mg 6MP were delivered by Hohenzollern-Apotheke, Münster, Germany.

A suspension (lot 0378n022) was obtained from Nova Laboratories. Another 1% suspension containing potassium sorbate, citric acid, sodium chloride, glucose, traganth, Ora-Sweet syrup vehicle (Paddock, Minneapolis, USA) and purified water was freshly prepared according to a formulation regularly used in the pharmacy of the University Hospitals of Saarland, Homburg, Germany. The 10 mg 6MP tablets newly developed by our cooperative project group were used in the quality of batches 43001, 43002 and 43003 (Medice, Iserlohn, Germany).

Water and methanol for the analytical assay were used in HPLC grades. All other materials were of pharmacopoeia grade and were used without further purification.

Analytical assay

The determination of the 6MP content in solutions, suspensions and tablets and the identification of its impurities were conducted by high-performance liquid chromatography (HPLC) using a diode-array detector (DAD). The HPLC apparatus (Knauer, Berlin, Germany) was equipped with a K-1001 pump, K-2001 detector and Eurochrom software. A Lichrospher® 100 column, RP18, 125 x 4 mm (5 µm particle size) from Merck (Darmstadt, Germany) was used as the stationary phase.

The isocratic mobile phase, a methanol/water mixture (20:80), was pumped at a constant flow rate of 1 ml/min through the column at a temperature of 25 °C. The DAD signals were fully recorded between 200 and 400 nm using a two-dimensional map. Key detection wavelengths for peak integration were determined as 250 nm (hypoxanthine) and 326 nm (6MP and 6MP disulfide) revealing best precisions in the validation procedure.

Standard solutions (6MP USP, hypoxanthine and 6MP disulfide in-house standards) were prepared using the mobile phase composition. The injection volume was 20 µl. Tablets were dissolved in a volumetric flask (500 ml), in 20 ml water and 100 ml methanol, and isothermally stirred by ultrasound for 45 minutes. Subsequently, the resulting suspension was diluted up to 500 ml by adding water.

The analytical method was fully validated according to ICH Guideline Q2A, validation of analytical methods. Robust, precise, and accurate quantification of 6MP and hypoxanthine is possible in single HPLC tracks above the quantification limit of 100 ng/ml. The detection limit was 50 ng/ml. Impurities were confirmed by storage of the substances under stress conditions in 0.1 M hydrochloric acid, 0.1 M sodium hydroxide, and 30% hydrogen peroxide solution. Hypoxanthine and MP disulfide were identified as impurities in the starting material and as degradation products. Both were clearly separated in the standard HPLC run from the main peak and do not interfere with the quantification of 6MP but they were not quantified for each run. The precision of the method for the quantification of 6MP was 1.2% and the accuracy, determined via the recovery rate, 100.3%. Linearity could be shown between 50% and 150% of the labelled claim of 6MP (r=0.9995) and between 0.1 and 1% of hypoxanthine related to the 6MP content (r=0.9999). An additional method for the accurate quantification of MP disulfide was developed separately.

Dissolution testing

The release of 6MP from tablets into 0.1 N hydrochloric acid and water (volume 1000 ml, temperature 37 °C) was studied using the fully automated AT70 Smart dissolution tester from Sotax (Basel, Switzerland) in the paddle setup. The paddle was rotating at 50 rpm. The dissolution medium was continuously pumped through a flow-through cell in the UV-VIS spectrometer. Absorption was measured at 328 nm and related to 6MP USP reference standard. Six objects (tablets, capsules or equivalent samples from suspensions) were tested for each batch under the defined conditions. The validation of the dissolution procedure revealed that the stability of the drug was better in water than in hydrochloric acid.

Microbiological assay

The microbiological assay was performed according to the requirements of Ph. Eur. Monograph 5.1.4 "Microbiological quality of pharmaceutical preparations", category 3 (preparations for oral and rectal administration). The total amount of bacteria and fungi was determined according to the Ph.Eur. 2.6.12 monograph "Microbiological examination of non-sterile products (total viable aerobic count)" using the pour-plate method. The identification and quantification of *Escherichia coli* was conducted according to the Ph.Eur. 2.6.13 monograph "Microbiological examination of non-sterile products (tests for specified micro-organisms)".

