Systemic preclinical studies of therapeutic substances can not answer the question how they behave when irregularly or occasionally administered. This kind of application is becoming more common due to increasing pace of life and growing number of drugs available without a prescription, a situation which favors self-treatment (1, 2). More drugs are permitted for use in an increasing number of ailments. Hence, the possibility of interactions between active drug compounds. These may be related to pharmacokinetic interactions connected with intestinal wall transporters and cytochrome P450 in liver and intestine. Complex influences between drugs or medicines together with the structures of the body - enzymes, transporters, cause great difficulty in predicting occurrence of possible interactions.

A variety of drug transporters present in the body control the fate of drugs by affecting absorption, distribution, and elimination processes. In clinical pharmacotherapy, ATP-dependent efflux transporters expressed on the apical membrane of the intestinal epithelial cells determine oral bioavailability, intestinal efflux clearance, and the site of drug-drug interaction of certain substances (3). P-glycoprotein (P-gp) belongs to the ABC transporters super family (4). P-gp is expressed on the luminal surface of the intestinal epithelia, the renal proximal tubule, the bile canalicular membrane of the hepatocytes, the placenta and the blood-brain barrier (5). This anatomical localization together with its broad substrate profile contributes to the significant role of P-gp in drug absorption and disposition. P-gp distribution within many organs and tissues results in the absorption or excretion of xenobiotics and drug-drug interactions with this protein may change the bioavailability of simultaneously administered active drugs. It must be considered that administration of
one drug can influence metabolism of another drug via modulation of P-gp activity (6) but also via modulation of the cytochrome P450 enzymes (7).

Quinidine (QD), an antiarrhythmic drug is a well-known cause of drug-drug interactions (8). It is P-gp (9) and P450 (10) as well as CYP2D6 substrate and inhibitor (11, 12). QD is listed on sixth place as substance involved in CYP-mediated drug-drug interaction by Kato et al. (10)

Domperidone (DOM) is a dopamine D2-receptor antagonist. Due to its high molecular weight and low lipid solubility DOM does not enter the CNS (13). It is rapidly absorbed after oral administration but with low bioavailability caused by intestinal P-gp activity (14). Both domperidone and quinidine interact with P450 enzymes (especially CYP2D6) and with P-gp in each phase of disposition of these pharmaceutical compounds within an organism (15, 16). QD has been shown in our previous paper to increase rat plasma DOM concentration during the first two hours after their single-dose administration (17). This study has investigated interaction between DOM and QD in multi-dosage (once a week repeated single disposition) administration to Wistar rat model. There also were examined kinetics of acetaminophen (PAM) administered (30 mg/kg) with domperidone as an indicator of gastric emptying, showing domperidone prokinetic activity, as well as quinidine anticholinergic activity (18, 19).

The aim of the study was to examine the changes of domperidone kinetics in rat serum assuming multiple administration at weekly intervals (during 7 weeks), while using different dosing regimens of quinidine. Among the different variants of medication, in addition to conventionally used doses of single and multiple, daily, administered at different times, the periodic, and/or occasional manner of medication, is virtually no examined. Raises the question whether dose used from time to time, separated by a longer period of absence of drug in the body, may interact as to lead to specific pharmacokinetic and pharmacological effects? In this model domperidone as occasionally used drug and quinidine as well-known cause of drug-drug interactions were used. We also have examined the course of the kinetics of paracetamol co-administered with domperidone as an indicator of gastric emptying, showing the prokinetic activity of domperidone.

MATERIALS AND METHODS

Domperidone (C_{22}H_{24}ClN_{5}O_{2}; ≥98%, PubChem CID: 44349952), quinidine hydrochloride monohydrate (C_{20}H_{24}N_{2}O_{2} HCl H_{2}O; ≥5.5% dihydroquinidine, PubChem CID: 16219921), acetaminophen (C_{6}H_{9}NO_{2}; ≥99%, PubChem CID: 1983), propranolol hydrochloride (C_{16}H_{21}NO_{2} HCl; ≥99%), 8-chlorotheophylline (C_{7}H_{7}ClN_{4}O_{2}; 98%), β-glucuronidase from Helix pomatia (type H-1, partially purified powder, ≥300,000 units/g solid) and carboxymethyl cellulose sodium salt (high viscosity, 1500-3000 cP, 1% in H_{2}O, in 25°C) were obtained from Sigma-Aldrich (Germany). Sodium phosphate monobasic (NaH_{2}PO_{4} ≥99%), phosphoric acid (H_{3}PO_{4} ≥85%), potassium phosphate monobasic monohydrate (KH_{2}PO_{4} H_{2}O ≥99.5%) and sodium chloride (NaCl ≥99.5%) were supplied by POCh Gliwice (Poland). HPLC grade dichloromethane and methanol were supplied by Labscan (Ireland). Water was obtained from Mili-Q purification system.

