

Assessment of interference of a CYP3A4 and CYP1A2 inhibitor (ciprofloxacin) with the MEGX in oral liver function test in rabbits

Edyta Szalek, Rafał Brzeziński, Edmund Grześkowiak, Marta Struś

Department of Clinical Pharmacy and Biopharmacy, Faculty of Pharmacy, Karol Marcinkowski University of Medical Sciences, Poznań, Poland

Abstract: Lidocaine is metabolised to its major metabolite monoethylglycinexylidide (MEGX) by the liver. The transformation is catalyzed by CYP3A4 and CYP1A2. The MEGX test is clinically used to estimate the liver function. The MEGX test by orally administered lidocaine is more comfortable for patients than the intravenous route. This study was designed to evaluate the effect of cytochrome P 450 (CYP) 1A2 and 3A4 inhibitor, ciprofloxacin, on the pharmacokinetics of orally administered lidocaine and its major pharmacologically active metabolite, MEGX. The study was carried out on 7 healthy rabbits. During the first phase the animals were orally given a dose of 3 mg/kg of lidocaine. During the second phase the rabbits received an injection of 25 mg/kg of ciprofloxacin and 3 mg/kg (p.o.) of lidocaine. Plasma concentrations of lidocaine and MEGX were measured by the HPLC method up to 240 minutes after the administration of lidocaine. There were no significant differences in lidocaine and MEGX pharmacokinetic parameters before and after inhibition with the exception of T_{0.5} of lidocaine. A single dose of ciprofloxacin had no influence on the MEGX test on rabbits.

Key words: lidocaine, monoethylglycinexylidide (MEGX) test, pharmacokinetics, ciprofloxacin, rabbits

INTRODUCTION

Lidocaine [2-(diethylamino)-N-(2,6-dimethylphenyl)acetamide] (CAS 137-58-6) is a commonly used local anaesthetic and antiarrhythmic agent. Lidocaine exhibits a significant first-pass effect in the liver, which causes variable levels of its concentration in human plasma. Lidocaine is rapidly deethylated in the liver to monoethylglycinexylidide (MEGX). MEGX is subsequently metabolized to glycinexylidide (GX) [1]. CYP1A2 and CYP3A4 are involved in the metabolism of lidocaine by human liver microsomes. Earlier studies suggested that lidocaine metabolism depends on CYP3A4, but Wang et al. [2] proved that CYP1A2 is the major isoform, which catalyzes lidocaine biotransformation.

Ciprofloxacin [1-cyclopropyl-6-fluoro-4-oxo-7-piperazine-1-yl-quinoline-3-carboxylic acid] (CAS 85721-33-1) is a broad-spectrum antibiotic that belongs to the group of fluoroquinolones. Ciprofloxacin is also known as a CYP1A2 and CYP3A4 inhibitor.

Routine liver tests (bilirubin, transaminases, alkaline phosphatase, glutamyl-transferase, prothrombin time) and several quantitative liver function tests (indocyanine green clearance, galactose elimination capacity, aminopyrine breath test, caffeine test) are insufficient to estimate the dynamic function of hepatocytes in patients with various forms of liver diseases and in patients before and after a liver transplantation [3, 4].

The traditional MEGX test was connected with an intravenous infusion of 1 mg/kg of lidocaine and determination of the concentration of MEGX in blood after 15 minutes. It is a useful marker of the hepatocyte function in numerous clinical cases [4]. Because of its ease of use and rapid analytical determination (FPIA or HPLC method), the MEGX test has found application for assessment of survival in chronic liver disease, for prognosis in patients before and after a liver transplantation, in critically-ill patients and for multiple organ failure (MOF) in trauma patients [5, 6, 7]. Some authors [8, 9] showed that the efficacy of the MEGX test could be improved by using the oral route. Assessing the concentration of MEGX in blood 60 minutes after a standard oral dose of 3 mg/kg of lidocaine could be helpful in estimating the liver function.

