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A novel 6-mercaptopurine oral liquid formulation for pediatric acute lymphoblastic leukemia patients – results of a randomized clinical trial

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Key words
mercaptopurine – drug formulation – acute lymphoblastic leukemia – pediatric pharmacology

Abstract. Objective: Pediatric patients with acute lymphoblastic leukemia (ALL) are treated with oral 6-mercaptopurine (6MP) for nearly 2 years, but no pediatric formulation has been available. In this study, an oral 6MP liquid suitable for pediatric use was developed and tested in the target population. Method: A randomized cross-over study was performed in 20 pediatric ALL patients (age 1.9 – 14.6 years), comparing pharmacokinetics and pharmacodynamics of a newly developed 6MP liquid formulation to 6MP capsules, both taken orally for 4 weeks. Results: Based upon trough levels of the principal active metabolite, 6-thioguanine nucleotides (6-TGN), a relative bioavailability of the liquid vs. capsules of 1.01 was found (90% CI 0.86 – 1.20), demonstrating bioequivalence. This was supported by the similarly observed 6MP dosages needed for leucocyte depletion, for both formulations (35 mg/day (range 10 – 115 mg)). 75% of the parents/patients (p = 0.005) preferred the oral liquid over the capsules because of the ease of administration. Conclusion: We conclude that the novel 6MP liquid is a promising treatment for ALL.

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Introduction

Despite the increased legislation and incentives to stimulate drug research in children, many drugs are currently licensed without an adequate pediatric formulation, resulting in off label use of – often untested – formulation [1].

6-Mercaptopurine (6MP) is a drug which is only available as a tablet containing a fixed amount of 50 mg 6MP. 6MP is used as an oral cytotoxic drug in pediatric acute lymphoblastic leukemia (ALL) patients and is an essential part of the maintenance treatment [2, 3]. 6MP exerts its cytotoxic action through incorporation of its active metabolites into the DNA of leucocytes [4]. After oral administration, 6MP has a poor and variable bioavailability of ~ 20%, due to the presence of xanthine oxidase in intestines and liver [4]. Xanthine oxidase inactivates 6MP to thiouric acid, which is excreted in the urine. After this first-pass metabolism, the remaining 6MP is taken up by blood cells and converted by two pathways to its metabolites 6-thioguanine nucleotides (6-TGN) and to 6-methylmercaptonucleotides (6-MMP) (Figure 1) [5]. The 6TGN metabolite is the main active metabolite causing the antileukemic as well as the myelosuppressive effect.

In the current ALL treatment protocols, the starting dosage of 6MP ranges from 25 mg/m^2 to 60 mg/m^2, once daily, to be continued for 1 – 2 years, depending on the block of treatment and on the risk group stratification. Genetic variation in metabolizing enzymes, especially thiopurine S-methyl transferase (TPMT), causes interindividual differences in sensitivity to 6MP with consequences for dosing 6MP [5]. To optimize the exposure to 6MP, the starting dose is based upon body surface and according to TPMT status, in most treatment protocols. Dosages are subsequently adjusted every 2 – 4 weeks if indicated, based on peripheral blood white blood cell counts (WBC).

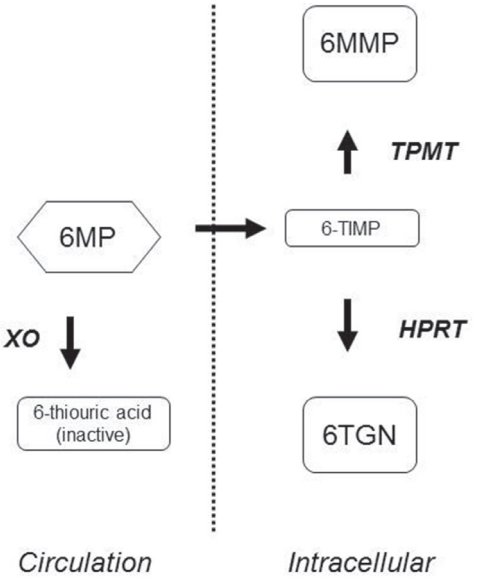

Figure 1. Schematic presentation of the metabolic pathway of 6MP. 6MP = 6 mercaptopine; XO = xanthine oxidase; 6TIMP = 6-thioinosine 5'monophoshate ; TPMT = thiopurine (S) methyltransferase; HPRT = hypoxanthine phosphoribosyl transferase; 6MMP = 6-methylmercaptonucleotides; 6TGN = 6-thioguanine nucleotides.

