BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902022e19674

Article

Free interstitial levels of metformin in the liver of healthy and diabetic Wistar rats

Andressa Braga[1], Jaqueline Schneider Izolan[1], Teresa Cristina Dalla Costa[1], Bibiana Verlindo de Araújo[1*]

[1]Pharmaceutical Sciences Graduate Program, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil

In the present study, free interstitial levels reached by metformin in the liver were investigated in control and diabetic rats by microdialysis. Firstly, a bioanalytical method using an HPLC-UV system to determine the drug concentration in microdialysis samples was validated. The blood glucose levels and biochemical parameters were investigated in control and diabetic animals. Following that, both groups received a dose of 50 mg/kg of metformin iv *bolus* and the free interstitial levels reached in the liver were assessed by microdialysis. The method was validated according to FDA guidelines being suitable to quantify free concentrations of metformin in the liver of control and diabetics rats. Free exposure to metformin was similar in control and diabetic animals: $AUC_{0-\infty}$ 118.50 ± 40.18 *vs* 112.93 ± 50.25 µg.h/mL, respectively. The half-life in tissue was similar to that described in the literature for plasma. Hence diabetes induced by streptozotocin after administration of nicotinamide in our study did not damage the renal and hepatic function of the animals. The levels reached in the liver were 1.6 times higher than the free plasma concentrations, demonstrating higher liver penetration of metformin. This is the first investigation in liver interstitial concentration of metformin in control and diabetic rats.

Keywords: Metformin. Microdialysis. Pharmacokinetics. Experimental diabetes.

INTRODUCTION

Metformin is the oral antidiabetic drug most prescribed worldwide to treat diabetic type 2 patients. In spite of its clinical use for decades, the pharmacokinetics (PK) and pharmacodynamics (PD) of the drug have been continuously investigated and the role of transporters in this scenario is a topic currently under investigation. This biguanide is a strong base with an acid dissociation constant value (pka) of 11.5, showing less than 0.01% at the nonionized form in the blood that, associated with its LogP equal to -0.82, hinders its permeability across membranes (Graham *et al*., 2011). As a result, metformin shows tissue permeability-limited and its absorption, distribution, and elimination are mediated by transporters. An interesting review of these transporters was recently published by Liang and Giacomini (2017). The organic cation transporter (OCT) is an influx transporter's family responsible for metformin tissue distribution with different subtypes expressed in the basolateral membrane of tissues. The liver, target tissue of metformin, expresses mainly OCT1 on the hepatocytes while the kidneys express OCT2 and OCT1 in the proximal tubular cells and OCT3 is mostly expressed in muscle and blood vessels (Slitt *et al*., 2002). Moreover, the multidrug and toxin extrusion protein (MATE) is responsible for metformin efflux transportation and the subfamily MATE1 is expressed in the apical membrane of proximal tubular cells in the kidney, being responsible for the drug tubular secretion into the urine (Terada *et al*., 2006; Graham *et al.*, 2011).

Currently, there is no study reporting metformin free concentration into the biophase (liver). Most studies published about metformin distribution employed tissues homogenates, showing high drug distribution in many tissues including

***Correspondence:** B V Araújo. Universidade Federal do Rio Grande do Sul. Programa de Pós-Graduação em Ciências Farmacêuticas. Av. Ipiranga, 2752. Porto Alegre - RS, 90.610-000, Brasil. Email: bibiana.araujo@ufrgs.br. Phone (+55) 51 3308 5418
ORCID: https://orcid.org/0000-0002-9706-0389

those enrolled on its response/exposition relationship such as liver, kidney, and muscle when compared to plasma concentration (Beckmann, 1969; Sogame *et al.*, 2011; Higgins, Bedwell, Zamek-gliszczynski, 2012). However, this technique presents some limitations as the measure of the total drug in tissue (unbound and bound drug's fractions) and blood residuals that could result in misleading information about drug distribution, overestimating the concentrations reached on tissues (Mouton *et al.*, 2008). An alternative and more appropriate method to investigate drug tissue distribution is microdialysis. This technique is useful in pre-clinical research to evaluate small endogenous or exogenous molecules in tissues, allowing the assessment of free interstitial drug levels reached in the biophase, which are responsible for the effect (Azeredo, Dalla Costa, Derendorf, 2014).

