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The role of organic cation transporter 2 inhibitor cimetidine, experimental *diabetes mellitus* and metformin on gabapentin pharmacokinetics in rats

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ABSTRACT

Purpose: We investigated the influence of diabetes mellitus (DM), glycemic control with insulin, cimetidine (Oct2 inhibitor) and metformin (Oct2 substrate) on the kinetic disposition of GAB in rats.

Main methods: Male Wistar rats were divided in five groups and all animals received an oral dose of 50 mg/kg GAB: (vehicle + GAB), cimetidine + GAB (single dose of cimetidine [100 mg/kg] intraperitoneally 1 h before GAB), metformin + GAB (single dose of metformin 100 mg/kg by gavage concomitantly with GAB), DM + GAB (single dose of 40 mg/kg streptozotocin (STZ) intravenously) and DM + GAB + insulin (single dose 40 mg/kg STZ intravenously and 2 IU insulin twice daily for 15 days). Pharmacokinetic analysis was based on plasma and urine data concentrations.

Key findings: No differences in pharmacokinetic parameters were observed between vehicle + GAB × cimetidine + GAB and vehicle + GAB × metformin + GAB groups. Diabetes increased the fraction of GAB excreted unchanged in urine (vehicle + GAB: 0.48 [0.38–0.58]; DM + GAB: 0.83 [0.62–1.04]; DM + GAB + insulin: 0.88 [0.77–0.93]) (mean [95% confidence interval]) without any changes in GAB exposure. Insulin treated diabetic animals showed higher renal clearance compared to control (vehicle + GAB: 0.25 [0.18–0.30] L/h·kg; DM + GAB + insulin: 0.55 [0.45–1.43] L/h·kg), which was attributed to the diabetes-induced glomerular hyperfiltration.

Significance: Glomerular filtration is the main mechanism of renal excretion of GAB without significant contribution of Oct2 active transport.

1. Introduction

Gabapentin (GAB) is used to treat epilepsy, hot flashes, restless legs syndrome or neuropathic pain associated with cancer, postherpetic neuralgia or with diabetes [1–4]. GAB is not metabolized in rats or humans and the renal excretion is the main pathway of elimination [5]. Its renal excretion occurs by glomerular filtration and clinical data suggest the contribution by renal active secretion via organic cation transporter 2 (OCT2: humans; Oct2: rodents) [6–8]. However, drug-drug interactions and disease-drug interactions mediated by OCT2 were not extensively investigated for gabapentin up to date.

OCT2/Oct2 is expressed mainly in the kidneys, but it is also found in the placenta, thymus, adrenal gland and choroid plexus [9]. In mice, rats and humans, Oct2/OCT2 is expressed in the basolateral membrane of the proximal renal tubules [9–12]. This transporter plays a relevant role in the excretion of the endogenous substances dopamine, histamine, serotonin and drugs including, cetirizine, cimetidine, imipramine, lamivudine and cisplatin [9,12–14].

Cimetidine was characterized as an OCT2/Oct2 inhibitor drug by in vitro, in vivo, and clinical studies [8,10,14,15] and there are no differences between human and rats in terms of the affinity of cimetidine for OCT2/Oct2 [16]. Cimetidine is recommended as OCT2/Oct2 inhibitor by The International Transporter Consortium, which developed organograms for drug-drug interaction studies [17]. Therefore, the investigation of the potential GAB × cimetidine interaction will explain the impact of Oct2 activity on GAB pharmacokinetics.

Experimental *diabetes mellitus* (DM) is involved in several drug × disease interactions since it alters the gastrointestinal

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absorption, distribution, metabolism and renal excretion of drugs [18]. In terms of P450 enzymes activity, experimental DM suppresses the expression of the isoforms CYP 1A2, 2C11, 2C13 and 3A2 and induces the expression of CYP 2A1, 2B1, 2C12, 4A1 and 2E1 [13,19,20]. DM also influences the activity of phase II enzymes [13] and drug transporters. A reduction of 50–70% reduction in Oct2 levels was observed in rats with DM induced by streptozotocin [21,22].

Metformin is considered the drug of choice for the treatment of DM type 2, due not only to glycemic control, but also to beneficial contributions related to vascular complications, body weight, lipid profile, diastolic pressure and reduced mortality [23]. OCT2 significantly contributed to renal excretion of metformin, as evidenced in clinical [24] and in vitro studies [25,26]. However, the influence of metformin on GAB kinetic disposition was not previously investigated.

Considering the potential role of OCT2/Oct2 in GAB kinetic disposition and the related effects of experimental DM on the expression of Oct2, the aims of the present work were: 1) to characterize the potential of Oct2 inhibition by cimetidine on GAB pharmacokinetics; 2) to evaluate the role of experimental DM and the glycemic control by insulin treatment on GAB pharmacokinetics; 3) to evaluate the impact of metformin on GAB pharmacokinetics. Therefore, an experimental study was conducted in rats treated with a single dose of GAB. Plasma and urine drug concentrations were evaluated for pharmacokinetic analysis.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing approximately 250 ± 20 g (7–8 weeks) were obtained from the *Campus de Botucatu - Universidade Estadual Paulista* and housed in controlled environmental conditions (temperature of 20 ± 1 °C and humidity 60 ± 20%). The experimental protocols were approved by the Committee for Ethics in Animal Experimentation from the School of Pharmaceutical Sciences, UNESP, Araraquara, São Paulo (CEUA/FCF/Car n° 75/2015).

2.2. Induction of diabetes

Diabetes mellitus was induced in 14-h fasting animals by a single intravenous dose of 40 mg/kg streptozotocin (STZ, > 98%, Sigma Aldrich, St. Louis, MO, USA) dissolved in 0.1 M sodium citrate buffer (pH 4.5) [30]. Four days after STZ administration, animals were considered diabetic if the glycemia values without fasting were higher than 300 mg/dL [31]. All non-diabetic groups received a volume of 0.2 mL of 0.01 M citrate buffer solution pH 4.5 at the same time as administration of STZ in the diabetic groups as a control of the vehicle.