Table 1. Composition of 6MP liquid 10 mg/mL.

Ingredient	Amount
6-mercaptopurine-1-water	1 g
Methylparabeen	74 mg
Aluminii et magnesia silicas colloidale	991 mg
Carboxymethylcellulose	991 mg
Acidum citricum 1H20	74 mg
Sirupus simplex	26 g
Aqua	77.87 g

Mg = milligram.

It has been shown that the cumulative administered dose of 6-MP influences the risk of leukemia relapse and therefore inadequate dosages, variation in bioavailability, or lack of compliance needs to be prevented [6, 7, 8]. Because of the frequent dosage adaptations, the need for a flexible oral dosage formulation is apparent. For young children, the age group with the highest prevalence of pediatric ALL, a liquid oral dosage form is preferred over a solid oral dosage form [9]. Apart from the need for dosage individualization, the acceptability of the formulation for pediatric patients is of major importance. Given the long duration of 6MP administration, it is remarkable that little interest so far has been given to drug formulation issues such as palatability, compliance and ease of administration of 6MP to children.

In the present paper, we report the development of a novel liquid formulation of 6MP for pediatric cancer patients. Given the importance of adequate intracellular uptake on long term survival, we analyzed the impact of the drug formulation of 6MP on its pharmacokinetics/dynamics in the target population. In this study, the liquid formulation of 6MP and 6MP capsules were administered to pediatric ALL patients (0 – 18 years), and pharmacokinetic data, safety and acceptability were compared between the two formulations in an open-label, crossover, randomized design.

Methods

Development of a 6-MP liquid formulation

A liquid formulation of 6-MP in a concentration of 10 mg/mL was developed by the Laboratory of Dutch Pharmacists. The liquid is formulated as a suspension because of the low aqueous solubility of 6-MP. The composition is shown in Table 1. All additives are approved for use in the pediatric population [9].

The composition of the liquid was initially based upon data in literature [10]. However, the addition of ascorbic acid as antioxidant as described by Aliabadi et al. [10], resulted in a decrease from 175 mPa.s to 100 mPa.s in viscosity upon storage, while having no additional anti-oxidizing effect. In the final formulation, ascorbic acid was therefore not included (Table 1). According to the methods of European Pharmacopoeia [11], appearance, re-suspendability, viscosity, concentration of preservatives, microbiological purity and pH were tested. The homogeneity of the suspension was tested according to the General Monographs Unlicensed Medicines of the British Pharmacopeia [12]. The formulations were stored at 25 °C, 60% relative humidity and were tested at different intervals. The influence of temperature and light was monitored.

For analysis of content and degradation products, a validated stability indicating HPLC based method was used, based on the

Table 2. Stability data of 6MP liquid 10 mg/mL.

Time after preparation[1]	Concentration of 6MP (active substance)[2] Specifications 95% ≤ Conc. ≥ 105%
Day of preparation	97.5%; 98.2%
3 months	101.7%; 102.1%
5 months	97.4%; 98.7%
9 months	100.5%; 99.2%
12 months	99.8%; 101.3%

[1]Preparation of 6MP – 1-water suspension 10 mg/mL (date of production 02-09-2008), stored at room temperature in PET bottles; [2]The 6MP concentrations were determined in duplo, using a reversed-phase HPLC with UV detection (see Methods).

monograph of the active substance 6MP of the European Pharmacopoeia [11]. In short, the method consisted of HPLC-UV detection at 320 nm with a specificity for related substances of 0.051% (< 0.1%) and a recovery of 101.6% (95 – 105%). HPLC Stability data confirmed a shelf-life stability of the 6MP suspension of up to 1 year at room temperature (Table 2). During the shelf-life, the suspension maintained a high viscosity, ensuring a homogeneous distribution of the active substance in the liquid.