<table>
<thead>
<tr>
<th>Number of administration</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>group I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAM</td>
<td>DOM</td>
<td>DOM</td>
<td>DOM</td>
<td>PAM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>group II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAM</td>
<td>DOM</td>
<td>DOM</td>
<td>DOM</td>
<td>DOM</td>
<td>DOM</td>
<td>DOM</td>
<td>DOM</td>
</tr>
<tr>
<td>group III</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAM</td>
<td>DOM</td>
<td>DOM</td>
<td>DOM</td>
<td>DOM</td>
<td>DOM</td>
<td>DOM</td>
<td>DOM</td>
</tr>
<tr>
<td>QD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PAM - acetaminophen, DOM - domperidone, QD - quinidine.
Male Wistar Cmd: (WI) WU rats weighing 191 ± 11 g at the beginning and 321 ± 25 g at the end of the experiment (Medical Research Center, Polish Academy of Sciences) were used. Rats were treated according to the Guiding Principles for the Care and Use of Laboratory Animals approved by the Local Animal Research Ethic Committee in an independent room with controlled temperature at 23°C, 12/12 light/dark cycle and 66% humidity. Animals had free access to water and food supplied as pellets. The general condition of the animals was checked daily. The animals were acclimated for 10 days before the beginning of the experiment. Thereafter, twelve rats were randomly divided into three groups, which were treated as shown in Table 1. Food was withdrawn for 12 h before drug administration, free access to water was allowed during the experiment.

DOM powder was suspended to concentration of 14 mg/mL in 1% water solution of carboxymethyl cellulose sodium salt. DOM was administered at the dose of 30 mg/kg b.w. The dose of DOM was chosen on the basis of doses used by Michiels and Heykants in their animal pharmacokinetic study (20, 21).

QD was dissolved in water to a final concentration of 8 mg/mL and given per os at the dose of 25 mg/kg (dose calculated as a free base). We decided on this dose due to the fact that in both studies the influence of quinidine on the metabolism of various compounds such as quinidine impact on P-glycoprotein in rats doses of 10 to 50 mg/kg were used (22, 23).

PAM was administered at the dose of 30 mg/kg b.w. (24), as a water suspension with 1% carboxymethyl cellulose sodium salt at a final concentration 24 mg/mL.

All compounds were given via gastric gavage, concomitant or alone with vehiculum (1% water suspension of carboxymethyl cellulose sodium salt or water) as a one shut.

Blood samples (250 µL) were collected without anticoagulants from tail vein at 30, 120, 240, 360, and 480 min after DOM treatment. The samples were centrifuged at 8000 × g for 5 min. to obtain the plasma which was stored at −20°C until quantitative determination of DOM and PAM.

For DOM extraction, 0.1 mL of each sample, 80 ng of propranolol hydrochloride as internal standard, 0.1 mL 0.1 M NaOH, and 3 mL of dichloromethane were added. The mixture was shaken for 10 min and centrifuged at 12000 × g for 15 min. Next, organic phase was transferred to another glass tube and evaporated to dryness under gentle nitrogen stream. Dried residue was dissolved in 200 µL of mobile phase, and 20 µL of the aliquot was injected into the HPLC column.

A recovery study was performed by spiking 0.1 mL of plasma with 20 µL DOM standard solutions and the samples underwent extraction procedure described above. For three tested concentration an average of 92.7% and 86.7% recovery was obtained, respectively, for DOM and propranolol hydrochloride.