Besides the impact of transporters on metformin distribution at the biophase, another point that must be evaluated in the context of drug exposure in diabetes is the influence of the disease on this process since it may change pharmacokinetic processes. Diabetes alters the physiology of several systems enrolled in the absorption, distribution, metabolism, and elimination as previously described for experimental models of diabetes in rodents induced by streptozotocin or alloxan (Srinivas, 2015). Considering the pharmacokinetics of metformin, distribution process may change due to decrease on OCT transporters expression (Grover *et al.*, 2004; Nowicki *et al.*, 2008) in diabetic animals and the excretion can be also affected by changes in drug biotransformation due to modifications on CYP expression or impairment of renal function (Lee *et al.*, 2010). In this context, the goals of this study are: 1) to validate a bioanalytical method to quantify metformin microdialysate samples; 2) to evaluate the free interstitial levels reached by metformin in the liver using microdialysis technique; and 3) to evaluate the influence of an experimental model of diabetes on drug concentrations reached in the biophase.

MATERIAL AND METHODS

Chemical and Reagents

Metformin hydrocloridrate (99%, molecular weight 165.625 g/mol) was purchased from LKT Laboratories (St Paul, MN, USA), octanosulfonic acid sodium salt, urethane (ethyl carbamate >99%) and nicotinamide (>99%) were obtained from Sigma Aldrich (St Louis, MO, USA). Streptozotocin was obtained from Adooq Bioscience® (Irvine, CA, USA), acetonitrile was obtained from Tedia (Fairfield, CT, USA), triethylamine was obtained from Merck (Darmstadt, Germany), and ultra-pure water was purified in a Milli-Q system from Millipore (Bedford, MA, USA). Ringer's solution consists of 149 mM NaCl, 2.46 mM CaCl2 and 4.02 mM KCl, pH = 7.4 ± 0.2 adjusted with NaOH 0.1M.

Chromatographic system

The metformin stock solution was prepared by dissolving 0.016 g of metformin hydrochloridrate in 25 mL of water ultra-pure, resulting in a 500 ug/mL metformin base (molecular weight 129.163 g/mol) as a stock solution, which was stored at -80ºC. This standard solution was used to spike Ringer's solutions to reach the following concentrations: 25, 50, 100, 250, 500, 1000, 2500 and 5000 ng/mL for the calibration curve; and the quality control samples (QC's): low (75 ng/mL), medium (2000 ng/mL) and high (4000 ng/mL).

The microdialysis samples were quantified by a Shimadzu® chromatographic system consisting of an isocratic LC-10AD VP pump, SIL-10AD VP auto-injector, SCL-10A VP system controller and DGU-14A degasser. The separation was performed using a Shim-pack C-8 column (150 mm x 4.6 mm, 5 µm; Shimadzu – Tokyo, Japan) as stationary phase, preceded by a Phenomenex guard column packed (3.0 x 4 mm; 5 µm; Torrance, CA, USA). The mobile phase consisted of acetonitrile: 10 mM sodium phosphate monobasic dihydrate ($NaHPO_4$. $2H_2O$) (10:90, v/v) containing 2.5 mM sodium octanesulfonate monohydrate and triethylamine (0.2% v/v) - pH adjusted to 3.04 ± 0.02 with *ortho*phosphoric acid - pumped at a flow rate of 1.0 mL/min, oven set at 40ºC, injection volume of 20 µL and UV detector at 236 nm. The chromatogram run time was performed in 8 min and the retention time of metformin was 6 min. The samples were quantified using the peak area of metformin. Data were recorded by Shimadzu Class VP software (version 6.12).

For the bioanalytical method of validation, the following parameters were evaluated: linearity, sensitivity, carryover effect, specificity, selectivity, accuracy, precision, dilution integrity and the stability of metformin stock solution and microdialysis samples.

The linearity was determined by six calibration curves in two different days. It was used eight concentrations ranging from 25 to 5000 ng/mL, which were prepared on the same day that analyses were conducted. The slope, intercept and correlation coefficient of the calibration curves were determined by the linear regression using weight equal to 1 (Scientist software – MicroMath, version 2.1).