As a reference product, 6MP capsules 1 mg, 5 mg, 10 mg, and 20 mg were prepared using lactose-monohydrate as excipient with 0.5% colloidal silicon dioxide.

The raw materials including 6MP were obtained from Spruyt Hillen, IJsselstein, NL in Ph. Eur. 5 analytical grade. All batches of study drugs were prepared and analyzed by the Hospital Pharmacy Erasmus MC, under GMP license. During the study no use of commercially available 6-MP was allowed.

Study population

Pediatric patients (age between 0 and 18 years), with confirmed diagnosis of ALL, were included in the study during treatment in the maintenance phase of the Dutch ALL treatment protocol DCOG ALL-10 or the treatment protocol Interfant-06 (for children < 1 year of age at diagnosis) at the Department of Pediatric Oncology, Erasmus MC – Sophia Children's Hospital in Rotterdam. During this phase, patients used 6MP once daily based on body surface area, in combination with oral methotrexate. Dosages were adapted, based on leucocyte counts, with the aim to keep peripheral WBC between 2 and 3×10^9/L.

Other inclusion criteria were Lansky play score > 60 or Karnofsky performance status > 60 [13,14] and no severe liver function disorders (defined as liver enzymes levels greater than 1,000 U/L for transaminases). Excluded were patients with pre-existent liver disease or pregnant patients.

Written informed consent according to ICH-EU-GCP regulations was obtained from all parents and/or patients before performing any study related procedures. The study was approved by the IRB of Erasmus MC and registered in the Dutch Clinical Trial database under number NTR 1633 and Eudract number 2008-000424-86.

Study design

Each patient was randomized for treatment for 4 weeks with 6MP once daily with either capsules or liquid and, after crossing over, for 4 weeks with the other formulation. Capsules were chosen as the reference formulation, because preparation and dispensing of capsules in individual dosages was current practice for pediatric 6MP treatment. A randomization list was prepared by the Department of Statistics of the Erasmus. Patients were assigned a randomization number after screening, by the Clinical Trial Unit of Pediatric Oncology, who were blinded to the allocated treatment sequence at randomization. The pharmacy was unblinded to the treatment and dispensed the allocated formulation for each period. Parents were instructed to administer 6MP at home in the evening, without milk products, 1 hour after a meal. Administration of the liquid or capsules (after dissolving the capsules in a 5 mL syringe with hand-warm water) through a nasogastric tube was allowed.

Initial dosage was the pre-trial dosage and subsequently the dosages were adapted based upon leucocyte counts. Every 2 weeks, patients visited the oncologists for efficacy and safety analysis, upon which the study drugs were dispensed.

Leucocyte counts were targeted at 2 – 3 $\times 10^9$/L, according to the standard practice of the running DCOG-ALL-10/Interfant 06-protocol. If the leucocyte counts were outside these specifications, adaptation of the dosage of 6MP was allowed.

If temporary discontinuation of 6MP was necessary according to the treating physician based upon causes other than direct 6MP toxicity (e.g., intermittent infections), the patient was allowed to re-enter the protocol at day 1 or day 28, depending on the moment of dose interruption. The two formulations were compared with respect for the need for temporarily discontinuation.

6MP treatment could be discontinued because of hematological toxicity or severe elevation of transaminases, according to the standard practice of the running DCOG-ALL-10 protocol. All patients, who had been randomized and included in the protocol, were included in the analysis.

Pharmacokinetic analysis

Blood samples were drawn from the indwelling venous catheter at each visit for analysis of the blood concentrations of the active metabolites of 6MP. If the dosage changed, blood levels were drawn at least 14 days after daily intake of the same dosage to assure steady-state concentrations in red blood cells (RBC).