Formulations studied

The prepared extemporaneous formulations with 6MP are of basically different nature. We received three different dosage forms (tablets, capsules and suspensions) from the hospital pharmacies and the "specials" manufacturer. Even the methods of preparing the same dosage forms vary dramatically which is an indication of the lack of knowledge about the best formulation of 6MP for children. Whereas the special manufacturer is preparing the capsules from the pure drug substance, the hospital pharmacies use the 50 mg Puri-Nethol® tablets because a pharmaceutical quality of 6MP is not available in small orders. In one hospital pharmacy, several tablets were mortared, diluted by a mixture of mannitol and anhydrous colloidal silica (Aerosil 200) and the calculated equivalent amount of powder was filled into hard gelatine capsule shells. In the other pharmacy, the 50 mg tablets were split into pieces and the complete tablet segments were filled into capsule shells.

Results

In the development of our 10 mg 6MP tablets we found that the dissolution of 6MP strongly depends on the matrix of excipients and the breaking strength of the tablets. It was therefore expected, that the dosage forms of the prepared extemporaneous formulations and their composition have a strong impact on the dissolution of the drug. Drug release is an important issue for 6MP formulations as the bioavailability of 6MP is only 16% with a high inter-individual variation between 5 and 35%[9].

Breaking the Puri-Nethol® tablet into segments results in highly variable doses of 6 MP. The span between the mass of tablet segments was between 15.96 mg and 47.15 mg which is 54 to 159% of the mean tablet mass (Figure 2). These results are consistent with earlier published data[8]. Cytotoxic dust of 6MP, up to 0.46% of the total tablet mass, is released by each splitting procedure. This might be a minor problem in preparing tablet pieces under protecting working benches in the hospital pharmacy, but it is of major concern in the domestic environment of the treated children.

The drug release from a solid drug formulation is determined by the dissolution test of the pharmacopoeias. The amount of drug released from the dosage form into an aqueous fluid, e.g. water or simulated gastric juice, is measured over time and is used as a predictor for the drug release *in vivo*. The dissolution profiles of individual segments yielded from Puri-Nethol® tablets varied significantly (Figure 3a). This could be due to the irregularity of the produced tablet segments but also to an inhomogeneous distribution of drug crystals within a tablet. In our study we could

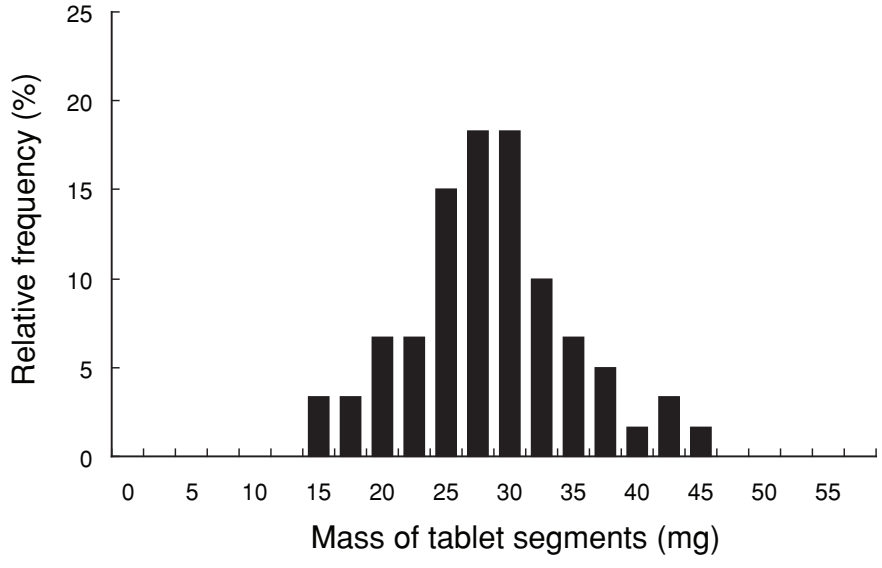

Figure 2 Mass distribution of Puri-Nethol® tablet quarters (n=60).

demonstrate that the differences in the release of 6MP were only related to the mass variation of the tablet segments as the normalisation of the individual dissolution profiles to the previously determined segment masses revealed high similarity of the drug release from each tablet segment (Figure 3b).