The lower limit of quantification (LLOQ) was determined by the lowest concentration of the calibration curve (imprecision ≤ 20%). The carryover effect was determined on six blank samples injected after the upper limit of quantification (ULOQ). Method selectivity was evaluated using six different microdialysate samples from rats. These were obtained before the drug administration in the animals and compared to the lower quality control (LQC). The intraday and interday precision and accuracy were calculated from data obtained in two consecutive days for the three quality controls: low (75 ng/mL), medium (2000 ng/mL) and high (4000 ng/mL) concentrations, assessed by six replicates each validation day. Since the first microdialysis sample exceeds the ULOQ, sample dilution was evaluated. For instance, six replicates of Ringer's solution spiked with metformin at a concentration of 12000 ng/mL were diluted fourfold to reach the concentration within the calibration curve range (3000 ng/mL).

The metformin stability in Ringer's solution was evaluated exposing three replicates of low and medium QC's to different conditions: 12 hours at ambient temperature (22 ± 3 ºC), 24 hours in the autosampler at 4 ºC, 7 days stored at 4 ºC, also 15 and 30 days stored at -80 ºC and after three freeze-thaw cycles. Moreover, metformin stock solution used to spike the calibration curves and stored at -80 ºC for 15 days were evaluated. The bioanalytical method was validated according to FDA guidance for bioanalytical methods (FDA, 2001).

Pharmacokinetic study

Animals

The male Wistar rats (200-360g) used in the pharmacokinetic study were obtained from the Reproduction and Experimental Center of Animals Laboratory (CREAL/UFRGS) – Porto Alegre/Brazil. They were kept under standard conditions of light/dark 12-hour cycles at room temperature of 21 ± 2ºC, 65% humidity with water and food allowed *ad libitum*. All experiments were approved by the Committee of Ethics in Animal Use – UFRGS (25780). Animal experiments were performed according to the principles of laboratory animal care (National Research Council, 2011) of National Institutes of Health (NIH)

Diabetes induction

Diabetes *mellitus* was induced in male Wistar rats with nicotinamide (100 mg/kg; i.p.) 15 minutes before streptozotocin administration (65 mg/kg; i.v.) according to the protocol previously described in the literature (Masiello *et al.*, 1998). Seventy-two hours later, the animals that showed blood glucose levels higher than 200 mg/dL were considered diabetic. After two weeks (day 14th) of induction, the diabetic animals were used for the pharmacokinetic experiments.

Microdialysis

Microdialysis experiments were performed using a CMA 20 probe (CMA, Stockholm, Sweden), syringe infusion pump (Harvard PHD 2000 – Holliston, MA, USA) and Hamilton 1750 syringes (Reno, NV, USA). The microdialysis probe resembles a capillary in the tissue inserted and in the extremity of the probe there is a semipermeable membrane constantly perfused with a fluid that mimics the extracellular physiological conditions (e.g. Ringer's solution), allowing the diffusion of the drug from the tissue into the probe. The constantly fluid perfusion establishes a *sink* condition; therefore, it is required to determine the drug relative recovery (RR) *in vitro* and *in vivo*. (Plock, Kloft, 2005; Lange, 2013).

The *in vitro* probe calibration evaluates aspects as the involvement of the drug with the membrane material, the influence of flow rate, and drug concentration on the probe's relative recovery (RR) by dialysis and retrodialysis techniques (Plock, Kloft, 2005; Lange, 2013). The *in vitro* relative recovery determined by dialysis and retrodialysis showed that metformin does not interact with the probe materials and is not dependent on the drug concentration. The best flow rate defined by these experiments was 1.5 µL/min.

The evaluation of the metformin RR *in vivo* determines the real drug concentration reached on the investigated tissue (Plock, Kloft, 2005; Lange, 2013) and was determined by retrodialysis. Control and diabetic rats were anesthetized with urethane (1.25 g/kg, i.p.). After complete anesthesia, the microdialysis probe (CMA 20) was inserted into the liver and Ringer's solution was infused for 1 hour as the equilibration period, then this perfusion solution was replaced by metformin (3000 ng/mL) solubilized in Ringer solution. Samples were collected every 30 min for 2 hours. The RR was calculated using the equation (Araujo *et al.,* 2008):

$$RR(\%) = \left(\frac{C_{perf} - C_{dial}}{C_{perf}}\right).100$$

Where RR is the relative recovery, C_{perf} is the drug concentration in the perfusate solution; C_{dial} is the drug concentration on the dialysate.