The goal of our study was to evaluate the effect of the CYP3A4 and CYP1A2 inhibitor ciprofloxacin on the pharmacokinetics of orally administered lidocaine in rabbits. The aims of the study were to determine the effect of ciprofloxacin on the formation kinetics of MEGX, to assess the possible interference of the CYP3A4 and CYP1A2 inhibitor with the MEGX liver function test.

MATERIALS AND METHODS

Materials. Lidocaine HCl was obtained from Sigma, Germany, and monoethylglycinexylidide (MEGX) from Astra-Zeneca, Poland Lidocainum hydrochloricum 10 mg/ml (Polfa Sp. z o.o. Pharmaceutical Company, Poland), ciprofloxacinum 100 mg/10 ml (Krka Sp. z o.o. Pharmaceutical Company, Poland). Acetonitrile, methanol, orthophosphoric acid and ethyl acetate HPLC grade were obtained from Merck, Germany.

Corresponding author: Dr. Edyta Szalek, Department of Clinical Pharmacy and Biopharmacy, Karol Marcinkowski University of Medical Sciences, Św. Marii Magdaleny 14, 61-861 Poznań, Poland.
E-mail: czechow73@wp.pl

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Kalium phosphoricum (analytical grade) was obtained from Xenon, Poland, sodium hydrophosphate was obtained from Hempur, Poland, NaOH (analytical grade) p.a. ACS>98 were obtained from Fluka, Germany.

Experimental design. Having obtained an approval from the Research Ethics Committee seven healthy rabbits entered the study.

The experiment was performed in the Department of Clinical Pharmacy and Biopharmacy, K. Marcinkowski University of Medical Sciences, Poznań, Poland.

Seven adult rabbits (2.8-4.9 kg) were used in the pharmacokinetic study. Before the experiment the rabbits were fasted for 24 h and given only water. During the first phase, the rabbits were orally given 3mg/kg of lidocaine. In the second phase along with lidocaine the rabbits received injections of 25 mg/kg of ciprofloxacin. Blood samples were collected before the application of the drug and within 1, 5, 10, 15, 30, 60, 90, 120, 150, 180, 240 minutes after the administration of the drug. After centrifugation and dividing the serum samples were frozen at -32°C.

Analytical method and chromatographic system. A sensitive, selective and accurate high-performance liquid chromatographic assay was developed and validated for the determination of lidocaine and its metabolite monoethylglycinexylidide (MEGX) in rabbit plasma. Briefly, lidocaine and MEGX were extracted from 1,0 ml of alkalized plasma to ethyl acetate and the organic phase was eliminated dried under a stream of nitrogen and injected with 0,1 ml phase on the HPLC system. The HPLC system equipped with an ultraviolet detector operated at a wavelength of 210 nm and with a C18 column (LioChroCART 250-4,6, Nucleosil). The mobile phase was acetonitrile, methanol and phosphate buffer (10:30:60) (pH 3.5) and the flow rate 0,7 mlmin⁻¹. Calibration curves (n=3) were linear in the concentration range of 10 to 5000 ng/ml for the lidocaine (r=0,9957) and MEGX (r=0,9994).

Pharmacokinetic analysis. The pharmacokinetic parameters were calculated according to standard methods using Microsoft Excel 97. Maximum plasma concentration and the time taken to reach C_{max} (t_{max}) were obtained directly from the concentration-time data. The terminal rate constant (k_t) was estimated by log-linear regression analysis on data points visually assessed to be on the terminal log-linear phase. At least three data points were used for fitting the terminal phase. The area under the concentration-time curve (AUC_t) was calculated using the linear trapezoidal method. The residual areas (from the last determined concentration C_t to infinity) were calculated as C_t divided by the terminal rate constant. The half-life of lidocaine and MEGX was calculated as Ln 2 divided by the terminal rate constant. The following variables were compared to assess changes before and after inhibition: the ratio of AUC-MEGX to lidocaine (M/L) extrapolated to infinity, the ratio of concentration MEGX to lidocaine at 1, 5, 10, 15, 30, 60, 90, 120, 150, 180, and 240 minutes after oral lidocaine ingestion.