It could be concluded from the results that if a Puri-Nethol® tablet could be split into equal pieces, accurate doses and drug release profiles would be obtained. However, it is impossible to yield equal segments, even if a commercial tablet splitter is used. Therefore, some hospitals avoid the splitting of tablets and prepare capsule formulations for individual use in children.

In our survey on the different preparation techniques it became obvious that there are three methods used. The capsule content may consist of a powder mixture of the pure drug compound and various excipients in order to increase the volume and the flowability of the powder. As the drug substance is often not available in pharmacopoeia quality at a reasonable price, some hospitals use the Puri-Nethol® tablets as a starting material. Some of them crush the tablets into a powder that is diluted by inert excipients like mannitol to yield a lower dose of the active drug in each capsule. Other pharmacies split the tablets into segments and fill the segments in hard capsules, in order to prepare single doses and prevent the caregivers and the environment from contamination with the cytotoxic drug substance. In almost all cases, the capsule is opened before application and the capsule's content is administered to the child.

The different methods in preparing capsules result in different dissolution profiles (Figure 4). There

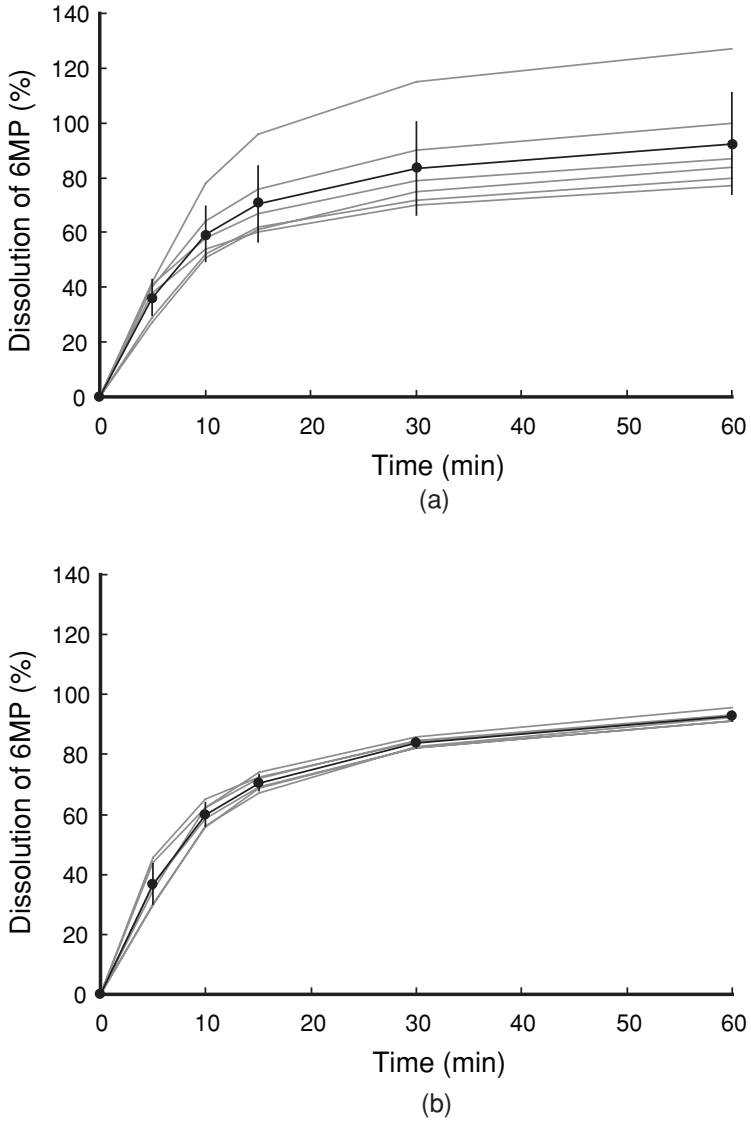

Figure 3 Dissolution of 6MP from upper left quarters of 50 mg tablets [Puri-Nethol®] (Paddle apparatus, 1000 ml purified water, 50 rpm). (a) Dissolved 6MP from tablet segments. (b) Dissolved 6MP, normalised by the mass of each tablet segment. –●– Mean dissolution from 6 upper left Puri-Nethol® quarters, lot. 4C073 (± SD).