For microdialysis experiments, the animals were anesthetized as described above. The microdialysis probe was inserted in the liver and continuously perfused with Ringer's solution for one hour before drug administration. Metformin (solubilized in saline solution) was administered at the dose of 50 mg/kg into the femoral vein to diabetic and non-diabetic rats (n= 5 animals/group). The microdialysis samples were collected up to 12 hours. The *in vivo* RR was used to correct the microdialysis concentrations in control and diabetic animals. The samples were collected and stored at -80 ºC until HPLC analysis (up to 30 days). Individual profiles were used to calculate the non-compartmental pharmacokinetic parameters using software Phoenix® (v.64, Pharsight).

Biochemical analysis

Serum samples of overnight fasting rats were collected from different times: basal (before diabetes induction), 7 and 14 days after administration of nicotinamide and streptozotocin. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine (n = 5/ test) were evaluated to infer the influence of experimental diabetes on hepatic and kidney function. Albumin levels were also evaluated. All biochemical information was measured by specific test kits (Labtest Diagnostica, MG, Brazil) and analyzed using BIO Plus 200® device (Bioplus Produtos para Laboratórios Ltda, SP, Brazil).

Statistical Analysis

The following pharmacokinetic parameters were calculated: the area under the curve ($AUC_{0-\infty}$), lambda (λ), half-time ($t_{1/2}$), clearance (CL) and volume of distribution (Vd). The parameters were compared using Student's *t*-test, and biochemical parameters were compared using one-way ANOVA at Sigma Stat 3.5 software (Jandel Scientific Corporation). Differences were considered statistically significant when α was <0.05.

RESULTS

The bioanalytical method showed specificity (Figure 1), linearity, accuracy, and absence of carryover effect, according to FDA criteria (FDA, 2001). The lower limit of quantification (LLOQ) was 25 ng/mL and the method was linear at ranging of 25 to 5000 ng/mL (r^2 = 0.999). The results of accuracy, precision, and stability are shown in Table I. Moreover, the dilution integrity presented imprecision (CV of 0.69%) and accuracy (88.86 to 90.36%), showing that the early time samples of the pharmacokinetic profile could be diluted with reproducible assurance.

Experimental diabetic animals showed high glycemic levels when compared to the control group (408.0 ± 123.36 *vs* 99.80 ± 4.32 mg/dL, p< 0.001). The diabetic animals showed no alteration in biochemical parameters evaluated as hepatic transaminases, creatinine

or albumin levels when compared to the animal baseline on the 14th day of diabetes induction (Table II).

The metformin showed a RR of microdialysis' probe in the liver of 6.92 ± 2.80% for diabetic and 8.29 ± 2.38% for non-diabetic rats, showing no impact of diabetes on probe's relative recovery. The microdialysis samples concentrations were corrected based on the probe RR *in vivo*. After metformin i.v. *bolus* administration (50 mg/kg) control and diabetic rats showed similar free metformin profiles in the liver (Figure 2). The non-compartmental parameters for control and diabetic groups in plasma were: λ of 0.36 ± 0.10 *vs* 0.36 ± 0.12 h^{-1}, $t_{1/2}$ of 2.04 ± 0.54 *vs* 2.14 ± 0.75 h, CL of 463.66 ± 162.82 *vs* 544.74 ± 312.37 mL/h/kg, Vd of 1314.46 ± 358.97 *vs* 1492.85 ± 453.24 mL/kg and $AUC_{0-\infty}$ 118.50 ± 40.18 *vs* 112.93 ± 50.25 µg.h/mL, respectively.