Statistical analysis. A statistical analysis was performed with the use of the InStat version 2.05 (GraphPad Software). The data was described with the arithmetic mean and standard deviation. Pharmacokinetic parameters of lidocaine and its active metabolite MEGX obtained before and after the administration of a single dose of ciprofloxacin were

compared by means of Student's paired t-test for determining the statistical significance of differences between the mean pharmacokinetic parameters.

RESULTS

The pharmacokinetic parameters of lidocaine and its active metabolite MEGX are presented in Table 1 and 2. Mean plasma concentration versus time profiles of lidocaine and MEGX measured after the oral administration of lidocaine alone and pretreatment with ciprofloxacin are shown in Fig. 1 and 2. The metabolic ratio of AUC-MEGX/AUC lidocaine is shown in Fig. 3. The ratio of mean concentration of MEGX/lidocaine after the oral administration of lidocaine in sampling time: 15, 30, 60, 90, 120, 150 and 180 minutes is presented in Fig. 4.

The administration of ciprofloxacin did not result in a significant increase in the AUC_{0-t} of lidocaine and MEGX. The mean AUC_{0-t} of lidocaine was 0.2165 ± 0.1075 µg × h/ml, which was changed to 0.2581 ± 0.0305 µg × h/ml upon pretreatment with a single dose of ciprofloxacin (p>0.05). The mean (±SD) AUC_{0-t} value for MEGX before and after the pretreatment with ciprofloxacin was 0.1301 ± 0.0375 and 0.1302 ± 0.0521 µg × h/ml respectively. There was no change in the t_{max} of lidocaine in the rabbits before and after the pretreatment. The mean (±SD) value of C_{max} of lidocaine before and after the pretreatment with cyprofloxacin was 0.3114 ± 0.1018 and 0.3280 ± 0.0688 µg/ml (p>0.05) respectively, and that of MEGX was 0.0919 ± 0.0482 and 0.0864 ± 0.0415 µg/ml (p>0.05) respectively. There was an increase in half-life of lidocaine from 7.1537 ± 2.0874 h to 11.2007 ± 3.8828 h. The effect was statistically significant (p<0.05). This effect was statistically insignificant for MEGX.

Table 1 Summary of lidocaine pharmacokinetic parameters before and after administration of ciprofloxacin in rabbits

Parameter	Before inhibition	After inhibition	p-value
Lidocaine			
AUC _{0-t} [µg × h/ml]	0,2165 ± 0,1075	0,2581 ± 0,0305	>0,05
AUC _{0-∞} [µg × h/ml]	0,3531 ± 0,1099	0,4288 ± 0,1634	>0,05
K _{el} [h ⁻¹]	0,1066 ± 0,0398	0,0692 ± 0,0257	>0,05
t _{0,5} [h]	7,1537 ± 2,0874	11,2007 ± 3,8828	<0,05*
C _{max} [µg/ml]	0,3114 ± 0,1018	0,3280 ± 0,0688	>0,05
T _{max} [h]	0,2619 ± 0,1121	0,2619 ± 0,1121	>0,05

AUC – area under the plasma concentration time curve; C_{max} – maximum plasma concentration; t_{max} – time to reach C_{max}; t_{0,5} – half-life; data represent mean ± SD (n=7), *p<0,05.

Table 2 Summary of MEGX pharmacokinetic parameters before and after administration of ciprofloxacin in rabbits

Parameter	Before inhibition	After inhibition	p-value
MEGX			
AUC _{0-t} [µg × h/ml]	0,1301 ± 0,0375	0,1302 ± 0,0521	>0,05
AUC _{0-∞} [µg × h/ml]	0,6624 ± 0,3115	0,5097 ± 0,2261	>0,05
K _{el} [h ⁻¹]	0,0337 ± 0,0219	0,0336 ± 0,0319	>0,05
t _{0,5} [h]	24,9960 ± 8,4445	29,2105 ± 11,2150	>0,05
C _{max} [µg/ml]	0,0919 ± 0,0482	0,0864 ± 0,0415	>0,05
T _{max} [h]	0,7857 ± 0,2673	0,9286 ± 0,3450	>0,05

AUC, area under the plasma concentration time curve; C_{max}, maximum plasma concentration; t_{max}, time to reach C_{max}; t_{0,5}, half-life; data represent mean ± SD (n=7).