PAM was isolated from plasma by solid phase extraction after enzymatic hydrolysis. In the Eppendorf tubes were placed 20 µL of serum, 20 µL β-glucuronidase solution in 0.2 M potassium phosphate monobasic solution and after thorough mixing the tubes were subjected to incubation at 37°C for 18 h. Afterwards, for the incubation of tubes, 20 mL methanol solution of 8-chlorotheophylline at concentration of 15 µg/mL as an internal standard and 440 µL 0.9% NaCl were added. Prepared samples were mixed and placed on the SPE columns (BAKER-BOND C18, 3 mL) for the extraction process. After final elution, methanol was evaporated to dryness under gentle nitrogen stream. Dried residue was dissolved in 200 µL of mobile phase, and 20 µL of the aliquot was injected into the HPLC column. Efficiency of the extraction process was 89.0% for acetaminophen and 104.9% for the 8-chlorotheophylline (average for three concentrations).

Concentration of DOM in plasma samples was measured by reversed phase HPLC method (Shimadzu, Germany) with fluorescent detection (RF-10Ax, Shimadzu, Germany) by use of the reported method with modifications (25). Chromatographic separation was performed using a Discovery HS PEG stainless steel column 150 × 4.6 mm I.D., 5 µm (Supelco, Bellefonte, PA, USA), preceded by a 20 × 4.6 mm I.D., Discovery HS PEG guard column. The column was kept at 40°C. Injection volume was 20 µL. The mobile phase consisted of 0.02 M water solution of natrium dihydrogen orthophosphate (adjusted to pH 3.5 with orthophosphoric acid) – methanol (82 : 18, v/v) and was delivered at a flow rate of 1.0 mL/min. The run time was 10 min. The mobile phase was ultrasonically degassed prior to use. Six point calibration curve was linear ranging between 10 and 1000 ng/mL with correlation coefficient (r²) 0.999. The precision was calculated as percent coefficient of variation (CV) and for each analyzed concentration did not exceed 6.5%. Accuracy of measurement for 6 different concentrations (bias) was 5%. The limit of detection for the assay was 7.97 ng/mL of plasma.
PAM plasma concentration was quantified by HPLC (Shimadzu, Germany). The method was based on the procedure developed and used in the Toxicology Department (26). Discovery C18 150 × 4.6 mm I.D., 5 µm column (Supelco, Bellefonte, PA, USA), preceded by a 20 × 4.6 mm I.D., Discovery C18 guard column were used for resolution. The column was kept at 40°C. The mobile phase consisted of 0.025 M water solution of potassium phosphate monobasic monohydrate – acetonitrile - methanol (85:10:5, v/v/v) and was delivered at a flow rate of 1.5 mL/min. Injection volume was 20 µL. PAM was detected by UV-detection (SPD Shimadzu, Germany) at a wavelength of 230 nm. The run time was 10 min. The mobile phase was ultrasonically degassed prior to use. The six point calibration curve was linear ranging between 2.5 and 100 µg/mL with correlation coefficient ($r^2$) 0.9921. Precision was calculated as percent CV and it was 2.5% (average for 5, 50 and 100 µg/mL of plasma concentration). Accuracy of measurement for 6 different concentration (bias) was 4.3%. The limit of detection for the assay was 2.35 µg/mL of plasma.

The peak serum concentration (Cmax) and the time to peak concentration (Tmax) were obtained from visual observation. Due to the limited number of measurement points, pharmacokinetic parameters were determined in real time and pharmacokinetic parameters of DOM and PAM were analyzed by noncompartmental method of the program Kinetica (version 5.0, Thermo Scientific, USA). The concentration-time profiles for domperidone and acetaminophen were analyzed for partial area under the concentration–time curve from zero to 6 h ($AUC_{0-6}$). The area under the serum concentration-time curve was calculated using linear trapezoidal rule.

All statistical analyses were conducted with Statistica (version 9.0, StatSoft, Poland). All results were tested for parametric assumptions (Shapiro-Wilk and Brown-Forsythe tests). For each parameter for which assumptions were satisfied the Repeated Measures ANOVA tests were used. When assumptions of the analysis of variance had been violated, the Friedman’s ANOVA test to determine significant differences within groups was used. The limit for statistical significance was set at $p < 0.05$.

**RESULTS**

Analysis of the results should be conducted based on experimental design scheme (Table 1).