The concentrations of the active metabolites 6TGN and 6MMP in RBCs were determined before start, and after 2 and 4 weeks of continuous use of each formulation. A validated HPLC method with UV detection [15] was used at the laboratory of the Hospital Pharmacy at the Free University of Amsterdam to determine 6TGN and 6MMP concentrations, which were expressed as pmol per 8×10^8 RBCs. Detection limits of both 6TG and 6MMP were 5 pmol, and accuracy and repeatability were within the accepted ranges.

The pharmacokinetics of the liquid formulation, compared to the capsules, was investigated by assessment of trough concentrations of 6TGN and 6MMP in RBCs. The pro-drug 6MP has a short half-life (1.2 hours), whereas the active metabolite 6TGN and the metabolite 6MMP exhibit half-lives of ca. 5 days [4, 16]. Once steady state is reached, the concentrations of the thiopurine metabolites in erythrocytes reflect accumulation of the active substance during the past period [17].

To formally determine bioequivalence, AUC and C_{max} need to be assessed of the parent drug 6MP and compared between the two formulations [18]. However, we considered determining the trough concentrations of the active metabolites more relevant with respect to the comparison of the efficacy and safety of both formulations, based upon the aforementioned accumulation of the metabolites in erythrocytes. Furthermore, the burden for pediatric patients to undergo an extensive blood-sampling schedule to obtain an AUC was not considered ethical, given the limited additive information.

Pharmacodynamic analysis

Based upon the white blood cell count, dosage changes were allowed during the study. The dosage differences between the two formulations were calculated for each patient. The formulations were considered equivalent if the targeted leucocytes counts could be obtained with similar dosages for both formulations.

Safety analysis

Safety parameters were determined by performing physical examinations, monitoring vital signs, and assessing adverse events (according to Common Terminology Criteria for Adverse Events (CTCAE v 3.0, http://ctep.cancer.gov) at each visit). The occurrence and grade of adverse effects were compared between the two formulations. Specifically, the occurrence of hematological toxicity or elevation of transaminases was compared between the two formulations.

Compliance and acceptance

Each parent or patient was asked to keep a diary to register the intake of the drug in order to assess adherence to the oral treatment. At each visit, the remaining study medication was returned by the patient/parents, and

drug reconciliation was performed by the pharmacy to confirm adherence. The patients were asked to score the taste by using an adapted hedonic face scale [19]. The preference of parents and patients for the solid or liquid formulation was determined at the end of the study period.

Statistical analysis

The primary goal of the study was to show that both formulations were equivalent with respect to their pharmacokinetics. Based upon the number of patients needed to evaluate bioequivalence between two formulations, inclusion of 20 patients was considered sufficient in a cross-over setting [18]. Evaluable patients were defined as patients who had fulfilled the eligibility criteria and who did not need to be excluded during the treatment phase because of hematological toxicity or severe elevation of transaminases, and of whom all requested laboratory values were available.

The pharmacokinetics of the liquid vs. the capsules were assessed by establishing the ratio of the steady state concentrations of the active metabolites after 2 and 4 weeks of continuous use of each formulation of all evaluable patients. Observed concentrations were divided by the administered dose since it was allowed to adjust the dose on basis of the leucocyte blood count. As a result, the ratio of dose corrected concentrations was evaluated as well.

Statistical differences between the concentrations of the two formulations were investigated with Wilcoxon signed rank tests using SPSS (IBM SPSS Statistics 20).

The relative pharmacokinetics of the liquid vs. the capsules were evaluated by calculation of the geometric mean (+ 90% CI) ratio of the concentrations of both formulations.

The differences in dosages between the two formulations were tested using the paired t-test. The preference of the parents or children for either formulation was tested using the McNemar exact significance probability test.

The occurrence of adverse events was tested between the two formulations by calculating the McNemar for each adverse event observed during the study period.

