




The Influence of Diabetes Mellitus on Glucuronidation and Sulphation of Paracetamol in Patients with Febrile Neutropenia

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Abstract

Background and Objectives Numerous studies have confirmed the influence of diabetes mellitus on the pharmacokinetics of drugs. Paracetamol (APAP) is an antipyretic that is commonly used in febrile neutropenia (FN) therapy. APAP is chiefly metabolised by glucuronidation and sulphation. This study assessed the influence of diabetes on the pharmacokinetics of paracetamol and its metabolites: glucuronide (APAP-glu) and sulfate (APAP-sulfate) in FN patients.

Methods Patients with FN received single intravenous dose 1000 mg of APAP. The FN patients were allocated to one of two groups: diabetics (DG, $n = 7$) or non-diabetics (NDG, $n = 11$). The plasma concentrations of paracetamol and its metabolites were measured with the validated high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection.

Results Pharmacokinetic parameters (mean [SD]) of APAP in the DG and NDG groups were as follows: C_{\max} (maximum concentration) = 21.50 [11.23] vs. 23.42 [9.79] mg/L, AUC_{0-t} (area under the concentration–time curve) = 44.23 [17.93] vs. 41.43 [14.57] mg·h/L, $t_{1/2\text{kel}}$ (elimination half-life) = 2.28 [0.80] vs. 2.11 [0.80] h. In both groups the exposure to APAP was comparable. The study did not reveal differences between the two groups in the pharmacokinetics of APAP-glu and APAP-sulfate. The C_{\max} and AUC_{0-t} ratio between the metabolites and APAP were similar.

Conclusions No differences in the pharmacokinetics of APAP, APAP-glu and APAP-sulfate in patients with FN indicates that diabetes does not influence glucuronidation and sulfation of paracetamol.

Key points

Diabetes mellitus does not influence glucuronidation of paracetamol.

Diabetes mellitus does not influence sulfation of paracetamol.

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1 Introduction

Patients with hematological malignancies usually undergo complex therapy, including antipyretic therapy. However, cytostatic therapy has the myelotoxic effect. The concentration of neutrophils decreases in most patients who undergo consecutive courses of chemotherapy. If they develop an infection, it usually results in febrile neutropenia (FN). Decreased concentration of neutrophils $< 500/\mu\text{L}$ favours rapid development of infections of different aetiologies. When the body temperature reaches $> 38.3\text{ }^{\circ}\text{C}$ in

one measurement or when it has been ≥ 38 °C for longer than one hour, the patient develops FN [1]. Moreover, diabetes mellitus may increase the risk of febrile neutropenia in cancer patients receiving chemotherapy [2]. Patients with hematological cancers usually undergo complex therapy, beginning with antipyretic therapy. Paracetamol (APAP) is a common antipyretic drug [3]. APAP is eliminated from the body in the form of inactive metabolites, mostly glucuronides (40–67%) and sulfates (20–46%). The analgesic is to a lesser extent (3–4%) metabolised by cytochrome P450 (CYP) 2E1—to hepatotoxic *N*-acetyl-*p*-benzoquinone imine (NAPQI) [4]. The metabolites are eliminated in urine. Only about 4–5% is eliminated in an unchanged form through the kidneys [4, 5]. Apart from that, diabetes is characterised by increased glycation of albumin. In consequence, the binding of the drug to the protein may be impaired and its volume of distribution may be altered [6, 7]. Little is known about the activity of enzymes participating in the second phase of metabolism of APAP in diabetic patients. Moreover significant differences were observed in pharmacokinetics of paracetamol between diabetic and non-diabetic rabbits [8]. Therefore, the aim of the study was to assess the influence of diabetes on the pharmacokinetics of paracetamol and its two metabolites: glucuronide and sulfate in FN patients with haematological malignancies.

2 Subject and Methods

2.1 Subjects

The research was approved by the Bioethics Committee, University of Medical Sciences, Poznan, Poland (437/16). The research was explained to the patients and those who signed written informed consent were enrolled in the study. It was conducted between July 2016 and February 2017 in diabetic and non-diabetic FN patients with hematological malignancies. Patients were included in the study if their age was > 18 years; they had haematological malignancy, neutropenic fever and no history of allergy to paracetamol. Patients with neutropenic fever and diagnosed diabetes mellitus were enrolled to diabetic group. Diagnosis of diabetes mellitus was made by the presence of classic symptoms of hyperglycemia and an abnormal blood test (fasting plasma glucose concentration ≥ 126 mg/dL). Diabetes were not controlled with medication in the diabetic patients. The exclusion criteria were as follows: previous exposure for paracetamol for about 16 h, liver insufficiency, pregnancy and lactation, or arterial hypertension, acute diarrhoea, gastrointestinal tract haemorrhage, ascites, pleural effusion and smokers. Eighteen patients (13 men, 5 women: 7 diabetics and 11 non-diabetics) were enrolled in the research.