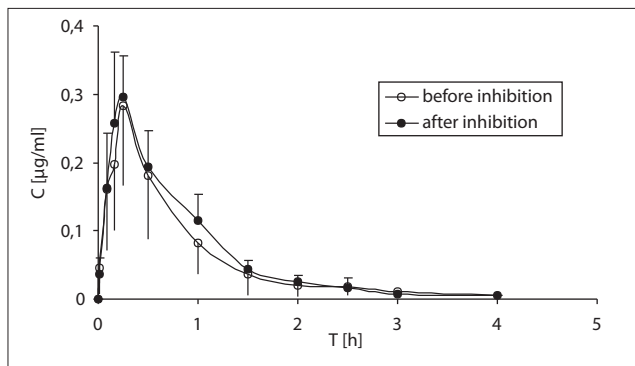


Figure 1 Mean plasma concentrations of lidocaine before and after inhibition by ciprofloxacin. Before inhibition (open circles): lidocaine was administered alone. After inhibition (filled circles): lidocaine was administered in combination with ciprofloxacin. Values are mean \pm SD.

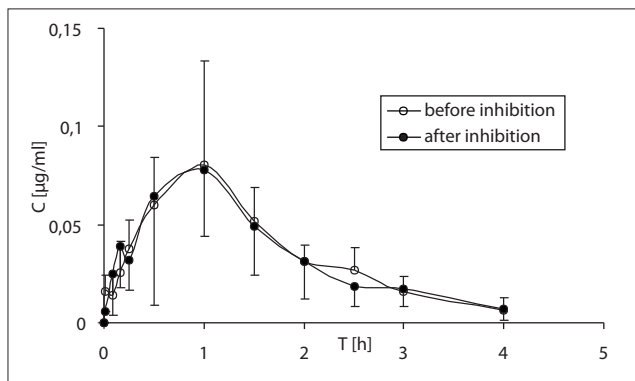


Figure 2 Mean plasma concentrations of MEGX before and after inhibition by ciprofloxacin. Before inhibition (open circles): lidocaine was administered alone. After inhibition (filled circles): lidocaine was administered in combination with ciprofloxacin. Values are mean \pm SD.

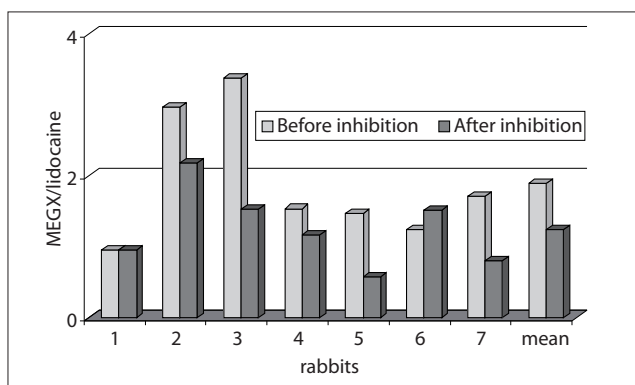


Figure 3 Ratio of $AUC_{0-\infty} \text{ MEGX} / AUC_{0-\infty} \text{ lidocaine}$ after oral administration of 1% lidocaine in rabbits.

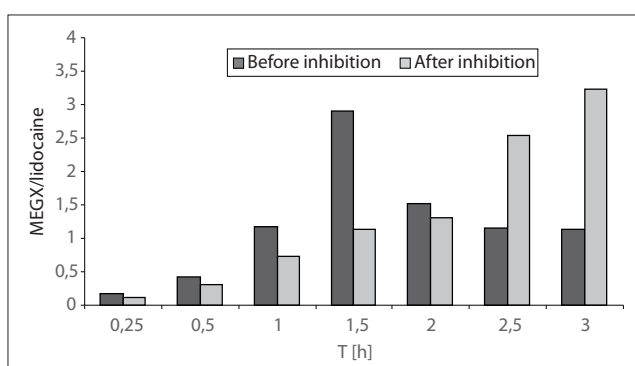


Figure 4 Ratio of mean concentration $\text{MEGX} / \text{lidocaine}$ after oral administration of 1% lidocaine in rabbits in sampling time: 0,25; 0,5; 1,0; 1,5; 2,0; 2,5; 3,0 h.