Results

Drug formulation

A liquid formulation of 6MP in a concentration of 10 mg/mL was developed as a suspension (see Table 1 for composition), using additives approved for use in the pediatric population [9]. The preparation of the liquid was straightforward, using a safety cabinet for compounding hazardous material in the pharmacy. The liquid complied with all relevant Pharmacopeia tests (see Methods). The concentrations of 6MP and of the preservative fell with the 95 – 105% range of the theoretical contents during a period of up to 1 year, proving a shelf-life stability of the 6MP liquid of 1 year at room temperature (Table 2). During the shelf-life, the suspension maintained a high viscosity, ensuring a homogeneous distribution of the active substance in the liquid.

Clinical study

Between June 2009 and July 2012, 24 patients provided informed consent and fulfilled the eligibility criteria after screening, and started with study treatment. For demographic and clinical characteristics of the 24 patients included in the study see Table 3.

Four patients could not be evaluated because they did not finalize the study treatment due to the occurrence of febrile neutropenia and/or infections necessitating discontinuation of 6MP (see safety section). Twenty patients were available for the pharmacokinetic evaluation of the two formulations.

Dosages and pharmacodynamics

All patients started with their pre-study dosage during regular maintenance therapy, and dosages were subsequently adapted if the leucocyte counts were outside the limits, according to the Dutch ALL treatment protocol DCOG All-10. The daily dosages of 6MP after 4 weeks use of either capsules or liquid were comparable (for both formulations the median dosage of 6MP was 35 mg (range 10 – 115 mg)). The median leucocytes count was 3.2 $\times 10^9$/L (range 1.7 – 10.8) for the capsules and 3.0 $\times 10^9$/L (range 1.3 – 7.1)

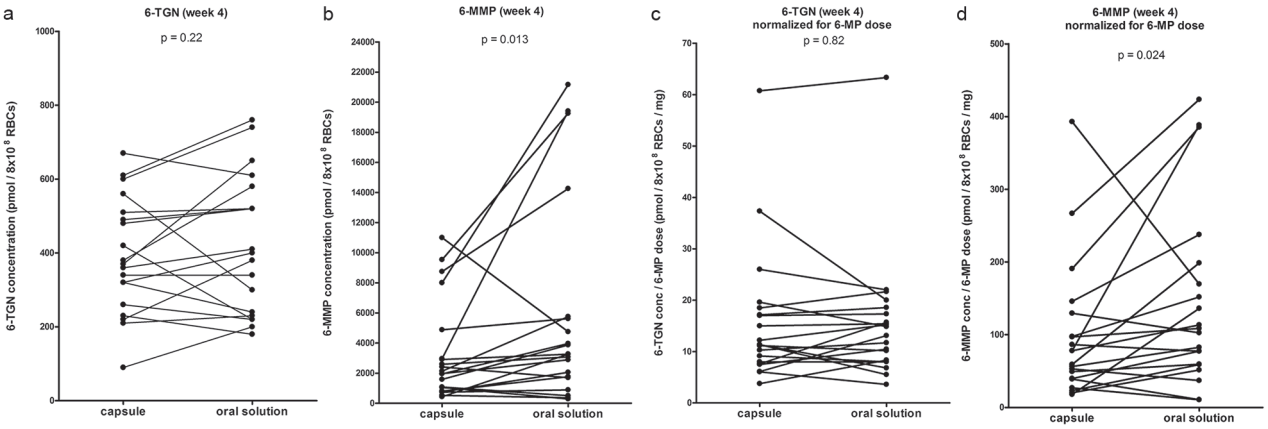

Figure 2. 6-TGN (a) and 6-MMP (b) trough levels (expressed as pmol/8 × 10^8RBC) and dose-corrected 6-TGN (c) and 6-MMP levels (d) (expressed as pmol/8 × 10^8RBC/mg) for the two formulations (n = 20) after 4 weeks treatment (n = 20).

Table 3. Demographic and clinical characteristics at start.