After conducting analysis in groups it should be noted that in group I (where quinidine was administered in 3rd week) the Cmax in this kinetics although less markedly significant ($p < 0.06$) was higher than in the 2nd and 4th week kinetics, where domperidone was given without quinidine. In 7th week kinetics, 5-fold increase in the values of the pharmacokinetic parameters for domperidone in relation to the initial data was observed. Mean values of parameters in the latter kinetics are up to 5 times higher than the initial ones (for example $AUC_{0-6}$). There also was statistically significant 2-fold increase in the partial $AUC$ values in relation to the 4th week kinetics. A general upward trend in values of these parameters over time was shown (Fig. 1).

![Figure 1 Values of domperidone area under the time-concentration curve and Cmax in group I. Doses of drugs: DOM 30 mg/kg, PAM 30 mg/kg and QD 25 mg/kg. Data represent the mean ± SD for n = 4. Administrations were compared by the Friedman’s ANOVA test, * $p < 0.05$ vs. 2nd administration; # $p < 0.06$ vs. 2nd administration](image-url)
Moreover, a rise in the value of domperidone AUC$_{0-2}$/AUC$_{4-6}$ ratio in 3rd week kinetics, when quinidine was administered, was observed.

In group II, during 6th administration there was a sharp increase in the AUC areas after first time quinidine co-administration with domperidone (especially for AUC$_{0-6}$, 2193 ng/mL ◊ h compared to 783 ng/mL ◊ h in 5th week kinetics). The remaining AUC: AUC$_{0-0.5}$, AUC$_{0-1}$ and AUC$_{0-2}$ after administration of quinidine increased by approximately 100%, as shown in Figure 2. In these kinetics, statistically significant increase in both parameters characterizing the absorption process - AUC$_{0-T_{max}}$, and other phases of the fate of the drug in the body - from the AUC$_{0-0.5}$ to AUC$_{0-6}$ and AUC$_{4-6}$, was revealed.

Group III, 6th week kinetics is characterized by a significant increase in partial AUC from 245% for AUC$_{0-4}$ to 467% for AUC$_{0-1}$ one week after QD disposition. Reduction of domperidone AUC$_{0-2}$/AUC$_{4-6}$ ratio in group III where quinidine was administrated every other week was observed in the 3rd, 5th and 7th administrations (Fig. 3).

In group I, quinidine administration in 3rd week kinetics resulted in a decrease in all AUC values but recorded decreases were not statistically significant. However a week after the administration of quinidine (4th administration) AUC areas were significantly higher and this effect persisted in the following 7th week kinetics, after three weeks (Fig. 4). Slight decrease in PAM AUC$_{0-2}$/AUC$_{4-6}$ ratio after
QD dosage was observed due to statistically significant rises of AUC4-6 values in 4th and 5th dispositions. Significant increases in PAM pharmacokinetic parameters in 7th week kinetics in both the group I (PAM administration with DOM after a two week break) and in group II - one week after administration of quinidine, are shown in Figures 4 and 5. In Group II - one week after quinidine administration, the highest values of the AUC in 7th week kinetics were observed.

Alternate administration of PAM with domperidone and PAM with domperidone and quinidine cause an increase in average AUC values in the week after the administration of quinidine, which is observed in 4th and 6th week kinetics and showed in Figure 6.

DISCUSSION

Among the different variants of drug administrations, apart from conventionally used single and multiple doses daily, administered at different periods of time, there is virtually unexplored way of irregular, occasional intake. The question is whether
Quinidine and domperidone interactions in the rat experimental model of... 1063

medicine applied from time to time, separated by a longer period of absence of drug in the body may interact so, as leading to the specific pharmacokinetic and pharmacological effects. According to the traditional approach, it is believed that the majority of pharmacokinetic interactions occurs due to drug biotransformation in the liver with the participation of cytochrome P450 enzymes (12, 27). P-glycoprotein may be perceived as a barrier due to the nature of its activities - regulated efflux of toxic substances and xenobiotics from the light outside the cell. Due to its characteristics, P-gp plays an important role in the processes of absorption and distribution of medicines (4, 28). Knowledge about the distribution and mechanisms of action of this membrane protein is crucial to understanding its impact on the kinetics of drugs, especially in the face of polytherapy and increasingly frequent phenomenon of self-medication. Therefore, an animal model was built by us.

