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Research Article

A Pharmacokinetic Analysis and Pharmacogenomic Study of 6-mercaptopurine

Abstract

Background: The efficacy and safety of 6-mercaptopurine (6-MP) therapy rely on the concentration of its metabolites. The aim of the current study is the pharmacokinetic analysis of 6-MP and the detection of its metabolites as well as the role of Thiopurine S-methyl transferase (TPMT), the enzyme associated with 6-MP metabolism, as a pharmacogenomics biomarker.

Materials and Methods: Data were collected from 19 patients with different types of leukemia and lymphoma who received 6-MP chemotherapy. Pharmacokinetic analysis was performed using an HPLC. The VNTR promoter polymorphisms of TPMT were detected.

Results and Conclusion: The pharmacokinetic analysis confirmed the heterogeneity of the 6-MP metabolism. The TPMT genotyping revealed a correlation between the TPMT*3C variant and increased levels of 6-thioguanine nucleotides (TGs). No methylation pattern was obtained.

Abbreviations

(TPMT): Thiopurine S-Methyl Transferase; (6-MP): 6-Mercaptopurine; (ALL): Acute Lymphoblastic Leukemia; (XO): Xanthine Oxidase; (HPRT): Hypoxanthine Phosphoribosyltransferase; (6-TU): 6-Thiouric Acid; (6-TIMP): 6-Thioinosine Monophosphate; (6-TGs): 6-Thioguanine Nucleotides; (6-mMPNs): 6-Methylmercaptopurine Nucleotides; (6-TIDP): Diphosphate; (6-TITP): Triphosphate; (ITPA): Inosinetriphosphatase; (BLL): B- Lymphoid Leukemia; (BL): Blastic Lymphoma; (AML): Acute Myeloid Leukemia; (ML): Myeloid Leukemia; (LL): Lymphoid Leukemia

Introduction

Thiopurine S-methyl transferase (TPMT) is an enzyme which affects the metabolism of 6-mercaptopurine (6-MP). Patients with lack or low levels of TPMT will not receive the appropriate therapy if a normal dose of 6-MP is used [1]. 6-MP, mainly used in treating acute lymphoblastic leukemia (ALL), is inactive and it requires metabolic activation to exert its cytotoxic effect. After 6-MP's administration, three enzymes are of significant importance for its metabolism; xanthine oxidase (XO), TPMT and hypoxanthine phosphoribosyltransferase (HPRT) [2]. XO metabolises 6-MP to 6-thiouric acid (6-TU) and TPMT methylates 6-MP to 6-mMP. HPRT catalyzes the production of 6-thioinosine monophosphate (6-TIMP) and subsequently, the active 6-thioguanine nucleotides (6-TGs). 6-TIMP can alternatively be methylated by TPMT, yielding 6-methylmercaptopurine nucleotides (6-mMPNs) [3]. Finally, 6-TIMP is converted successively into 6-thioinosine diphosphate (6-TIDP) and triphosphate (6-TITP) to form 6-TIMP once again by the action of the enzyme inosinetriphosphatase (ITPA) [4]. TGs incorporate into DNA causing further DNA damage by single-strand breaking, inter-strand crosslinking and DNA-protein crosslinking [5]. Despite this knowledge, the pharmacokinetic profile of 6-MP remains partial understood. A better understanding of the disposition of 6-TGs and 6-MMPs would be of extreme value towards an improved design of 6-MP dose regimens, diminishing drug toxicity [2]. In this context, the application of pharmacogenetics that relies on studying sequence variations in candidate genes that probably affect drug response could serve for patient monitoring and stratification [6].

In this study, the pharmacokinetic profile of 6-MP was investigated in patients with different types of leukemia and lymphoma under 6-MP maintenance chemotherapy. Also, the TPMT polymorphisms and gene methylation were examined, both retrospectively and prospectively. Understanding the metabolism of 6-MP along with the detection of TPMT variants in patients with different types of leukemia and lymphoma will contribute to the improvement of the 6-MP therapeutic regimen eliminating the associated toxicity.