Parameter	n = 24 (patients included)	n = 20 (evaluable patients)
Age (median (range)) years	4.1 (1.9 – 14.6)	4.3 (1.9-14.6)
Gender (F:M)	11 : 13	10: 10
Weight (median (range)) kg	18 (12 – 53)	19 (12 – 52)
Body surface area (median (range)) m^2	0.72 (0.54 – 1.52)	0.73 (0.54 – 1.52)
TPMT genotype (n (%))		
Normal	22 (92%)	18 (90%)
*1/*3A	1 (4%)	1 (5%)
Not determined	1 (4%)	1 (5%)
Treatment protocol [n (%)]		
ALL-10 Standard Risk	7 (30%)	7 (35%)
ALL-10 Median Risk	15 (62%)	12 (60%)
Interfant 2006 study	2 (8%)	1 (5%)
6MP daily dose([median (range)) mg (prestudy)	32 (15 – 100)	33 (15 – 60)
6MP daily dose/BSA * (prestudy) (median (range)) mg/m^2	47 (13 – 66)	46 (13 – 60)

*BSA = body surface area.

for the liquid. After analyzing the difference in dosages between the two formulations for each individual patient, no statistically significant differences were found (mean difference 3.6 mg ± 8.2, p = 0.06), indicating a similar myelosuppressive effect of both capsules and liquid. These comparable pharmacodynamic effects of both formulations support the equivalence of the two formulations.

Pharmacokinetic results

The RBC concentrations of the active metabolites 6-thioguanine nucleotide (TGN) and methylmercaptopurine (MMP) were determined in a total of 115 blood samples taken as trough levels, both pre-study and at 2 weekly-intervals during the study. Pre-study concentrations of 6TGN and 6MMP were 381 ± 181 pmol/8 × 10^8 RBCs and 4,709 ± 5,560 pmol/8 × 10^8 RBCs (mean ± SD, n = 24), respectively.

Administration of both the liquid formulation and the capsules resulted in all patients in detectable RBC concentrations of 6TGN and 6MMP. None of the patients received a red blood cell transfusion during the study period, excluding any influence of the blood transfusion on the analysis of the thioguanine metabolites in RBC's.

The results of the 20 evaluable patients who had samples taken after 4 weeks use of either formulation are shown in Figure 2.

For the liquid formulation and the capsules, 6TGN concentrations at week 4

Table 4. Adverse events (n = 24). (Expressed as number of adverse events documented during the study period).

Adverse event Grade[1]	Capsule	Liquid	p-value
Febrile neutropenia			
Grade 1	6	9	
Grade 3	1	1	0.375
Infection			
Grade 1	2	1	
Grade 2	3	1	
Grade 3	1	1	0.375
Fatigue			
Grade 1	7	8	1
Fever (without neutropenia)			
Grade 1	1	1	
Grade 2	5	0	
Grade 3	1	0	0.0703
Rash			
Grade 1	2	5	
Grade 2	3	2	0.625
Vomiting			
Grade 1	5	5	1
Constipation			
Grade 1	2	2	1
Cough			
Grade 1	9	6	
Grade 2	1	0	0.3438
Elevated liver enzymes			
Grade 2	9	6	
Grade 3	5	11	0.4531

[1]Grading according to CTC grade CTCAE v 3.0.

Table 5. Preferences of parents and children for either formulation (n = 20).

	Parents	Child
Liquid	15* (p = 0.005)	9
Caps	3	5
No preference	2	2
Not determinable	0	4