We examined rat plasma domperidone kinetics changes employing multiple dosing at weekly intervals, while using different dosing regimens of quinidine. Expected interactions between domperidone and quinidine may have its origin both in the ability of quinidine to inhibit P-gp activity as well as cytochrome P450-mediated metabolism of the two compounds. The kinetics of acetaminophen were also investigated when administered concomitantly with domperidone as an indicator of gastric emptying, showing domperidone prokinetic activity, as well as quinidine anticholinergic activity.

In our investigation, we used DOM, the drug administered in many disease entities but not with repeated dosage regimen and with occasionally repeated dosage as in dyspepsia, nausea and vomiting treatment or travel sickness. Domperidone was administered in various systems with acetaminophen and quinidine at weekly intervals for 5-7 weeks.

Animal study shows that domperidone undergoes rapid and extensive hepatic metabolism by hydroxylation and N-dealkylation (20). In vitro metabolism in human liver microsomes with inhibitors revealed that CYP3A4 is a major form of cytochrome P-450 involved in the N-dealkylation of domperidone, whereas CYP3A4, CYP1A2 and CYP2B6, CYP2C8 and CYP2D6 are involved in domperidone aromatic hydroxylation (16). Domperidone is a very good substrate for MDR1 and mdr1a P-gp (4, 5). Quinidine is metabolized by CYP3A superfamily of cytochrome P450 enzymes to hydroxylated derivatives excreted with urine. QD is known as a good P-gp inhibitor (9, 29).

There is no data tied to pharmacokinetics after occasionally repeated dosage. However, the importance of CYP3A and P-glycoprotein in limiting oral delivery is suggested by: their joint presence in small intestinal enterocytes, the significant overlap in their substrate specificities, and by the poor oral bioavailability of drugs that are substrates for both CYP3A and P-gp, e.g., cyclosporine (30) and examined in this trials QD (10). Additionally, enzymes and drug transporter proteins may be induced or
inhibited by the same compounds - QD is found to be an inhibitor and substrate for CYP2D6 (8, 11, 12) or CYP3A4 (10).

The increase in value of DOM AUC_{0-2}/AUC_{4-6} ratio after first quinidine and domperidone co-administration at 3rd week of experiment was the result of QD-DOM interactions at the level of absorption (17). This effect is visible even in spite of overall upward trend in concentrations and AUC levels in subsequent administrations (Fig. 1). This augmentation is consistent with quinidine inhibitory action on intestinal P-gp. However, in group II, when first QD and DOM co-administration was preceded by four domperidone dispositions much greater growth of DOM pharmacokinetic parameters was observed. DOM AUC_{0-6} was 3-fold higher and others were 100% higher (Fig. 2). In this case, the action of QD as an inhibitor of P-glycoprotein is evident by a statistically significant increase in parameters characterizing the absorption - AUC_{0-t_{max}}, AUC_{0-0.5} and other phases of ADME - AUC_{0-6} and AUC_{4-6} (data not shown), which may indicate an inhibition of domperidone hepatic biotransformation by cytochrome P450. Note that where DOM and QD co-administration was done after one DOM premedication only, an increase in Cmax took place, while the other examined parameters did not show statistically significant changes.

When considering acetaminophen, it should be noted that in contrast to group I PAM, DOM and QD co-administration (in the 6th kinetics, Fig. 4 and Fig. 5) caused statistically insignificant increase in PAM AUC values. A reduction of domperidone AUC_{0-2}/AUC_{4-6} ratio when quinidine was administered every other week, at 3, 5 and 7 weeks (in group III) demonstrated the inhibitory action of quinidine on domperidone metabolism presumably by inhibiting the activity of hepatic CYP3A4. Moreover, in this group at 6th week, statistically significant increase in absorption of domperidone was noted. The fact that the largest increases in domperidone AUC values in this drug delivery system (Fig. 3) took place in the week after the quinidine administration is also worth mentioning. The largest increases in PAM pharmacokinetic parameters one week after quinidine administration in group I (4th disposition, Fig. 4) and II (7th disposition, Fig. 5) was observed as well. Analysis of changes in the value of AUC_{0-2} at the initial three weeks of experiment suggests intensity of domperidone absorption processes. The following week increase in the value AUC_{4-6} suggests inhibition of domperidone hepatic biotransformation.