2.3. Experimental protocol

The experiment involved a total of 105 animals divided in five groups (vehicle + GAB, cimetidine + GAB, metformin + GAB, Experimental *diabetes mellitus* (DM) + GAB and DM + GAB + insulin). Serial blood samples and urine collection were required for pharmacokinetic analysis of GAB. To minimize the contamination of the urine with blood, animals were maintained singly in metabolic cages for urine sampling. Each group had 21 animals, of which 15 were used for blood and 6 for urine sampling, in order to achieve n = 6 per sampling time.

All animals received a single dose of 50 mg/kg GAB by gavage after 14-h fasting fifteen days after vehicle (citrate buffer) administration or diabetes confirmation. Cimetidine group received a single dose of 100 mg/kg cimetidine intraperitoneally, 60 min before receiving a single dose of GAB [14]. Metformin group animals receive a dose of 100 mg/kg metformin by gavage concomitantly with GAB dose [29]. Fifteen days after diabetes mellitus (DM) induction, DM + GAB group received 50 mg/kg GAB (gavage). DM + GAB + insulin group was treated with 2 UI NPH insulin twice daily for fifteen days after diabetes induction and then received 50 mg/kg GAB (gavage).

Blood samples were obtained through circumcision of 0.3 mm of the tail distal end, after local heating at 42 °C. Considering the total blood volume of animals and to avoid hypovolemic shock without fluid replacement, which could cause hemodilution [27,28], the maximum of five blood collections of 500 μL were drawn per animal. Individual blood samples were collected at times 0.25 h; 0.5 h; 0.75 h; 1 h; 2 h; 3 h; 4 h; 5 h; 6 h; 8 h; 10 h; 12 h after GAB administration, in heparinized tubes. Thus, sparse blood samples were collected at predefined times randomly combined to reach the sample size of 6 (n = 6), per sampling time. Urine samples were collected at time intervals 0–4 h; 4–8 h; 8–12 h. All samples were kept at −70 °C until analysis.

2.4. Analysis of GAB in plasma by HPLC-MS and in urine by HPLC-UV

The determination of GAB in rat plasma was performed by HPLC-MS (Perkin Elmer, Flexar SQ 300 MS, Shelton, USA). Phenacetin (Sigma-Aldrich, Missouri, USA) was used as internal standard (IS), at a concentration of 10 μg/mL in methanol. GAB and the IS were resolved on LiChrospher® C18 RP column (125 × 4.0 mm, 5 μm, Merck, Darmstadt, Germany) kept at 18 °C and mobile phase consisting of 5 mM ammonium acetate solution (pH = 4) and methanol (40:60, v/v) and flow rate of 0.4 mL/min [32] The mass spectrometry was performed on positive ion mode and the protonated molecular ions $[M\text{-}H]^+$ with *m/z* 172 and 180 were used for monitoring GAB and IS, respectively. The determination of GAB in rat urine was performed by HPLC-UV at λ = 360 nm. GAB and the IS (amlodipine besylate (99,7%, European pharmacopeia Reference Standard, Sigma-Aldrich, São Paulo) at a concentration of 200 μg/mL in water, were resolved in the same column, and the mobile phase consisting of 0.05 M sodium monobasic phosphate buffer (pH 3.9) and methanol (27:73, v/v), in a flow rate of 1.2 mL/min.

Shortly, 100 μL aliquots of plasma samples were added to 5 μL of IS solution (phenacetin), 50 mg of Na_2SO_4 and 1000 μL of dichloromethane:n-butanol (1:1, v/v) [33]. The microtubes were shaken and centrifuged (15 min, 15,000 × *g*) and 900 μL of the supernatant was separated and evaporated up to dryness. The residue was reconstituted in 120 μL of mobile phase and 10 μL was inject in the chromatographic system. Similarly, 100 μL aliquots of urine samples were added to 30 μL of IS solution (amlodipine), 50 mg of Na_2SO_4 and 1000 μL of dichloromethane:n-butanol (1:1, v/v). The microtubes were shaken and centrifuged (15 min, 15,000 × *g*) and 800 μL of the supernatant was separated and evaporated up to dryness. Aliquots of 40 μL of borate buffer (pH 8.2), 12 μL of derivatization agent FDNB 0.06 M and 400 μL of acetonitrile [34] were added to the residues. After homogenization, the microtubes were heated at 65 °C for 10 min for derivatization reaction. After cool, 10 μL of 1 M HCl were added and aliquots of 50 μL were injected in the system.

2.5. Pharmacokinetic and statistical analysis

The areas under the plasma concentration versus time curves in the interval zero to infinity ($AUC^{0-\infty}$) were calculated by Gauss-Laguerre Quadrature. The concentrations for the noncoincident times to the nodes of the quadrature were estimated by polynomial interpolation [35]. Differently from the trapezoidal rule, the Gauss-Laguerre Quadrature approach leads to more accurate results and its reliable in pharmacokinetics study designs when more than one sample (but not all of them) is taken from each animal [35,36]. The apparent total clearance (CL_T/F) was calculated using the equation $CL_T/F = Dose/AUC^{0-\infty}$. The maximum plasma concentrations (C_{max}) and the time to reach maximum plasma concentrations (T_{max}) were obtained from the observed data. Elimination half-life ($t_{½}$) was calculated by the equation: $t_{½} = (0.693 \times Vd)/Cl_T/F$, were Vd is the apparent volume of distribution calculated as C_{max}/dose. The fraction excreted unchanged in urine