Materials and Methods

Patients

Nineteen patients with different types of leukemia and lymphoma (Table 1) upon 6-MP treatment were enrolled for this study. The majority of patients (18) were hospitalized at the University Hospital of Patras, Greece and 1 patient was hospitalized at the University Hospital of Ioannina, Greece. Samples were collected according to the ethics rules and upon patients' approval. Eleven subjects have completed their therapy before this study and 8 subjects were on treatment. Peripheral blood samples (1.5 ml) were collected in ethylene-diaminetetraaceticacid (EDTA) tubes at the appropriate

Table 1: The demographic data of the patients treated with 6-MP that were included in the current study.

No	Sex	Age	Time of diagnosis (years)	Disease	Therapy status	Methotrexate
1	F	63	60	BLL	On therapy	-
2	M	21	17	BL	On therapy	-
3	F	33	28	ALL	Completed therapy	-
4	M	31	27	BL	On therapy	-
5	F	18	14	BLL	Completed therapy	-
6	F	53	48	BLL	Completed therapy	-
7	M	83	78	AML	Completed therapy	-
8	F	50	45	ML	Completed therapy	-
9	M	45	42	AML	Completed therapy	-
10	F	46	37	BLL	Completed therapy	-
11	M	26	13	LL	Completed therapy	-
12	M	26	16	ALL	Completed therapy	-
13	F	55	54	LL	On therapy	40mg/m^2
14	M	20	14	ALL	Completed therapy	-
15	F	77	76	AML	Completed therapy	-
16	F	65	56	AML Myelodisplastic	On therapy	40mg/m^2
17	M	63	63	ALL	On therapy	40mg/m^2
18	M	20	19	AL	On therapy	40mg/m^2
19	F	76	75	AMLM3	On therapy	40mg/m^2

time point's pre and post (1h, 1.3h, 1.45h, 5h, 8h, and 24h) 6-MP administration and centrifuged at 1000 g for 10 min to separate plasma from red blood cells (RBCs). The RBCs were washed twice with a Hank's balanced salt solution (HBBS) and then suspended at a density of 8x108 RBCs per 200ml and kept frozen at -20°C until required for further processing. An extra peripheral blood sample (1 ml) from all patients was collected in an EDTA tube and keptat-20°C without centrifugation for TPMT genotyping.

Reagents and chemicals

HPLC-grade acetonitrile (CH3CN) and methanol (CH3OH) (Honeywell Burdick & Jackson, Seelze, Germany), mono-potassium phosphate (KH2PO4), phosphoric acid (H3PO4) and potassium hydroxide (KOH) (Merck KgaA, Darmstadt, Germany) and ultra-pure water from a MilliQ® instrument (Millipore, Billerica-USA) were used.

Bench-top stability and freeze and thaw studies of 6-MP, TG and 6-MMP

The bench-top stability of the analytes of interest (6-MP and its major metabolites) in human RBCs was evaluated at room temperature. Two concentrations per analyte were tested (low and high) at t=1h and t=24h. A freeze and thaw experiment was also performed for the evaluation of the analytes' stability in RBCs at the same concentrations.

Preparation of standards and calibration curve for the detection of 6-MP and its metabolites

Stock solutions of 6-MP (1.6 mg/ml), 6-TG (1 mg/ml) and 6-MMP (2.5 mg/ml) were prepared in 0.1 NaOH. Two sets of standard solutions were then prepared for the analysis in the RBCs of patients. The standard solutions prepared were in the concentration range of 8.5-170 ng/ml, 0.025-1.25 μg/ml and 0.20-20 μg/ml for 6-MP, 6-TG and 6-MMP, respectively. Concentrations were chosen as such in order to exhibit clinical relevance. A different set of solutions at three different concentrations (low, medium and high) were prepared depending on the analyte tested, serving as quality control (QC) samples. Calibration curves and QC samples were prepared daily in RBCs.