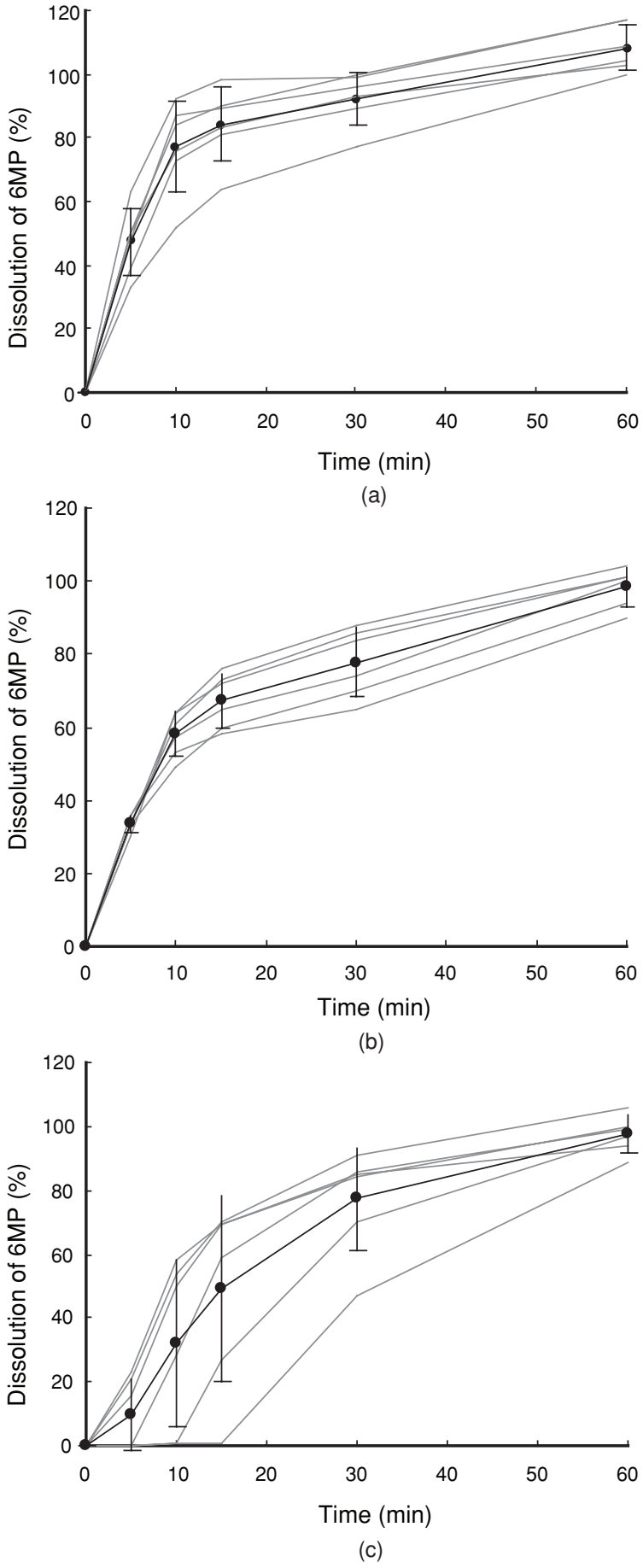

Figure 4 Dissolution of 6MP from capsule formulations (Paddle apparatus, 1000 ml purified water, 50 rpm, *n*=6, ± SD). Ensembles of six individual capsules and mean (–●–) dissolution profile (± SD). (a) Capsules made from 6MP starting material (Nova Laboratories, lot 0458n018). (b) Capsules made from crushed and mortared 50 mg 6MP tablets (lot 408E09). (c) Capsules filled with segments from 50 mg 6MP tablets (lot 030904).

seems to be no difference in the drug dissolution when using either a powder of excipients and drug obtained by crushing and mortaring tablets (Figure 4a) or the pure drug substance in a mixture with excipients (Figure 4b). However, highly variable dissolution profiles are obtained when the tablet segments are filled into gelatine capsule shells (Figure 4c). Lag-times up to 15 minutes were recorded. The content of 6MP in all capsule formulations was stable during the proposed usage time which is in accordance with recent stability data of other 6MP capsules[10].