2.2 Drug administration and blood sampling

A dose of 1000 mg of paracetamol (100 mL of solution; Paracetamol B.Braun 10 mg/mL, address of manufacture: 34212 Melsungen, Germany) was administered to the patients by intravenous infusion lasting 15 min. Blood samples (1 mL) were collected before the drug administration (0) and 0.083; 0.25; 0.5; 1; 2; 4; 6; 7; 8 h after termination of the infusion. The blood samples were transferred into heparinised tubes and centrifuged at 2880 g for 10 min at 4 °C. Next the plasma was transferred to propylene tubes and stored at -80 °C until analysis (max. 1 month).

2.3 Bioanalytical assay

The reagents used in the research were purchased from the following suppliers: paracetamol, theophylline and perchloric acid (Sigma Aldrich, Poland), paracetamol glucuronide and paracetamol sulfate (LGC Standards, Poland), HPLC (high-performance liquid chromatography) grade acetonitrile, methanol and orthophosphoric acid (Merck, Poland), sodium sulfate anhydrous (POCH S.A., Poland). Water used in the mobile phase was deionised, distilled and filtered through a Millipore system (Direct-Q UV3, Millipore) before use. Paracetamol B.Braun® 0.01 g/mL (batch: 16156451, expiration date: September 2017) was purchased from B.Braun®, Melsungen, Germany.

The concentrations of paracetamol, paracetamol glucuronide and paracetamol sulfate were assayed using the HPLC method with UV detection [9]. Separation was achieved by isocratic elution of the mobile phase, sodium sulfate 0.05 M pH 2.2 (adjusted with 85% orthophosphoric acid)—acetonitrile (93:7, v/v), at a flow rate of 1.0 mL/min through a Hypersil ODS C18 column (150 mm, 4.6 mm, 5.0 μ m particle size) (Thermo Electron Corporation®). The column temperature was maintained at 25 °C. The UV detection wavelength was set at 254 nm, and the injection volume was 50 μ L. The total analysis time for each run was 10 min. The lower limit of quantification (LLOQ) for paracetamol, paracetamol glucuronide and paracetamol sulfate were 0.1, 0.5 and 0.1 mg/L, respectively. Intra- and inter-day accuracy and precision of the LLOQ, low quality control (0.25, 1.0, 0.25 mg/L), medium quality control (20.0, 30.0, 10 mg/L), and high quality control (40.0, 50.0, 15.0 mg/L) were well within the acceptable limit of 15% coefficient of variation (CV%) for paracetamol, paracetamol glucuronide and paracetamol sulfate. The calibration was linear within the range of 0.1–45 mg/L ($r=0.999$) for paracetamol, 0.5–60 mg/L ($r=0.999$) for paracetamol glucuronide and—0.1–20 mg/L ($r=0.998$) for paracetamol sulfate.

2.4 Pharmacokinetic analysis

Pharmacokinetic parameters (C_{\max} , $t_{1/2\text{kel}}$, V_d/kg —volume of distribution per kilogram, CL clearance, AUC_{0-t} , AUMC_{0-t} —area under first moment concentration time profile, MRT_{0-t} mean residual time) were estimated by non-compartmental methods, using Phoenix™ WinNonlin® v. 6.3; Certara L.P., USA software (Certara L.P., USA) and ThothPro 4.1 (ThothPro Sp. z o.o., Poland).

2.5 Statistical analysis

No study power calculation was performed. The number of subjects to be included in the study was based on previous similar studies in severely ill patients. Descriptive analysis of study results was performed. The results are expressed as mean \pm SD.