DISCUSSION

Lidocaine metabolism is determined by MEGX formation after intravenous and oral administration in the MEGX test. The intravenous route of lidocaine administration is better known and more often tested in numerous experiments. The MEGX test is a quantitative index of the hepatic function used in many patients. The results of Gremse's et al. [3] study revealed that within 15 min after an injection of lidocaine (1 mg/kg) the peak monoethylglycinexylidide concentration in healthy children was 97 ± 12 micrograms/L and in children with chronic liver disease it was 32 ± 5 micrograms/L. They summarized the findings stating that the rate of formation of the lidocaine metabolite monoethylglycinexylidide was decreased in patients with chronic liver disease and it is related with disease severity. Another study [10] proved that low MEGX test results < 10 micrograms/L obtained 30 min after an intravenous administration of lidocaine (1 mg/kg body weight) are a useful prognostic sign in paediatric transplant candidates – non-survivors. Shiffman and co-workers in the review article [11] about the use of hepatic lidocaine metabolism to monitor patients with chronic liver disease concluded that severe life-threatening complications of cirrhosis were observed only in patients with MEGX production below 20 ng/ml and one-year survival for patients with an MEGX value of < 10 ng/ml was only 50%. Recent studies have demonstrated that the MEGX test can predict pretransplant survival. Shiffman et al. [12] revealed that the MEGX test could also predict pretransplant complications. Patients with MEGX concentrations greater than 30 ng/ml did not have a major complication and all patients who died had MEGX values below 10 ng/ml. The effects of Huang's et al. study [13] revealed that the mean MEGX concentration in healthy controls was 67 ng/ml and was higher than the mean 43 ng/ml in patients with chronic hepatitis and the mean 24 ng/ml in those with cirrhosis. The MEGX test has also found application in predicting multiple organ failure (MOF) in trauma patients. Lehmann et al. show that all patients who developed MOF displayed a sharp decrease in their MEGX values between days 1 and 3 after the trauma [5]. The disadvantage of the MEGX test is connected with the fact that MEGX formation is affected by gender and several medications, especially CYP3A4 and CYP1A2 inhibitors and inducers. The values of MEGX concentration in blood are well determined, but its changes connected with additional therapy should be more defined. Many interactions can be theoretically predicted by the known mechanism. Some interactions are confirmed *in vitro*, but they have no clinical implications. We should know more about drug interactions with lidocaine to estimate the clinical value of the dynamic liver function test in patients. Its power will be reduced in patients with concomitant therapy including drugs which can inhibit or induce CYP3A4 and CYP1A2. Olkkola et al. [14] studied the effect of coadministration of the CYP1A2 inhibitor fluvoxamine and CYP3A4 inhibitor erythromycin on lidocaine pharmacokinetics in healthy volunteers. During the combination of fluvoxamine and erythromycin, the lidocaine clearance was by 53% smaller than during the placebo. Fluvoxamine alone decreased the clearance of lidocaine by 41% and prolonged its elimination half-life from 2.6 to 3.5 h. Orlando et al. [15] proved that the effect of pharmacokinetic changes after fluvoxamine-lidocaine interaction depends on the liver condition. The lidocaine clearance was decreased by 60% on average in healthy subjects and by 44% in patients with