Liquid formulations of 6MP are also promising drug formulations as they offer the possibility of individual dosing from a multiple-dose container. Due to the poor solubility of 6MP in water, suspensions have to be prepared. However, suspensions tend to physical and microbiological instability over time and should only be used after accurate investigations on the shelf-life stability and in-use stability. Unfortunately, only few data have been reported on extemporaneously prepared suspensions of 6MP yet.

In our survey, we found at least two different suspension formulations in use. Both suspensions showed good content uniformity when aliquots were withdrawn from the bottle after shaking. The drug release into the dissolution medium was fast (Figures 5a and 5b). However, the suspension from the "specials" manufacturer (Figure 5b) exhibited a lower content of the drug substance than a suspension (Figure 5a) that was produced from 50 mg Puri-Nethol® tablets, basic suspension medium and Ora-Sweet® for the University Hospital of Saarland.

It was unknown whether there was a lower content of the drug substance in the "specials" suspension at the time point of manufacturing or whether this was due to drug instability in the formulation. Therefore, we tested the stability of the self-prepared suspensions according to the formulation of the pharmacy of the University of Saarland. We used 50 mg Puri-Nethol® tablets from GSK and, for a second batch, 50 mg generic tablets. Neither significant differences in the content of 6MP nor variations in the HPLC chromatograms were found in either batch one month after production. The prepared suspensions were stored at room temperature. The drug substance shows high in-use stability for at least one month, the proposed usage time of a bottle for a paediatric patient. Up to now, drug stability of only 14 days had been demonstrated for storage stability of 6MP suspensions[11], but the stability of this formulation had not been investigated under in-use conditions.

Improved stability can be obtained by formulating a solid dosage form. Therefore, we developed small-sized, child-appropriate 10 mg 6MP tablets. They showed almost complete drug dissolution within 60 min and a very high dose uniformity (Figure 5c).

The microbiological investigation of the prepared suspensions revealed no growth of bacteria and fungi according to the test of the European Pharmacopoeia for oral drug formulations. The amount of colony-forming units (CFU) was below 10 per ml suspension both for bacteria (limit Ph.Eur.: 10^3/ml CFU) and fungi (limit Ph.Eur. 10^2/ml CFU). *Escherichia coli* bacteria were absent in the samples. Therefore, it can be concluded that the prepared extemporaneous suspensions made from 6MP tablets are chemically, physically and microbiologically stable and safe for use for at least one month.

Discussion

Different formulations of 6MP are used for the treatment of children with ALL. The only product with a marketing authorisation is a 50 mg tablet which is unsuitable for more than 90% of the affected children. There is an urgent need for alternative formulations for children that provide correct single doses and the protection of the caregivers from the cytotoxic drug.

In our study, we investigated five formulations (three capsule formulations, two suspensions) from different hospital pharmacies and "special manufacturers" and additionally a newly developed tablet formulation with 10 mg 6MP per tablet. The variety of dosage forms and drug formulations used in the pharmaceutical practice indicate the lack of availability of an ideal 6MP formulation for children. The different formulations exhibit highly variable results regarding stability and content uniformity. The best results were obtained for a suspension prepared extemporaneously from commercial tablets for adults. However, for a longer period of storage time, e.g. at the pharmaceutical manufacturer, the wholesaler, the pharmacy shop and at home, the tablet formulation with 10 mg 6MP seems to be advantageous. The tablets may have also some additional advantages in the clinical setting, as the dose uniformity of the suspensions in our study was obtained under optimal conditions and by experienced pharmacists. Dosing errors may occur by insufficient shaking before use or false measuring of single doses. If a child cannot swallow the tablets, even though they are very small-sized and rapidly disintegrating, the suspension may be an effective alternative.