HPLC analysis

HPLC was performed with an Ultimate 3000 Pump system (Dionex Corporation, Sunnyvale, CA, USA), sharing an infusion valve 8125 (Rheodyne, Rohnert Park, CA, USA) with a 20 μL loop and an Ultimate 3000 Photodiode Array Detector (Diode Array Detector, DAD). The Chromeleonv.6.80 software was employed for the sample and data analysis. Resolution was achieved using a reverse phase analytical column, C18 (250 × 4.6 mm, i.d. 5 μm, Xterra) at a flow rate of 0, 9 mL/min. The mobile phase consisted of solvents A: KH2PO4 (pH=2.25) 0.02M, B: acetonitrile (HPLC grade), and C: methanol (HPLC grade). A 16 min-isocratic run was developed (A:B:C, 96:3:1). Typical injection volume was 20 μl. A stable temperature of 25°C was used towards peak shape optimization. Three absorption UV-V is wavelengths were selected, being specific and optimum for each analyte; 322 nm (6-MP), 303 nm (6-MMP) and 342 nm (TG).

For the construction of calibration curves with spiked samples the method of constant addition was used. The calibration curves of peak areas (in mAU x min-the peak areas of blank samples were subtracted from those of the spiked samples) versus concentration (ng/mL) were linear in the concentration range studied. Blank samples were analyzed with each calibration curve. Then a random sample of RBCs

was analyze during HPLC (data not shown). In order to verify the detection capacity of the method, standard concentrations of 6-MP, 6-TG and 6-MMP were added into a healthy volunteer's RBCs, as described in "Materials and Methods" section. An HPLC analysis was performed and an indicate chromatograph is demonstrated in Figure 1A. The performance of analysis for the standard solutions was followed by the analysis of samples from patients treated with 6-MP.

Detection of VNTR promoter polymorphisms and methylation pattern in the TPMT gene

All subjects were genotyped in the TPMT gene promoter VNTR and coding regions, as described previously [7]. Genomic DNA was isolated from blood specimens using QIAamp® DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany). The number and type of tandem repeats were determined by PCR amplification followed by direct re-sequencing, as previously described [7], using the Big Dye® Terminator Version 3.1 Ready Reaction Kit (Applied Biosystems, CA, USA) and by capillary electrophoresis using an automatic sequencer (3130 Genetic Analyzer, Applied Biosystems) according to the manufacturer's instructions. The sequences of the primers employed were; TPMT_VNTR_F: AGGACTAGGGATGGGTAGGG and TPMT_VNTR_R: ACCTCGCTTACAGCTGGTTG. To investigate TPMT methylation, we focused on V4/V7, V5/V5, V5/V7, V6/V6 and V6/V8. A pyrosequencing-based methylation assay was used, according to the Pyromark Q24 software quick start guide and Pyromark Q24 Vacccum workstation quick start guide. Ten samples were analyzed.

Results

HPLC analysis of patients' samples undergoing therapy, to detect 6-MP and its metabolites

The patients that were on treatment with 6-MP during this study and the patients that have completed their treatment are shown in Table 1. Samples were collected at the indicated time points as described in the "Material and Methods" section. The sample of patient 4 was subjected to haemolysis and therefore, further analysis wasnot performed. Table 2 shows the 6-MP levels in the RBCs' of patients (13, 16, 17, 18 and 19) prior and post their standard treatment at various time points. In all cases, except patient 18, the inactive form of 6-MP was not detected prior drug administration. In patients 13 and 16, the levels of inactive 6-MP were decreased in a time dependent manner, as expected. Patients 17 and 19 exhibited a similar pharmacokinetic profile as 6-MP was detected only 1h after drug administration and its levels decreased gradually with time resulting in no 6-MP detection. The major metabolites of 6-MP, namely 6-TG and 6-MMP were also analyzed (Table 2). In patients 13 and 16, the levels of both metabolites increased in a time dependent manner, as expected. Interestingly, only 6-MMP was detected in patient 17 within 1h after 6-MP treatment. On the contrary, in patients 18 and 19, only 6-TG was detected prior and 1h post treatment. At this point, it should be noted that patients 17, 18 and 19 demonstrated a very steep drug elimination profile. Representative chromatographs for 6-MP, 6-TG and 6-MMP of patient 13 are depicted in Figure 1B.