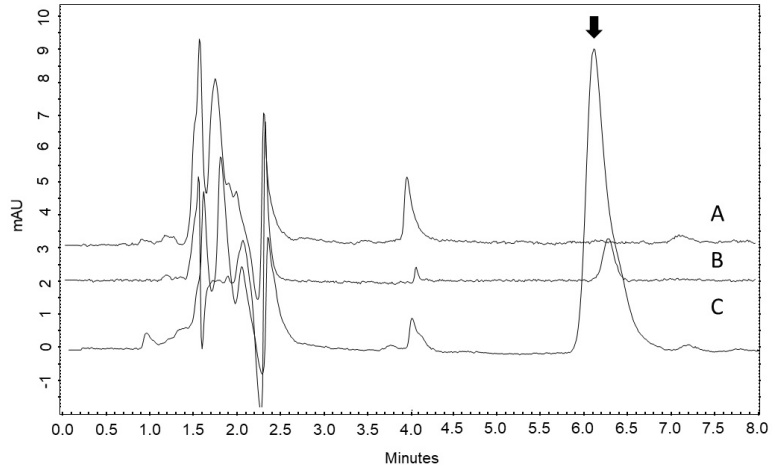

FIGURE 1 - Representative chromatograms of (a) microdialysate blank diabetic rat sample (b) metformin low QC sample (75 ng/mL) and (c) liver microdialysate sample (1172 ng/mL) 1h after i.v. administration of metformin (50 mg/kg) to diabetic rat. The arrow indicates the metformin peak.

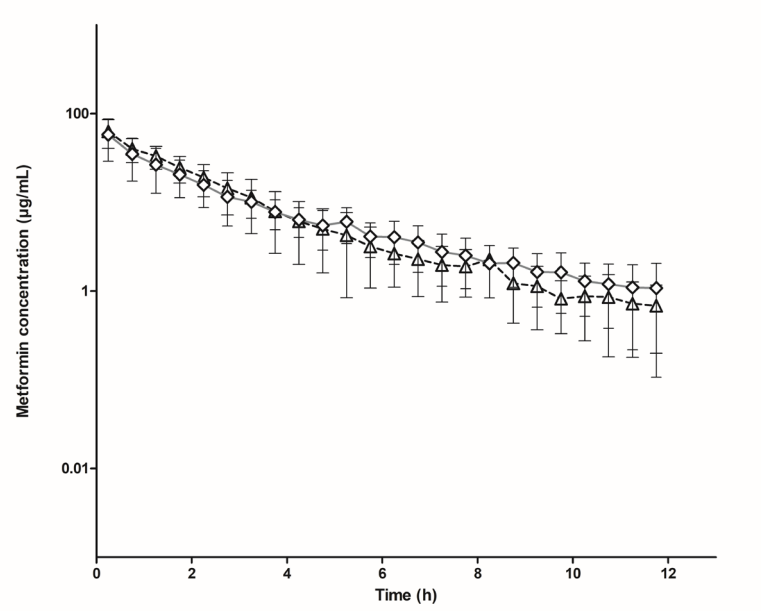

FIGURE 2 - Mean metformin interstitial free concentration versus time profiles after i.v. bolus dose of 50 mg/kg to control (triangle) and diabetic (diamond) rats induced by streptozotocin and nicotinamide. Each point represents mean SD (n = 5/group).

TABLE I - Accuracy and precision on intraday and interday runs and stability of metformin in Ringer's solution

Nominal concentration (ng/ml)		**Run**	**Mean ± SD**	**CV (%)**	**Accuracy (%)**
Intraday					
QC`s	75	1	74.24 ± 2.84	3.82	97.67
		2	73.37 ± 2.54	3.46	97.83
	2000	1	2082.51 ± 25.90	1.24	104.73
		2	2189.21 ± 11.83	0.54	109.46
	4000	1	3779.48 ± 111.36	2.95	95.07
		2	4181.29 ± 55.06	1.32	104.53
LLOQ	25	1	21.85 ± 1.52	6.94	90.95
		2	23.63 ± 3.13	13.23	94.52
Interday					
QC`s	75		73.81 ± 2.61	3.53	97.75
	2000		2135.86 ± 58.93	2.76	107.10
	4000		3980.37 ± 225.92	5.68	99.80
LLOQ	25		22.74 ± 2.52	11.09	92.74

Nominal concentration (ng/ml)	**Stability condition**	**CV (%)**	**Accuracy (%)**
75 (ng/mL)	12 hours at room temperature	11.13	103.89
	24 hours at autosampler (4ºC)	2.23	93.67
	30 days stored at -80ºC	4.30	102.08
	Freeze-thaw (three cycles): -80ºC	4.35	100.02
4000 (ng/mL)	12 hours at room temperature	0.89	96.77
	24 hours at autosampler (4ºC)	0.78	98.84
	30 days stored at -80ºC	0.51	98.50
	Freeze-thaw (three cycles): -80ºC	0.75	99.67

Intraday run mean of six replicates; Interday run (two days) of twelve replicates. Metformin stability mean of three replicates. SD = Standard Deviation; CV= Coefficient of Variation (SD/mean x 100); QC's = Quality Controls; LLOQ = Low Limit of Quantification.