mild liver dysfunction, with proportional increases in terminal half-lives. No pharmacokinetic changes were calculated in patients with severe liver dysfunction. Similar effects were observed on MEGX and GX formation kinetics, which were drastically impaired in healthy subjects and patients with mild liver cirrhosis but unaffected in patients with severe cirrhosis [15]. The results of pharmacokinetic studies are different for various routes of lidocaine administration. Isohanni et al. revealed the pharmacokinetic implications of CYP3A4 inhibitor itraconazole, which significantly increases plasma concentrations and toxicity of oral lidocaine [16], but for the inhaled route of lidocaine the same author shows no statistically significant changes in any of the pharmacokinetic parameters (peak concentrations, concentration peak times or elimination half-lives) of lidocaine or monoethylglycinexylidide [17]. The interaction between amiodarone and lidocaine may be explained by the inhibition of CYP3A4 by amiodarone and/or by its main metabolite DEA (N-monodesethylamiodarone). The analysis of lidocaine pharmacokinetics showed an increase in the area under the curve (AUC), a decrease in the systemic clearance, but the elimination half-life ($t_{1/2}$) and the distribution volume at the steady state of lidocaine remained unchanged when amiodarone was administered [18]. Reichel et al. [19] claims that the MEGX test is not a sensitive marker of P-450 induction in a healthy human liver. The conclusion was an effect of investigation of the lignocaine metabolite (MEGX) liver function test and P-450 induction by rifampicin. MEGX test results before and after the induction with rifampicin were compared. The activity of CYP enzymes can be modified even by herbal drugs like: St John's wort, milk thistle, echinacea, garlic and others.

In our study a visual and statistical comparison of the concentration-time curves reveals that the coadministration of ciprofloxacin as a single dose had no effect on the lidocaine and MEGX concentrations. As shown in Table 1, ciprofloxacin had no statistically significant modifications of lidocaine and MEGX pharmacokinetic parameters with the exception of lidocaine $T_{0.5}$. The coadministration of ciprofloxacin produced no significant interference with the results of the MEGX liver function test, irrespective of the sampling time (15, 30, 45, or 60 minutes; $p > 0.05$). Isohanni et al. [20] proved that a multiple dose of ciprofloxacin in volunteers increased the mean peak concentration and area under the plasma concentration-time curve of intravenous lidocaine by 12% and 26% respectively. The mean plasma clearance of lidocaine was decreased by ciprofloxacin by 22%. Ciprofloxacin decreased the area under the plasma concentration-time curve of MEGX by 21%. The authors suggest that ciprofloxacin may increase the systemic toxicity of lidocaine [20]. A single-dose administration of ciprofloxacin did not result in statistically significant changes in the lidocaine pharmacokinetics in rabbits. However, in most animals a decrease in the $AUC_{0-\infty}$ MEGX/ $AUC_{0-\infty}$ lidocaine ratio can be observed after the administration of ciprofloxacin (Fig. 3). The MEGX/lidocaine mean concentration ratio in time points: 0.25, 0.5, 1.0, 1.5, 2.0 h is also reduced after the application of ciprofloxacin (Fig. 4). A multiple administration of the antibiotic would probably enhance the observed changes.

Further studies are needed to determine the role of CYP enzymes and their inhibitors and inductors in the biotransformation of lidocaine. Although the MEGX test is a useful prognostic index in people with liver diseases, clinicians should be conscious that this test may have several limitations connected with the administration of other drugs.