Analysis of the TPMT gene promoter VNTR polymorphism and gene methylation

The patients enrolled in this study were either upon 6-MP

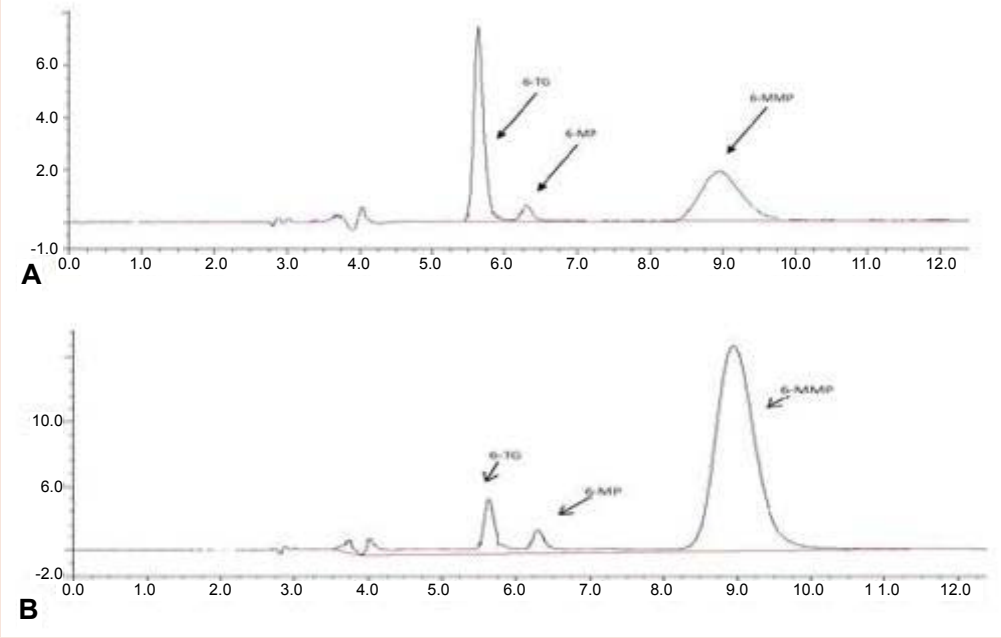

Figure 1: An HPLC analysis of A) an indicative chromatograph of a 6-MP, 6-TG, 6-MMP mixture in the RBCs of a healthy volunteer. The analysis was conducted at 342 nm, the optimum wavelength for the 6-TG. B) A representative chromatograph of sample No 13, 1.45h post 6-MP treatment, the peaks indicate the levels of 6-MP, 6-TG and 6-MMP. 6-MP: 6-mercaptopurine, 6-TG: 6-thioguanine, 6-MMP: 6-methyl-mercaptopurine.

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treatment or treatment completion. The genotyping of the VNTR promoter of the TPMT gene was conducted on samples of all patients enrolled. The results of the VNTR analysis are summarized in Table 3. All patients were found to be wild-type (wt), except patient 18 where the TPMT*3C polymorphism was detected. No methylation was obtained (data not shown).