TABLE II - Biochemical parameters observed in Wistar rats (n = 5) at day 0, 7th and 14th after diabetes induction with streptozotocin and nicotinamide administration (65 mg/kg iv and 100 ip mg/kg)

Parameter	Basal	7 days	14 days
Serum Creatinine (mg/dL)	0.26 ± 0.11	0.40 ± 0.17	0.28 ± 0.08
AST (IU/L)	146.80 ± 54.69	116.80 ± 39.44	130.60 ± 40.88
ALT (IU/L)	63.00 ± 17.80	66.40 ± 17.53	65.60 ± 6.11
Albumin (g/dL)	2.76 ± 0.36	2.42 ± 0.19	3.05 ± 0.56

Results expressed by mean ± standard deviation (SD); One Way ANOVA; AST (aspartate aminotransferase); ALT (alanine aminotransferase).

DISCUSSION

The present study describes a bioanalytical method able to assess the free interstitial concentration of metformin in the liver of rats by microdialysis. By this fast and simple method, it was possible to characterize the free interstitial concentration of metformin reached in the liver of control and diabetic rats induced by streptozotocin and nicotinamide. The levels of the drug attained in the animal's liver were similar (Figure 2) showing no influence of disease on drug distribution. Metformin presents some chemical characteristics that difficult its permeability by diffusion across the membranes and the role of transporters is crucial to its distribution. Using microdialysis it was possible to observe that the concentration reached by the drug in the liver are higher than the free concentration in the plasma for the same dose and route of administration ($fAUC_{0-\infty}$ 26.78 µg.h/mL, fu = 0.90) (Choi, Kim, Lee, 2006) to rats.

Considering that there are no previous studies about metformin tissue pharmacokinetics, we compared our results to reports of metformin plasma pharmacokinetics in rats in the same dose and route of administration. The pharmacokinetics of metformin in rats is described by a two-compartmental model and the drug presents low protein binding in rats (0.10 ± 0.05). Our results demonstrate a half-life of 2 hours for non-diabetic and diabetic groups, similar to the plasma parameter ($t_{½}$ = 2.4 h) (Choi, Kim, Lee, 2006). The free interstitial levels reached on the liver ($AUC_{0-\infty}$ 118.50 ± 40.18 µg.h/mL) was 4 times higher than the free plasma levels described by literature (Choi, Kim, Lee, 2006) which reports an $AUC_{0-\infty}$ of 31.5 ± 3.0 µg.h/mL for healthy rats. This is the first report about the free interstitial levels reached by metformin in the liver. The influx transporters OCT3, expressed in the blood vessels and the hepatic physiology, could explain the high metformin concentration in the interstitial liver fluid (Slitt *et al.,* 2002; Pries, Kuebler, 2006). Based on these results, further investigations about the concentration/effect relationship of metformin using the actual concentrations reached on the target tissues will be possible, allowing a more precise data analysis.

Another significant finding of our study is the absence of diabetes influence on metformin concentrations in the liver of rats induced by streptozotocin and nicotinamide. Previously studies using more aggressive protocols for diabetes induction in rats demonstrated important differences in metformin plasma concentration between control and diabetic animals (Choi *et al.*, 2008; Lee, Choi, Lee, 2008). These protocols of induction showed the influence of diabetes on metformin exposition in different ways. While streptozotocin resulted in lower $AUC_{0-\infty}$ 74.33 ± 11.67 *vs* 57.67 ± 9.42 µg.h/mL for control and diabetic animals respectively (Choi *et al*. 2008), alloxan showed increase on $AUC_{0-\infty}$ from 85.17 ± 12.1 to 113.5 ± 26 µg.h/mL for control and diabetic rats, respectively (Lee, Choi, Lee, 2008). According to the authors, these results are due to changes in total clearance (CL) associated with impaired drug renal elimination in the diabetic animals, since metformin is mostly excreted