3 Results

In both groups, the mean BMIs (body mass index) of the subjects were similar. However, the diabetic patients were slightly older (52 [15] vs. 42 [14] years) and their fasting serum glucose concentration were significantly higher (137.4 vs. 96.1 mg/dL) (Table 1). The creatinine clearance estimated by the Cockcroft-Gault formula was below the reference values (75–115 mL/min) in 6 patients, but it was >30 mL/min, so it did not indicate renal failure and was not a contraindication against APAP. The concentrations of hepatic enzymes, i.e. aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were too high in 4 and 6 patients, respectively. 17 patients had hypoalbuminaemia,

which is characteristic of patients treated for haematological malignancies.

There was considerable intersubject variability in the pharmacokinetic parameters of APAP, APAP-glu and APAP-sulfate, as evidenced by the CV% [10] (Table 2).

Figures 1 and 2 show mean plasma concentration–time profiles for APAP and its metabolites, respectively, in both groups during the 8 h period after the administration of APAP. Table 2 shows the pharmacokinetics of APAP, APAP-glu and APAP-sulfate. We observed no changes in the exposure to intravenous APAP, what was reflected by similar values of C_{\max} , AUC_{0-t} in the groups (Table 2). There were also no differences in the following pharmacokinetic parameters of APAP: $t_{1/2\text{kel}}$, V_d/kg , CL, AUMC_{0-t} , MRT_{0-t} . Similarly, the groups under analysis did not differ in the C_{\max} , t_{\max} , AUC_{0-t} , $t_{1/2\text{kel}}$, AUMC_{0-t} , MRT_{0-t} of APAP-glu and APAP-sulfate. The C_{\max} and AUC_{0-t} ratio between the metabolites and APAP were similar.

4 Discussion

Diabetes can cause pathophysiological changes in the body and affect the pharmacokinetics and pharmacodynamics of drugs. These changes include reduction in gastric emptying time, albumin glycation, changes in P-gp expression and CYP activity [11–13].

The influence of diabetes on the enzymatic activity at the second phase of the drug metabolism has not been fully investigated. In order to assess APAP glucuronidation and sulphation the pharmacokinetic parameters of APAP and its glucuronide and sulfate were compared in patients with FN between the groups of diabetic and non-diabetics.

Table 1 The characteristics of patients with febrile neutropenia

Parameter	Value ^a	
	Diabetic group	Non-diabetic group
Males/females [n]	4/3	9/2
Age [years]	52 \pm 15 (33–73)	42 \pm 14 (19–57)
Weight [kg]	80 \pm 24 (49–120)	84 \pm 17 (60–107)
BMI [kg/m ²]	27.1 \pm 7.3 (18.2–36.2)	26.2 \pm 5.4 (19.3–36.1)
Fasting glucose [mg/dL]	137.4 \pm 45.9 (98.8–235.1)	96.1 \pm 12.8 (83.3–119.0)
C_{cr} [mg/dL]	1.11 \pm 0.45	0.99 \pm 0.72
CL_{CR} [mL/min]	109.4 \pm 77.8 (24.7–262.7)	104.8 \pm 66.3 (49.9–297.4)
Total bilirubin [$\mu\text{mol/L}$]	11.0 \pm 6.4 (3.7–19.4)	11.6 \pm 6.4 (4.9–21.7)
Albumin [g/L]	27.5 \pm 5.6 (20.3–32.6)	26.8 \pm 6.0 (19.6–39.6)
AST [U/L]	28 \pm 15 (12–52)	18 \pm 10 (8–41)
ALT [U/L]	85 \pm 71 (15–234)	46 \pm 37 (11–118)

BMI body mass index, C_{cr} creatinine concentration, CL_{CR} creatinine clearance estimated by the Cockcroft-Gault formula, AST aspartate aminotransferase, ALT alanine aminotransferase

^aValues are expressed as the mean \pm standard deviation

Table 2 Pharmacokinetic parameters for paracetamol (APAP) and its metabolites—glucuronide (APAP-glu) and sulfate (APAP-sulfate) in patients with neutropenic fever