Discussion

The efficacy and safety of 6-MP therapy rely on the concentration of the cytotoxic TGs metabolites, which in turn depends on the metabolism of 6-MP by TPMT [8,9]. Herein, the pharmacokinetic analysis of 6-MP confirms the heterogeneity that accompanies the metabolism of this drug. In particular, 6-MP as well as its major metabolites (6-TG and 6-MMP) were detected in patients 13 και 16, while only 6-MP and 6-TG were detected in patients 18 and 19 and 6-MMP was only detected inpatient 17. 6-MP detection at several time points after drug administration- especially in the case of patient 13 – should be noted. The slow rate of drug elimination might be correlated with methotrexate that was also included in patient treatment along with 6-MP. Methotrexate is a potent inhibitor of dihydrofolatereductase (DHFR), a key enzyme for intracellular folate metabolism, and functions to regenerate tetrahydrofolate from dihydrofolate, a product of thymidylatesynthase.As a consequence of DHFR inhibition, intracellular levels of tetrahydrofolate coenzymes are decreased, resulting in the inhibition of thymidylate and consequently DNA biosynthesis, as well as purine biosynthesis [10]. Previous in vitro and in vivo studies have shown that the simultaneous treatment with 6-MP and methotrexate affects 6-MP metabolism. De novo inhibition of purine biosynthesis by methotrexate causes increased intracellular levels of phosphoribosyl pyrophosphate which is a cofactor of HPRT enzyme in 6-MP metabolism [11-13]. It has been found that the combination of 6-MP with methotrexate leads in a synergistic conversion of 6-MP to 6-TG. Furthermore, XO is inhibited and the levels of 6-MP increased in plasma. In addition, data showed that increased accumulation of polyglutamate derivatives of methotrexate in red blood cells is correlated with increased 6-TG levels during all maintenance therapy using low dose methotrexate (40 mg/m2) and daily treatment with 6-MP (75 mg/m2). However, when high dose of methotrexate (1g/m2) are used, 6-TG accumulation is disrupted after treatment with 6-MP (1g/m2) during induction phase [11,14]. Indeed, patients 13, 16, 17, 18 and 19 participated in the current study were also treated with methotrexate (Table 1) and this might contributed to 6-MP metabolites' accumulation in red blood cells resulting in increased toxicity.

Table 2: The demographic data of the patients treated with 6-MP that were included in the current study.

	6-MP (ng/ml) levels						
No	**prior to treatment**	**1h**	**1,3h**	**1,45h**	**5h**	**8h**	**24h**
1	ND	ND	ND	ND	ND	-	ND
2	ND	ND	ND	ND	ND	ND	-
13	-	229,12	ND	99,68	ND	ND	ND
16	-	203,24	120,61	ND	89,59	ND	ND
17	-	-	ND	ND	ND	ND	ND
18	13,07	80,90	ND	ND	ND	ND	ND
19	-	50,55	ND	ND	ND	ND	ND
	6-TG (ng/ml) levels						
1	ND	ND	ND	ND	ND	-	ND
2	ND	ND	ND	ND	ND	ND	-
13	-	153,83	ND	347,30	ND	ND	ND
16	-	219,52	318,80	ND	440,67	ND	ND
17	-	-	ND	ND	ND	ND	ND
18	30,75	380,25	ND	ND	ND	ND	ND
19	87,09	260,098	ND	ND	ND	ND	ND
	6-MMP (ng/ml) levels						
1	ND	ND	ND	ND	ND	-	ND
2	ND	ND	ND	ND	ND	ND	-
13	212,06	1195,78	ND	3124,44	ND	ND	ND
16	-	1137,78	32160,72	ND	3360,00	ND	ND
17	-	2012,67	ND	ND	ND	ND	ND
18	-	-	ND	ND	ND	ND	ND
19	-	-	ND	ND	ND	ND	ND

Table 3: The analysis of VNTR promoter in patients enrolled in this study.