as unchanged drug via renal clearance (CL_R) (Choi *et al*., 2008; Lee, Choi, Lee, 2008). Another hypothesis to explain this event is the potential nephrotoxicity and hepatotoxicity associated with alloxan and streptozotocin exposure (Radenković, Stojanović, Prostran, 2016), which can alter the drug's elimination in diabetic animals. For instance, diabetic rats induced by streptozotocin showed an increase of AST and ALT (Choi *et al*., 2008) and the diabetic rats that received either alloxan or streptozotocin presented an increase of urea nitrogen and kidney weight (Choi *et al*., 2008; Lee, Choi, Lee, 2008), which could be an indicator of nephrotoxicity. In the present study, the diabetes induction by streptozotocin and nicotinamide produced high levels of blood glucose compatible with diabetes without changes in the biochemical parameters investigated (Table II). This model was firstly described by Masiello and coworkers (1998) and consists of the administration of nicotinamide previously to streptozotocin. Nicotinamide prevents the extensive damage triggered by streptozotocin in the β-pancreatic cells, resulting in diabetics animals with moderate blood glucose levels without nephrotoxicity and hepatotoxicity (Masiello *et al.,* 1998; Szkudelski, 2012; Ghasemi, Khalifi, Jedi, 2014). In our study, as streptozotocin did not damage the elimination pathways of metformin and tissue exposure was similar in control and diabetic animals. This result is especially important in PK/PD analysis of anti-diabetic drugs since these protocols of diabetes induction in rats by chemicals are widely described in the literature. However, the changes associated with nephrotoxicity and hepatotoxicity are not considered in most cases and, thus, differences observed in effect can be associated with tissue exposure and not with receptor-binding events.

CONCLUSION

The bioanalytical method described in the present study was able to characterize the free interstitial levels reached by metformin in the liver of rats using microdialysis. This technique was feasible to assess the drug concentration in the target tissue of control and diabetic rats. This is the first report about metformin directly *in vivo* demonstrating the free drug concentrations in the target tissue (liver). The results described in this study showed that diabetic animals with no kidney injury have similar metformin liver concentrations to non-diabetic animals. Moreover, this anti-diabetic drug presented high liver concentration when compared to plasma concentration reported in the literature.

ACKNOWLEDGMENTS

This study was supported by FAPERGS and CNPq (# processes 159722/2013-6 and 0450-2251/14-6). The authors report no conflict of interest.

REFERENCES

Araujo BV, Silva CF, Haas SE, Dalla Costa T. Microdialysis as a tool to determine free kidney levels of voriconazole in rodents: a model to study the technique feasibility for a moderately lipophilic drug. J Pharm Biomed Anal. 2008;47(4-5):876–81.

Azeredo FJ, Dalla Costa T, Derendorf H. Role of microdialysis in pharmacokinetics and pharmacodynamics: Current status and future directions. Clin Pharmacokinet. 2014;53(3):205-12.

Beckmann R. Absorption, distribution in the organism and elimination of metformin. Diabetologia. 1969;5(5):318-24.

Choi YH, Kim SG, Lee MG. Dose-independent pharmacokinetics of metformin in rats: Hepatic and gastrointestinal first-pass effects. J Pharm Sci. 2006;95(11):2543-52.

Choi YH, Lee DC, Lee I, Lee MG. Changes in metformin pharmacokinetics after intravenous and oral administration to rats with short-term and long-term diabetes induced by streptozotocin. J Pharm Sci. 2008;97(12):5363-75.

Food and Drug Administration. Guidance for industry: Bioanalytical method validation. Available from: http://www.fda.gov/downloads/Drugs/Guidance/ucm070107.pdf

Ghasemi A, Khalifi S, Jedi S. Streptozotocin-nicotinamide-induced rat model of type 2 diabetes (review). Acta Physiol Hung. 2014;101(4):408-20.

Graham GG, Punt J, Arora M, Day RO, Doogue MP, Duong JK, et al. Clinical pharmacokinetics of metformin. Clin Pharmacokinet. 2011;50(2):81-98.