Pharmacokinetics parameters	Diabetic group (n = 7) Value ^c	Non-diabetic group (n = 11) Value ^c
APAP		
C_{max} [mg/L]	21.50 ± 11.23 (52.2)	23.42 ± 9.79 (41.8)
$t_{1/2kel}$ [h]	2.28 ± 0.80 (35.1)	2.11 ± 0.80 (38.0)
V_d/kg [L/kg]	0.87 ± 0.36 (41.2)	0.80 ± 0.36 (45.7)
CL [h]	24.56 ± 11.08 (45.1)	29.24 ± 21.28 (72.8)
AUC_{0-t} [mg·h/L]	44.23 ± 17.93 (40.5)	41.43 ± 14.57 (35.2)
$AUMC_{0-t}$ [mg·h ² /L]	104.23 ± 51.55 (49.5)	93.12 ± 37.60 (40.1)
MRT_{0-t} [h]	2.23 ± 0.38 (17.0)	2.15 ± 0.41 (19.2)
APAP-glu		
C_{max} [mg/L]	24.03 ± 10.49 (43.7)	20.05 ± 14.48 (72.2)
t_{max} [h]	2.00 ± 1.41 (70.7)	1.03 ± 0.71 (69.4)
$t_{1/2kel}$ [h]	13.21 ± 25.87 (195.9)	4.54 ± 3.42 (69.4)
AUC_{0-t} [mg·h/L]	117.52 ± 41.43 (35.3)	110.06 ± 105.71 (96.1)
$AUMC_{0-t}$ [mg·h ² /L]	387.93 ± 144.51 (37.3)	384.78 ± 408.27 (101.6)
MRT_{0-t} [h]	3.20 ± 0.48 (14.9)	3.17 ± 0.49 (72.2)
APAP-sulfate		
C_{max} [mg/L]	6.29 ± 5.63 (89.5)	6.99 ± 3.02 (43.2)
t_{max} [h]	1.40 ± 0.55 (39.1)	1.33 ± 0.75 (56.5)
$t_{1/2kel}$ [h]	2.68 ± 0.56 (21.0)	2.07 ± 0.48 (23.3)
AUC_{0-t} [mg·h/L]	34.23 ± 30.65 (89.5)	35.73 ± 21.04 (58.9)
$AUMC_{0-t}$ [mg·h ² /L]	119.19 ± 110.67 (92.9)	115.70 ± 72.57 (62.7)
MRT_{0-t} [h]	3.08 ± 0.71 (23.2)	3.03 ± 0.23 (8.1)
APAP-glu/APAP^a		
AUC_{0-t}	2.93 ± 1.34 (42.4)	1.87 ± 0.69 (105.4)
C_{max}	1.19 ± 0.33 (25.87)	0.76 ± 0.31 (110.8)
APAP-sulfate/APAP^b		
AUC_{0-t}	0.77 ± 0.55 (89.6)	0.98 ± 0.37 (62.1)
C_{max}	0.32 ± 0.27 (106.2)	0.35 ± 0.15 (70.0)

AUC_{0-t} area under the plasma concentration–time curve from zero to the time of last measurable concentration, C_{max} maximum observed plasma concentration, t_{max} time to first occurrence of C_{max} , $t_{1/2kel}$ half-life in elimination phase, Cl clearance (Cl), V_d/kg volume of distribution per kilogram, $AUMC_{0-t}$ area under the first moment curve from zero to the time of last measurable concentration, MRT_{0-t} mean residence time, M Arithmetic mean, SD standard deviation, CV coefficient of variation

^aRatio of paracetamol glucuronide/paracetamol

^bRatio of paracetamol sulfate/paracetamol

^cValues are expressed as the mean ± standard deviation (%CV)

Pharmacokinetic parameters of APAP and its metabolites were comparable between diabetic and non-diabetic patients with FN. It might indicate that the diabetes does not influence the pharmacokinetics of paracetamol and its metabolites in patients with FN. Apart from that, the similar values of APAP-glu/APAP and APAP-sulfate/APAP ratios between the groups show that the disease seems not influence glucuronidation and sulphation of the analgesic (see Table 2).

Studies have shown that FN patients may exhibit hypoalbuminaemia, and in consequence increased volume of distribution and clearance of the drugs [8, 14]. Therefore, the pharmacokinetic parameters of APAP observed in the FN

patients were compared with the data published in the literature [15, 16]. The pharmacokinetic parameters of APAP observed in the FN patients ($AUC_{0-t, FN} = 42.5$ mg·h/L; $C_{max, FN} = 22.7$ mg/L; $t_{1/2, FN} = 2.2$ h; $V_{d, FN} = 0.83$ L/kg) were comparable with the values observed in healthy volunteers ($AUC_{0-t} = 42.5$ mg·h/L; $C_{max} = 21.6$ mg/L; $t_{1/2} = 2.2$ h; $V_d = 1$ L/kg) [12, 16]. Only the drug clearance was slightly greater (27.4 vs. 20.7 L/h).