No	VNTR promoter	2	3A	3B	*3C	4	7	8	20	25
1	*6a/*7a (A2B3C/A5BC)	wt	wt	wt	wt	wt	wt	wt	wt	wt
2	*4a/*5a (A2BC/A2B2C)	wt	wt	wt	wt	wt	wt	wt	wt	wt
3	*4a/*5a (A2BC/A2B2C	wt	wt	wt	wt	wt	wt	wt	wt	wt
4	*4a/*5a (A2BC/A2B2C)	wt	wt	wt	wt	wt	wt	wt	wt	wt
5	*4a/*4a (A2BC/A2BC)	wt	wt	wt	wt	wt	wt	wt	wt	wt
6	*4a/*4a (A2BC/A2BC)	wt	wt	wt	wt	wt	wt	wt	wt	wt
7	*4a/*5a (A2BC/A2B2C)	wt	wt	wt	wt	wt	wt	wt	wt	wt
8	*6a/*6a (A2B3C/A2B3C)	wt	wt	wt	wt	wt	wt	wt	wt	wt
9	*4a/*5a (A2BC/A2B2C)	wt	wt	wt	wt	wt	wt	wt	wt	wt
10	*5a/*6a (A2B2C/A2B3C)	wt	wt	wt	wt	wt	wt	wt	wt	wt
11	*4a/*4a (A2BC/A2BC)	wt	wt	wt	wt	wt	wt	wt	wt	wt
12	*4a/*4a (A2BC/A2BC)	wt	wt	wt	wt	wt	wt	wt	wt	wt
13	*4a/*4a (A2BC/A2BC)	wt	wt	wt	wt	wt	wt	wt	wt	wt
14	*5a/*5a (A2B2C/A2B2C)	wt	wt	wt	wt	wt	wt	wt	wt	wt
15	NA	-	-	-	-	-	-	-	-	-
16	NA	-	-	-	-	-	-	-	-	-
17	*5a/*6a (A2B2C/A2B3C)	wt	wt	wt	wt	wt	wt	wt	wt	wt
18	*4b/*5a (AB2C/A2B2C)	wt	wt	wt	A/G	wt	wt	wt	wt	wt
19	*4a/*5a (A2BC/A2B2C)	wt	wt	wt	wt	wt	wt	wt	wt	wt

Herein, the highest levels of 6-TG were only observed in patient 18. This might be correlated with the polymorphism TPMT*3C (A/G heterozygous) that was revealed after the analysis of the TPMT gene promoter. The TPMT gene promoter VNTR polymorphisms are known to affect the activity of the enzyme. A recent study also shows that the number and the type of the repeats affect gene transcription levels [7]. Interestingly, no gene methylation was observed.

Besides the genetic factors that affect TPMT activity, nongenetic factors might be implicated, too. In fact, nationality seems to be an important factor [15,16]. TPMT is an enzyme with great interest because of its implication in drug metabolism. Many studies show a worldwide attempt for mapping the TPMT polymorphisms [17-25] that affect its activation and hence, determine drug dosage in the clinic. Previous data demonstrated that the frequency of TPMT polymorphisms is 1 to 300 cases or 1 to 200 cases [26]. These studies refer to Caucasians and non-Caucasians or describe nations. Furthermore, there is a detailed study in South India that considers people migration from other countries [19]. Genetic analyses show that the frequency of TPMT polymorphisms is higher in Caucasians compared to North Asians. Also, their frequency is similar between black and white Americans [26]. Differences in TPMT variants across different populations are increasingly being recognized and a more detailed TPMT screen should be conducted to better define the clinical implications of TPMT heterogeneity with respect to thiopurine treatment. However, the genotyping of TPMT is not adequate for determining the activity of TPMT. In the UK, there were some cases of low TPMT activity that were detected independently of TPMT genotyping [27]. Besides the frequency of TPMT polymorphisms, the type of the polymorphism is important, too. TPMT*3A και *3C are detected in Caucasians [28] at a percentage of 95%, while type *2 is less frequent. The same pattern is observed in European people, with the exception of TPMT*3B that is also detected, yet less frequently in Swedish people [30]. In Italy, the most frequent variant is the TPMT*3A, although the TPMT*3B and *3C variants are equally detected [29]. This difference might be correlated with the influence of other populations like Sardinians [30] that have been affected by several invaders in their history. Greece is another typical example that has been influenced by many invaders in its history. In the current project, noteworthy, the only polymorphism that was detected was TPMT*3C, although the number of patients was small and more samples should be analyzed for a robust outcome.