Many studies have demonstrated that in response to increased intestinal metabolism caused by the interaction of P-gp and metabolizing enzyme substrate concentration is stabilized at a lower level, thus affecting the AUC and Cmax (4, 31). This has occurred in our study - domperidone as a substrate for CYP3A, CYP2D and P-gp undergoes extensive metabolism associated with the induction of intestinal cytochrome P450 system, which is a response to the previously given quinidine. This results in low levels of pharmacokinetic parameters, lower than implied by the simultaneous administration of quinidine with domperidone, especially with regard to high levels of pharmacokinetic parameters in the week after the QD administration. Induction of cytochrome may depend on many factors, such as the accumulation of an inducer or the enzyme capacity synthesis de novo. According to Shapiro et al., induction of CYP3A with a model inducer - rifampicin can take several weeks (12).

Wandel et al. describes the ability of quinidine to inhibit CYP3A4 activity, thereby inhibiting the metabolism of substrates of cytochrome P450 isoforms. They studied the activity of CYP3A in the metabolism of nifedipine to dehydronifedipine in human microsomes in the presence of 14 different compounds, which are inhibitors of P-glycoprotein (32). All the increases in pharmacokinetic parameters in conducted experiment may be partially due to inhibitory action of quinidine on domperidone metabolizing enzymes.

P-glycoprotein is a widely recognized cause of reduced bioavailability of orally administered drugs due to its active role in limiting absorption from the intestinal lumen. A classic example of it is the interaction of quinidine-digoxin. Digoxin as a model P-glycoprotein substrate (as also is domperidone studied in this work), undergoes no further metabolism by cytochrome P450 and has greatly limited oral bioavailability. Quinidine given orally to patients with digoxin causes total increase of examined compound absorption by 15%, while increasing the AUC by 77% and the maximum concentration by 81%. These data suggest that inhibition of P-gp activity by quinidine in the intestine at the stage of absorption, which significantly affects the growth of the pharmacokinetic parameters of digoxin, takes place in our study in the group I, II and III with different intensities after quinidine co-medication (33). Weekly domperidone administration causes increases in its AUC maximum at 4 and 5 week of study. These growths are visible in all AUC bands. They are the most marked in the range of 0-0.5 h - 6-fold, while for
Quinidine and domperidone interactions in the rat experimental model of... 1065

AUC0-6 is a 4.7-fold increase observed. Because significant increases in the parameters values describing all phases of drug present in the system took place, there is difficulty in classifying the reasons for the observed effect. Given that DOM bioavailability is approximately 20% and after several domperidone administration absorption system as a result of compensating effects can operate more efficiently or start a new factor responsible for its absorption, DOM concentrations have the right to grow. Moreover, vehicle, in which the test substances were administered is sodium carboxymethyl cellulose. Sodium carboxymethyl cellulose salt, as shown by Clausen et al., has the ability to increase the permeability of fluorescein, bacitracin and insulin in guinea pig small intestine (34). One percent addition of carboxymethyl cellulose significantly increased absorption within 180 min of fluorescein dose of 4.93 to 6.42%. This feature was additionally confirmed by measurements of TEER (Transendothelial Electrical Resistance). Sodium carboxymethyl cellulose salt causes 10% decrease in transepithelial electrical resistance compared to baseline.

CONCLUSION

Based on statistical analysis of the results, it cannot be unambiguously determined what mechanisms are responsible for the observed effects. Clarification of the mechanisms of this phenomenon requires additional study despite the fact that there are not documents which take into account repeated administration during occasional irregular administrations. Especially it is worth to remember that irregular intake of drugs with concomitant treatment of chronic disease can lead to unpredictable effects of interactions.

Acknowledgment

Authors would like to thanks Ms. Sylwia Sztermel for help in acetaminophen isolation and determination.

All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted which are consistent with European Communities Council Directive of 24 November 1986 (86/609/EEC). The research was financed by: the Statutory Funds of the Medical University of Warsaw (FW13/N/14) and received no special grant from any founding agency in the public, commercial or not-for-profit sectors.

REFERENCES


Received: 23. 06. 2015