The research was limited by the small number of participants. Therefore, it should be continued on larger groups of patients. It is acceptable to administer APAP to patients with liver failure if a dose of ≤ 3000 mg is not

Fig. 1 Paracetamol (APAP) plasma concentration vs. time profiles following single intravenous administration of paracetamol to patients with febrile neutropenia (diabetic vs. non-diabetic group). Plots represent the arithmetic mean with standard deviation

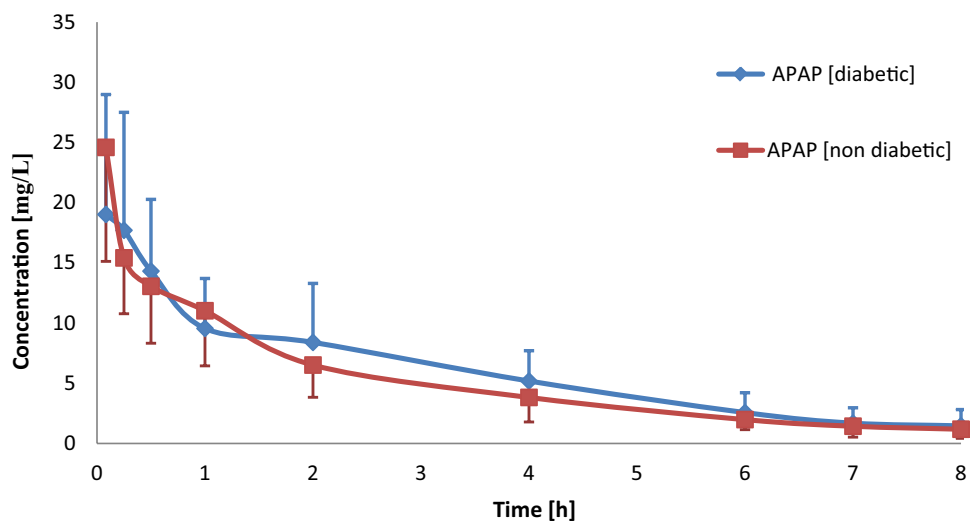
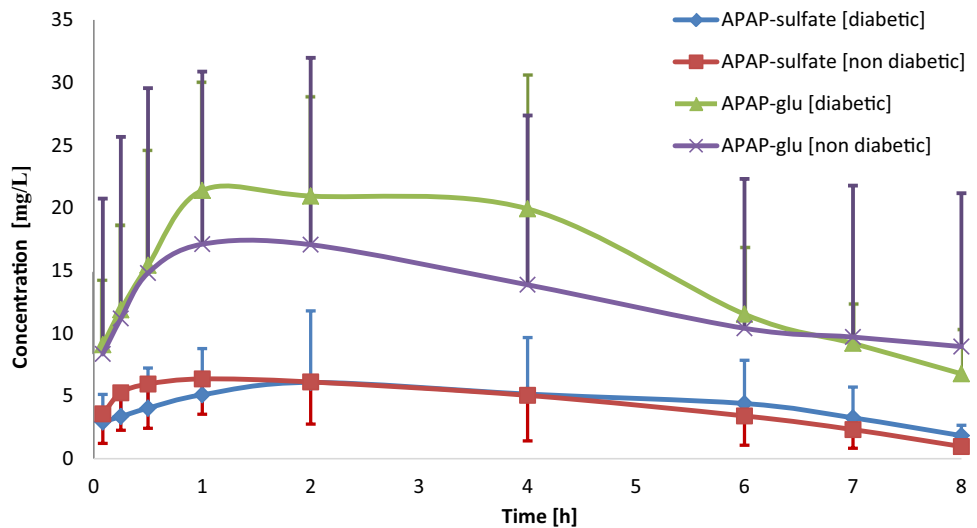


Fig. 2 Paracetamol glucuronide (APAP-glu) and paracetamol sulphate (APAP-sulfate) plasma concentrations vs. time profiles following single intravenous (*i.v.*) administration of paracetamol to patients with febrile neutropenia (diabetic vs. non-diabetic group). Plots represent the arithmetic mean with standard deviation



exceeded [11]. Another limitation is that we did not measure NAPQI, which is a hepatotoxic APAP metabolite.