We believe that the understanding of the pharmacokinetic profile of 6-MP along with the detection of the TPMT gene promoter VNTR polymorphisms in patients with different types of leukemia and lymphoma will result in an improved therapeutic strategy and patient stratification.

Statement of human rights/ Informed consent

The studies have been approved by the appropriate institutional research ethics committee and have been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

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References

1. Ford LT, Berg JD (2010) Thiopurine S-methyltransferase (TPMT) assessment prior to starting thiopurine drug treatment; a pharmacogenomic test whose time has come. J Clin Pathol 63: 288-295.
2. Hawwa AF, Collier PS, Millership JS, McCarthy A, Dempsey S, et al. (2008) Population pharmacokinetic and pharmacogenetic analysis of 6-mercaptopurine in paediatric patients with acute lymphoblastic leukaemia. Br J Clin Pharmacol 66: 826-837.
3. Sahasranaman S, Howard D, Roy S (2008) Clinical pharmacology and pharmacogenetics of thiopurines. Eur J Clin Pharmacol 64: 753-767.
4. Derijks LJ, Gilissen LP, Hooymans PM, Hommes DW, et al. (2006) Review article: thiopurines in inflammatory bowel disease. Aliment Pharmacol Ther 24: 715-729.
5. Kotur N, Stankovic B, Kassela K, Georgitsi M, Vicha A, Leontari I, et al. (2012) 6-mercaptopurine influences TPMT gene transcription in a TPMT gene promoter variable number of tandem repeats-dependent manner. Pharmacogenomics 13: 283-295.
6. Zhou S (2006) Clinical pharmacogenomics of thiopurine S-methyltransferase. Curr Clin Pharmacol, 1: 119-128.
7. Zukic B, Radmilovic M, Stojiljkovic M, Tosic N, Pourfarzad F, et al. (2010) Functional analysis of the role of the TPMT gene promoter VNTR polymorphism in TPMT gene transcription. Pharmacogenomics 11: 547-557.
8. Karas-Kuzelicki N, Mlinaric-Rascan I (2009) Individualization of thiopurine therapy: thiopurine S-methyltransferase and beyond. Pharmacogenomics 10: 1309-1322.
9. Coulthard S, Hogarth L (2005) The thiopurines: an update. Invest New Drugs 23: 523-532.
10. McGuire JJ ((2003) Anticancer antifolates: current status and future directions. Curr Pharm Des 9: 2593-2613.
11. Adam de Beaumais T, Dervieux T, Fakhoury M, Medard Y, Azougagh S, et al. (2010) The impact of high-dose methotrexate on intracellular 6-mercaptopurine disposition during interval therapy of childhood acute lymphoblastic leukemia. Cancer Chemother Pharmacol 66: 653-658.
12. Bokkerink JP, Bakker MA, Hulscher TW, De Abreu RR, Schretlen ED, et al. (1986) Sequence-, time- and dose-dependent synergism of methotrexate and 6-mercaptopurine in malignant human T-lymphoblasts. Biochem Pharmacol, 35: 3549-3555.
13. Tan CT, Wollner N, Trippett T, Goker E, Tong WP, et al. (1994) Pharmacologic-guided trial of sequential methotrexate and thioguanine in children with advanced malignancies. J Clin Oncol, 12: 1955-1962.
14. Dervieux T, Hancock ML, Pui CH, Rivera GK, Sandlund JT, et al. (2003) Antagonism by methotrexate on mercaptopurine disposition in lymphoblasts during up-front treatment of acute lymphoblastic leukemia. Clin Pharmacol Ther 73: 506-516.
15. Chowbay B, Zhou S, Lee EJ (2005) An interethnic comparison of polymorphisms of the genes encoding drug-metabolizing enzymes and drug transporters: experience in Singapore. Drug Metab Rev 37: 327-378.