Grover B, Buckley D, Buckley AR, Cacini W. Reduced expression of organic cation transporters rOCT1 and

rOCT2 in experimental diabetes. J Pharmacol Exp Ther. 2004;308(3):949–56.

Higgins JW, Bedwell DW, Zamek-gliszczynski MJ. Ablation of Both Organic Cation Transporter (Oct) 1 and Oct2 Alters Metformin Pharmacokinetics but Has No Effect on Tissue Drug Exposure and Pharmacodynamics. Drug Metab Dispos. 2012;40(6):1170-7.

Lange ECM. Recovery and calibration techniques: toward quantitative microdialysis. In: Müller M (Ed.) Microdialysis in Drug Development. New York: Springer-Verlag; 2013; p.13-33.

Lee JH, Yang SH, Oh JM, Lee MG. Pharmacokinetics of drugs in rats with diabetes mellitus induced by alloxan or streptozocin: comparison with those in patients with type I diabetes mellitus. J Pharm Pharmacol. 2010;62(1):1-23.

Lee MG, Choi YH, Lee I. Effects of diabetes mellitus induced by alloxan on the pharmacokinetics of metformin in rats: restoration of pharmacokinetic parameters to the control state by insulin treatment. J Pharm Pharm Sci. 2008;11(1):88-103.

Liang X, Giacomini KM. Transporters Involved in Metformin Pharmacokinetics and Treatment Response. J Pharm Sci. 2017;106(9):2245-2250.

Masiello P, Broca C, Gross R, Roye M, Manteghetti M, Hillarire-Buys D, et al. Development of a new model in adult rats administered streptozotocin and nicotinamide. Diabetes. 1998;47(February):224-9.

Mouton JW, Theuretzbacher U, Craig WA, Tulkens PM, Derendorf H, Cars O. Tissue concentrations: do we ever learn? J Antimicrob Chemother. 2008;61(2):235-7.

National Research Council (US) Guide for the Care and Use of Laboratory Animals. 8th Ed. Washington: The National Academies Press; 2011. Available from: http://www.ncbi.nlm.nih.gov/books/NBK54050/

Nowicki MT, Aleksunes LM, Sawant SP, Dnyanmote AV, Mehendale HM, Manautou JE. Renal and hepatic transporter expression in type 2 diabetic rats. Drug Metab Lett. 2008;2(1):11-7.

Plock N, Kloft C. Microdialysis--theoretical background and recent implementation in applied life-sciences. Eur J Pharm Sci Off J Eur Fed Pharm Sci. 2005;25(1):1-24.

Pries AR, Kuebler WM. Normal endothelium. In: Moncada S, Higgs, A (Ed). The vascular endothelium I. Berlin, Heidelberg:Springer; 2006;p.1-40.

Radenković M, Stojanović M, Prostran M. Experimental diabetes induced by alloxan and streptozotocin: The current state of the art. J Pharmacol Toxicol Methods. 2016;78:13-31.

Slitt AL, Cherrington NJ, Hartley DP, Leazer TM, Klaassen CD. Tissue distribution and renal developmental changes in rat organic cation transporter mRNA levels. Drug Metab Dispos. 2002;30(2):212-9.

Sogame Y, Kitamura A, Yabuki M, Komuro S. Liver uptake of Biguanides in rats. Biomed Pharmacother. 2011;65(6):451-5.

Srinivas NR. Strategies for preclinical pharmacokinetic investigation in streptozotocin-induced diabetes mellitus (DMIS) and alloxan-induced diabetes mellitus (DMIA) rat models: case studies and perspectives. Eur J Drug Metab Pharmacokinet. 2015;40(1):1-12.

Szkudelski T. Streptozotocin-nicotinamide-induced diabetes in the rat. Characteristics of the experimental model. Exp Biol Med. 2012;237(5):481-90.

Terada T, Masuda S, Asaka J-I, Tsuda M, Katsura T, Inui K. Molecular cloning, functional characterization and tissue distribution of rat H+/organic cation antiporter MATE1. Pharm Res. 2006;23(8):1696-701.

Received for publication on 12th September 2019
Accepted for publication on 20th November 2019