5 Conclusion

Based on the results of this study, it seems that diabetes does not influence glucuronidation and sulfation of paracetamol in patients with FN. Therefore, paracetamol may be given to these patients without any dose adjustment.

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Compliance with Ethical Standards

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Conflicts of interest Anna Stachowiak, Edyta Szalek, Agnieszka Karbownik Joanna Porazka, Iwona Przewozna, Tomasz Grabowski, Anna Wolc, Edmund Grzeszkowiak have no conflict of interest.

Ethics approval The research was approved by the Bioethics Committee, University of Medical Sciences, Poznan, Poland (437/16). All procedures in this study were in accordance with the 1964 Helsinki declaration (and its amendments).

Informed consent Written informed consent was obtained from all patients participating in the study.

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References

- Villafuerte-Gutierrez P, Villalon L, Losa JE, Henriquez-Camacho C. Treatment of febrile neutropenia and prophylaxis in hematologic malignancies: a critical review and update. *Adv Hematol.* 2014;2014:986938.
- Alenzi EO, Kelley GA. The association of hyperglycemia and diabetes mellitus and the risk of chemotherapy-induced neutropenia among cancer patients: a systematic review with meta-analysis. *J Diabetes Compl.* 2017;31:267–72.
- Weinkove R, Clay J, Wood C. Temperature management in haematology patients with febrile neutropenia: a practice survey. *N Z Med J.* 2013;126:62–73.
- Bertolini A, Ferrari A, Ottani A, Guerzoni S, Tacchi R, Leone S. Paracetamol: new vistas of an old drug. *CNS Drug Rev.* 2006;12:250–75.
- Mattia C, Coluzzi F. What anesthesiologists should know about paracetamol (acetaminophen). *Minerva Anesthesiol.* 2009;75:644–53.
- Kim YC, Lee AK, Lee JH, Lee I, Lee DC, Kim SH, et al. Pharmacokinetics of theophylline in diabetes mellitus rats: induction of CYP1A2 and CYP2E1 on 1,3-dimethyluric acid formation. *Eur J Pharm Sci.* 2005;26:114–23.
- Dostalek M, Akhlaghi F, Puzanovova M. Effect of diabetes mellitus on pharmacokinetic and pharmacodynamic properties of drugs. *Clin Pharmacokinet.* 2012;51:481–99.
- Bienert A, Kamińska A, Olszewski J, Gracz J, Grabowski T, Wolc A, et al. Pharmacokinetics and ocular disposition of paracetamol and paracetamol glucuronide in rabbits with diabetes mellitus induced by alloxan. *Pharmacol Rep.* 2012;64:421–7.
- Brunner LJ, Bay S. Simple and rapid assay for acetaminophen and conjugated metabolites in low-volume serum samples. *J Chromatogr B Biomed Sci Appl.* 1999;732:323–9.
- Faria Filho DE, Dias AN, Veloso ALC, Bueno CFD, Couto FAP, Matos Júnior JB, et al. Classification of coefficients of variation in experiments with commercial layers. *Braz J Poult Sci.* 2010;12:255–7.
- Summary of product characteristics *Perfalgan®* V6.0.
- Tran M, Elbarbry F. Influence of diabetes mellitus on pharmacokinetics of drugs. *MOJ Bioequiv Availab.* 2016;2:3–4.
- Nawa A, Fujita-Hamabe W, Tokuyama S. Altered intestinal *P*-glycoprotein expression levels in a monosodium glutamate-induced obese mouse model. *Life Sci.* 2011;89:834–8.
- Sime FB, Roberts MS, Warner MS, Hahn U, Robertson TA, Yeend S, et al. Altered pharmacokinetics of piperacillin in febrile neutropenic patients with hematological malignancy. *Antimicrob Agents Chemother.* 2014;58:3533–7.
- Singla NK, Parulan C, Samson R, Hutchinson J, Bushnell R, Beja EG, et al. Plasma and cerebrospinal fluid pharmacokinetic parameters after single-dose administration of intravenous, oral, or rectal acetaminophen. *Pain Pract.* 2012;12:523–32.
- Atkinson HC, Stanescu I, Frampton C, Salem II, Beasley CP, Robson R. Pharmacokinetics and bioavailability of a fixed-dose combination of ibuprofen and paracetamol after intravenous and oral administration. *Clin Drug Investig.* 2015;35:625–32.