were comparable with average values of 487 ± 342 pmol/8 × 10^8 RBCs (mean ± SD, n = 20) and 454 ± 318 pmol/8 × 10^8 RBCs, respectively (p = 0.22). Corresponding values for dose corrected 6TGN concentrations were 15.5 ± 12.5 pmol/8 × 10^8 RBCs/mg and 15.8 ± 13.2 pmol/8 × 10^8 RBCs/mg (p = 0.82). In comparison with the capsules the liquid formulation produced higher 6MMP concentrations; average values were 5,910 ± 6,770 pmol/8 × 10^8 RBCs vs. 3,300 ± 3,300 pmol/8 × 10^8 RBCs for the capsules (p = 0.01). Corresponding values for dose corrected 6MMP concentrations were 144 ± 124 pmol/8 × 10^8 RBCs/mg and 97 ± 93 pmol/8 × 10^8 RBCs/mg (p = 0.024). One patient with a TPMT*1*3A allele (Table 1) showed, both initially and during the study, high blood levels of 6TG (1,160 – 1,860 pmol/8 × 10^8 RBCs) and low blood levels of 6MMP (290 – 1,140 pmol/8 × 10^8 RBCs), most probably caused by the decreased TPMT enzyme activity.

With respect to the relative bioavailability, a geometric mean ratio of 1.07 (90% CI 0.93 – 1.23) was found for the 6TGN concentrations. The dose corrected ratio of 6TGN concentrations was 1.01 for the liquid vs. the capsules (90% CI 0.86 – 1.20).

6MMP concentrations were higher after administration of the liquid vs. the capsules. The geometric mean ratios of the 6MMP concentration and the 6MMP dose corrected concentration liquid/capsule were 1.48 (90% CI 1.07 – 2.06) and 1.40 (90% CI 1.02 – 1.93), respectively.

The 6TGN and 6MMP concentrations and relative pharmacokinetics at 2 and 4 weeks were similar (data not shown).

Safety evaluation

Three serious adverse events (SAE) have been reported; 2 patients with grade 3 febrile neutropenia, and 1 patient with a grade 3 infection with normal ANC; all necessitating hospitalization. One patient used 6MP capsules when the SAE occurred, 1 patient used 6MP liquid and in 1 patient the SAE occurred during a treatment interruption period due to an infection, after using 6MP liquid. All three patients went off study. The 4th patient who went off study, had several episodes of febrile neutropenia during intake of 6MP capsules, necessitating frequent treatment interruption. No blood levels were drawn in these patients after discontinuation of the study, but the last RBC concentrations taken before the premature discontinuation did not show significantly higher levels of 6TGN or 6MMP. No suspected unexpected serious adverse reactions (SUSARs) occurred.

No differences were found in the occurrence of temporarily halting of 6MP due to

fever or infection (4× during capsule intake, 3× during liquid intake).

In Table 4 the most frequently reported adverse events are shown for each formulation, with corresponding CTC grades (CTCAE v 3.0). The occurrence of adverse events did not differ between the two formulations. In 7 of the 24 patients (30%), elevated liver enzymes (CTC grade 2: n = 3, CTC grade 3: n = 4) were already present before start of the study medication

Patient-acceptance

Questionnaires on taste, handling and formulation preference were presented to parents and patients at the end of the study. These showed that most parents preferred the oral liquid over the capsules because of the ease of administration as compared to the capsules (p = 0.005, McNemar test) (Table 5). The capsules were reported by the parents of 9 patients as difficult to administer, especially in children with a nasogastric tube. Taste assessment with the hedonic scale was not feasible in 13 of the 24 patients, because of young age or administration through a nasogastric tube. In 3 of the 11 remaining patients the taste was scored in the lower quartile (n = 2 after intake of capsules, n = 1 after intake of liquid and capsules). No patient refused the liquid because of the taste. 15 (75%) patients continued with the 6MP liquid on compassionate use base after finishing the study, at their own request.

Discussion

In this study a novel liquid formulation of 6MP with high pharmaceutical quality and a simple compounding technique, was developed, with a shelf-life of 1 year.

The pharmacokinetic results showed that the geometric mean ratio's ± 90%CI of the steady state concentrations of the main cytotoxic metabolite 6TGN are within 0.80 and 1.25, which means that the liquid can be considered bioequivalent to the capsules in the pediatric population. Moreover, both compounds resulted in similar leucocyte depletion with equal dosages. No differences in adverse effects were found, indicating that the liquid is an effective and safe alternative to capsules.