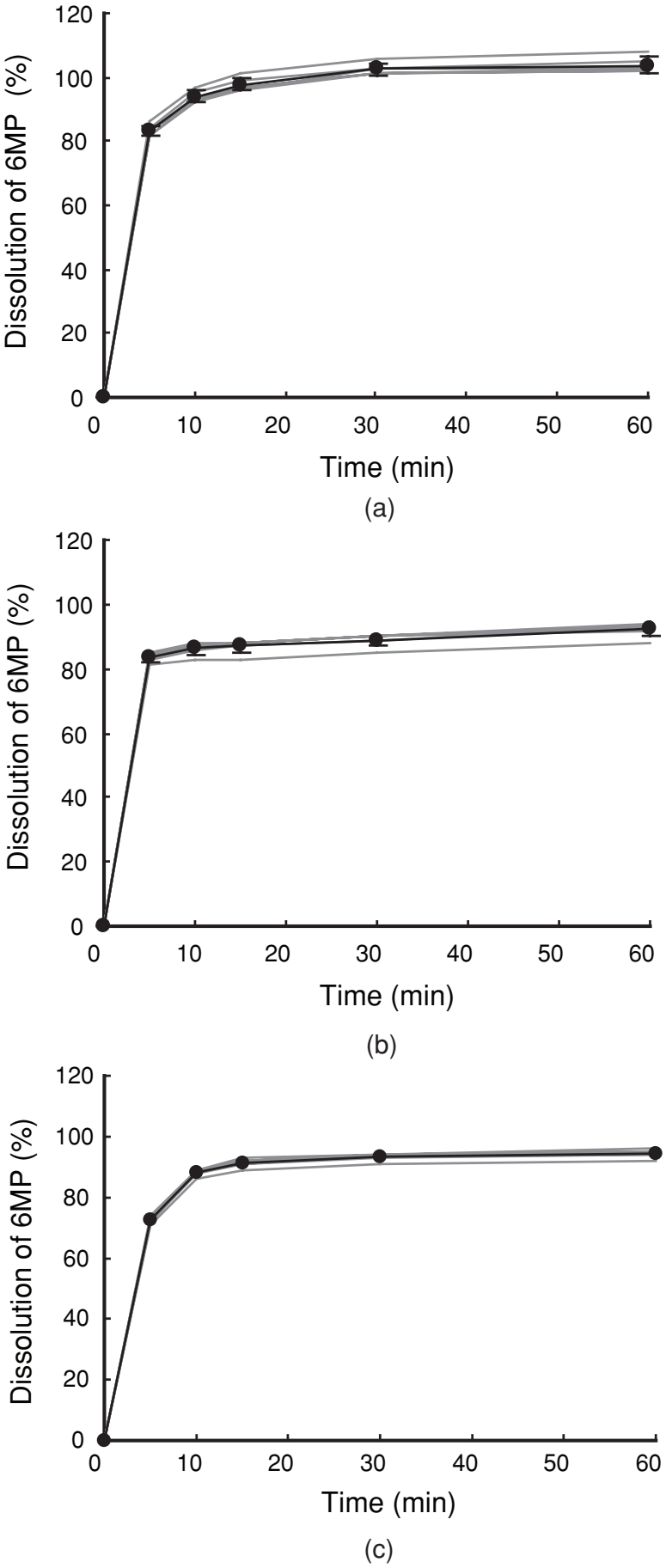

Figure 5 Dissolution of 6MP from suspensions and the new child-appropriate tablet (Paddle apparatus, 1000 ml purified water, 50 rpm, *n*=6, ± SD). Ensembles of 6 individual capsules and mean (–●–) dissolution profile (± SD). (a) Extemporaneously prepared suspension (lot PL 4110), 10 mg/ml 6MP. (b) "Special" product (Nova Laboratories, lot 0378n022), 20 mg/ml 6MP. (c) Newly developed small-sized tablets (lot 43001), 10 mg 6MP.

The newly developed 10 mg 6MP tablet has become available in the UK as a "special" medicine. Splitting tablets for adults into pieces and filling tablet segments into capsule shells should be avoided in the future. It is likely that the delayed drug dissolution may affect the systemic availability of the drug as the poor bioavailability of 6MP is attributed to both poor solubility and limited absorption, probably restricted by the presence of an active carrier transport system located in the upper intestine. The impact of delayed dissolution on the varying bioavailability of 6MP in humans has not been investigated yet.

The results of our study also indicate the urgent need for scientific based standard procedures for the preparation of extemporaneous formulations and for the development of drugs appropriate for children. An application for the marketing authorisation of the newly developed tablets has been submitted to make the new formulation available for the other children in Europe affected by ALL.

Acknowledgements

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