16. Kramer SD, Testa B (2008) The biochemistry of drug metabolism--an introduction: part 6. Inter-individual factors affecting drug metabolism. Chem Biodivers, 5: 2465-2578.
17. Ouerhani S, Cherif N, Bahri I, Safra I, Menif S, et al. (2013) Genetic polymorphisms of NQO1, CYP1A1 and TPMT and susceptibility to acute lymphoblastic leukemia in a Tunisian population. Mol Biol Rep 40: 1307-1314.
18. Iyer SN (2012) Genotype frequencies of drug-metabolizing enzymes responsible for purine and pyrimidine antagonists in a healthy Asian-Indian population. Biochem Genet 50: 684-693.
19. Umamaheswaran G, Krishna Kumar D, Kayathiri D, Rajan S, Shewade DG, et al. (2012) Inter and intra-ethnic differences in the distribution of the molecular variants of TPMT, UGT1A1 and MDR1 genes in the South Indian population. Mol Biol Rep 39: 6343-6351.
20. Hakooz N, Arafat T, Payne D, Ollier W, Pushpakom S, et al. (2010) Genetic analysis of thiopurine methyltransferase polymorphism in the Jordanian population. Eur J Clin Pharmacol 66: 999-1003.
21. Murugesan R, Vahab SA, Patra S, Rao R, Rao J, et al. (2010) Thiopurine S-methyltransferase alleles, TPMT (*)2, (*)3B and (*)3C, and genotype frequencies in an Indian population. Exp Ther Med 1: 121-127.
22. Desire S, Balasubramanian P, Bajel A, George B, Viswabandya A, et al. (2010) Frequency of TPMT alleles in Indian patients with acute lymphatic leukemia and effect on the dose of 6-mercaptopurine. Med Oncol 27: 1046-1049.
23. Silva MR (2008) Thiopurine S-methyltransferase (TPMT) gene polymorphism in Brazilian children with acute lymphoblastic leukemia: association with clinical and laboratory data. Ther Drug Monit 30: 700-704.
24. Samochatova EV, Chupova NV, Rudneva A, Makarova O, Nasedkina TV, et al. (2009) TPMT genetic variations in populations of the Russian Federation. Pediatr Blood Cancer 52: 203-208.
25. Dokmanovic L, Urosevic J, Janic D, Jovanovic N, Petrucev B, et al. (2006) Analysis of thiopurine S-methyltransferase polymorphism in the population of Serbia and Montenegro and mercaptopurine therapy tolerance in childhood acute lymphoblastic leukemia. Ther Drug Monit 28: 800-806.
26. Cooper SC (2008) Ethnic variation of thiopurine S-methyltransferase activity: a large, prospective population study. Pharmacogenomics, 9: 303-309.
27. Winter JW, Gaffney D, Shapiro D, Spooner RJ, Marinaki AM, et al. (2007) Assessment of thiopurine methyltransferase enzyme activity is superior to genotype in predicting myelosuppression following azathioprine therapy in patients with inflammatory bowel disease. Aliment Pharmacol Ther 25: 1069-1077.
28. Wang L, Weinshilboum R (2006) Thiopurine S-methyltransferase pharmacogenetics: insights, challenges and future directions. Oncogene, 25: 1629-1638.
29. Serpe L, Calvo PL, Muntoni E, D'Antico S, Giaccone M, et al. (2009) Thiopurine S-methyltransferase pharmacogenetics in a large-scale healthy Italian-Caucasian population: differences in enzyme activity. Pharmacogenomics 10: 1753-1765.
30. Rossino R, Vincis C, Alves S, Prata MJ, Macis MD, et al. (2006) Frequency of the thiopurine S-methyltransferase alleles in the ancient genetic population isolate of Sardinia. J Clin Pharm Ther 31: 283-287.