The higher 6MMP RBC concentrations found after administration of the liquid were unexpected. These higher concentrations might imply that a shunting from the formation of 6TGN towards overproduction of 6MMP occurs as the two metabolic pathways are correlated. However, the concentrations of 6TGN were not found to be decreased in the patients during treatment with the liquid formulation, making a shift from 6TGN metabolic pathway to the 6MMP pathway unlikely. If the formation of 6TGN is comprised of a saturable process, then an increased availability of 6MP may result in increased 6MMP levels, without a change in 6TGN. However, no evidence was found in the literature for a saturable pathway in 6TGN formation. Therefore we currently have no ready explanation for this phenomenon.

6MMP is not considered primarily responsible for the cytotoxic effect (as opposed to 6TGN), but higher concentrations of 6MMP have been reported to correlate with hepatotoxicity, although literature has not been conclusive [20]. In our study, no significant difference in hepatotoxicity, defined as elevated liver enzymes, could be found between the two formulations. In 1/3 of the patients, elevated liver enzymes (grade 2 or 3, according to CTCAE v 3.0, http://ctep.cancer.gov) were already present before the start of the study medication, which is a well-known side effect of the chemotherapeutic agents used during ALL treatment [21].

Due to the cross-over setting in the study, the variability in TPMT activity based upon the individual phenotype has been minimized. The co-administration of MTX, an inhibitor of xanthine oxidase (XO), is standard practice in ALL treatment protocols, but this may influence 6MP pharmacokinetics [22]. In this study the co-administration of MTX and other supportive care drugs, as part of the DCOG ALL-10 treatment protocol remained constant during the study period, and therefore co-medication is not expected to have influenced the results.

Most patients belonged to the age group of 2 – 6 years, which is the most prevalent

age for patients with ALL. Especially in this age group, the choice of drug formulation is very important with respect to acceptance of the drug and compliance. Our study showed that the liquid formulation was well tolerated in this age group, and the parents expressed an evident a preference for the liquid.

When this study started, the only licensed formulation of 6MP was Purinethol® 50 mg tablets, which is not suitable for children due to the fixed dosage and the solid drug formulation. Pharmacies therefore need to prepare individual capsules or non-standardized liquid formulations. Very recently, a 6MP suspension 20 mg/mL (Xaluprine®, Nova laboratories) has received Marketing Authorization from the European Medicines Agency for treatment of ALL in adults, adolescents and children and it has come available in some European countries. However, no research has been conducted in pediatric patients with Xaluprine® with regard to pharmacokinetics/dynamics or tolerability in children. Its registration is based upon a bioequivalence study of the inactive pro-drug 6MP, conducted in adult volunteers, in comparison to Purinethol® tablets as a reference formulation, which is not used in young children for reasons stated above. In general, to assess bioequivalence between a new formulation and a reference formulation, a study in adult volunteers is considered sufficient for the Regulating Authorities. If bioequivalence has been shown and the reference formulation is already licensed for pediatric use, no additional studies are necessarily in the pediatric population to obtain market approval (www.ema.europa.eu). However, apart from the ethical discussion whether volunteers should be asked to participate in a study with a cytotoxic compound, we considered the data obtained from performing this study in children with ALL, as essential information. Differences in bioavailability and/or differences in acceptation of either formulation in the actual target population may affect blood levels and thus the outcome of this potentially life-saving treatment. A follow-up study may be needed to assess the safety of the formulation after long-term use, especially with respect to the effect on 6MMP concentrations in relation to hepatotoxicity.

Conclusion

In conclusion, the results of this study showed that the novel 6MP liquid was bioequivalent to 6MP capsules with respect to the main active metabolite 6TGN, and resulted in higher acceptability. Also, the intended pharmacodynamic effect on leucocytes depletion did not differ between the two formulations, which support the conclusion that the novel 6MP liquid is suitable as antileukemic treatment.

Due to the ease of administration and the dosing flexibility of this liquid formulation, this new liquid formulation is a promising treatment for ALL.

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Conflict of interest

The authors declare no conflicts of interest.

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