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REFERENCES

- Orlando R, Piccoli P, De Martin S, Padriani R, Floreani M, Palatini P: Cytochrome P450 1A2 is a major determinant of lidocaine metabolism in vivo: effects of liver function. *Clin Pharmacol Ther* 2004, **75**(1), 80-88.
- Wang JS, Backman JT, Wen X, Taavitsainen P, Neuvonen PJ, Kivisto KT: Fluvoxamine is a more potent inhibitor of lidocaine metabolism than ketoconazole and erythromycin in vitro. *Pharmacol Toxicol* 1999, **85**(5), 201-205.
- Gremse DA, A-Kader HH, Schroeder TJ, Balistreri WF: Assessment of lidocaine metabolite formation as a quantitative liver function test in children. *Hepatology* 1990, **12**(3), 565-569.
- Wang JS, Backman JT, Taavitsainen P, Neuvonen PJ, Kivisto KT: Involvement of CYP1A2 and CYP3A4 in lidocaine N-deethylation and 3-hydroxylation in humans. *Drug Metab Dispos* 2000, **28**(8), 959-965.
- Munoz AE, Miguez C, Rubio M, Bartellini M, Levi D, Podesta A, Niselman V, Terg R: Lidocaine and monoethylglycinexylidide serum determinations to analyze liver function of cirrhotic patients after oral administration. *Dig Dis Sci* 1999, **44**(4), 789-795.
- Oellerich M, Burdelski M, Lautz HU, Schulz M, Schmidt FW, Herrmann H: Lidocaine metabolite formation as a measure of liver function in patients with cirrhosis. *Ther Drug Monit* 1990, **12**(3), 219-226.
- Olkkola KT, Isohanni MH, Hamunen K, Neuvonen PJ: The effect of erythromycin and fluvoxamine on the pharmacokinetics of intravenous lidocaine. *Anesth Analg* 2005, **100**(5), 1352-356.
- Corpataux JM, Munafo A, Buclin T, Biollaz J, Mosimann F: A preliminary evaluation of the discriminative power of the monoethylglycinexylidide formation test after intravenous and oral administration of lidocaine. *Transplant Proc* 2001, **33**(4), 2557-2562.
- Oellerich M, Armstrong VW: The MEGX test: a tool for the real-time assessment of hepatic function. *Ther Drug Monit* 2001, **23**(2), 81-92.
- Burdelski M, Schutz E, Nolte-Buchholtz S, Armstrong VW, Oellerich M: Prognostic value of the monoethylglycinexylidide test in pediatric liver transplant candidates. *Ther Drug Monit* 1996, **18**(4), 378-82.
- Shiffman ML, Fisher RA, Sanyal AJ, Edinboro LE, Luketic VA, Purdum PP 3rd, Raymond P, Posner MP: Hepatic lidocaine metabolism and complications of cirrhosis. Implications for assessing patient priority for hepatic transplantation. *Transplantation* 1993, **55**(4), 830-835.
- Tanaka E, Inomata S, Yasuhara H: The clinical importance of conventional and quantitative liver function tests in liver transplantation. *J Clin Pharm Ther* 2000, **25**(6), 411-419.
- Huang YS, Lee SD, Deng JF, Wu JC, Lu RH, Lin YF, Wang YJ, Lo KJ: Measuring lidocaine metabolite-monoethylglycinexylidide as a quantitative index of hepatic function in adults with chronic hepatitis and cirrhosis. *J Hepatol* 1993, **19**(1), 140-147.
- Orlando R, Piccoli P, De Martin S, Padriani R, Palatini P: Effect of the CYP3A4 inhibitor erythromycin on the pharmacokinetics of lignocaine and its pharmacologically active metabolites in subjects with normal and impaired liver function. *Br J Clin Pharmacol* 2003, **55**(1), 86-93.
- Reichel C, Skodra T, Nacke A, Spengler U, Sauerbruch T: The lignocaine metabolite (MEGX) liver function test and P-450 induction in humans. *Br J Clin Pharmacol* 1998, **46**(6), 535-539.
- Isohanni MH, Neuvonen PJ, Olkkola KT: Effect of erythromycin and itraconazole on the pharmacokinetics of oral lignocaine. *Pharmacol Toxicol* 1999, **84**(3), 143-146.
- Isohanni MH, Neuvonen PJ, Olkkola KT: Effect of itraconazole on the pharmacokinetics of inhaled lidocaine. *Basic Clin Pharmacol Toxicol* 2004, **95**(3), 120-123.
- Ha HR, Candinas R, Stieger B, Meyer UA, Follath F: Interaction between amiodarone and lidocaine. *J Cardiovasc Pharmacol* 1996, **28**(4), 533-539.
- Shiffman ML, Luketic VA, Sanyal AJ, Thompson EB: Use of hepatic lidocaine metabolism to monitor patients with chronic liver disease. *Ther Drug Monit* 1996, **18**(4), 372-377.
- Lehmann U, Armstrong VW, Schutz E, Regel G, Pape D, Oellerich M: Monoethylglycinexylidide as an early predictor of posttraumatic multiple organ failure. *Ther Drug Monit* 1995, **17**(2), 125-132.