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Research Paper

Effects of insulin on CYP3A activity and nicardipine disposition in streptozotocin-induced diabetic rats

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Abstract

Objectives The aim of the study was to clarify the effect of insulin treatment on drug metabolism and disposition.
Methods We investigated the mRNA expression and activity of cytochrome P450 (CYP) 3A, which is involved in the metabolism of several drugs, by using a rat model of diabetes and insulin-treated diabetes. In addition, we investigated the mRNA expression of the nuclear receptors reported to regulate the transcription of CYP3A, pregnane X receptor (PXR) and constitutive androstane receptor (CAR). We also assessed the disposition of nicardipine, which is mainly metabolised by CYP3A, using both rat models to evaluate the influence of insulin treatment on drug disposition.
Key findings We noted that alterations in the serum bile acid concentration in both rat groups were related to the changes in CAR mRNA expression, CYP3A mRNA expression and CYP3A activity. Furthermore, although the enhanced CYP3A activity in the diabetic rat accelerated the elimination of nicardipine, insulin administration decreased the enhanced CYP3A activity in the diabetic group and delayed the elimination of nicardipine to the same level as that in the control group. However, the steady-state volume of distribution was increased in the insulin-treated diabetic group as compared to the control and diabetic groups. We further noted that although the CYP3A activity in the diabetic group returned to the same level as in that in the non-diabetic group by insulin treatment, other values, such as the distribution volume of nicardipine, did not show a similar return.
Conclusions Based on our results, we suggest that alterations in the drug disposition in diabetes and insulin-treated diabetes should be taken into consideration in order to provide safe and effective drug therapy.
Keywords CYP3A; disposition; insulin; nicardipine

Introduction

Diabetes type 1 is a metabolic disorder caused by the absence of insulin induced by the destruction of β cells of the pancreas. It causes high levels of glucose, hyperlipemia and ketosis by significantly disturbing the metabolism of glucose, lipids and amino acids.

It has been reported that the expressions of cytochrome P450 (CYP),[1–3] conjugation enzymes (e.g. UDP-glucuronosyltransferase, sulfotransferase, glutathione S-transferase, etc.),[4–7] and transporters (e.g. multidrug resistance protein, bile salt export pump, multidrug resistance-associated protein (MRP), etc.) are altered in the livers of rats with diabetes type 2,[8] therefore it is possible that drug dispositions might be altered in diabetes type 1.

Certain studies have reported alterations in the expression of CYP in diabetes type 1. Shimojo demonstrated that the expression of CYP1A2, 2B1 and 4A was altered in the rat model liver of diabetes type 1 induced by streptozotocin (STZ) treatment.[1] In addition, Matzke *et al*.[9] and Sotaniemi *et al*.[10] reported that enhanced activity of CYP1A2 accelerated the metabolism of antipyrin in diabetes type 1.

Emergency treatment (e.g. continuous intravenous use of insulin, fluid replacement, etc.) is often required as episodic therapy for diabetes type 1. After that, intensive conventional insulin therapy is introduced as a sequential therapy.[11] In intensive conventional insulin therapy, the patient with diabetes type 1 is treated with multiple injections or continuous subcutaneous infusions of insulin and blood glucose levels are self-measured by the patient.

The Diabetes Control and Complication Trial (DCCT) clarified that stringent control of blood glucose levels by intensive conventional insulin therapy delays the onset of microvascular complications.[12] However, the effect of insulin treatment on the expression and function of CYPs, conjugation enzymes and transporters in the liver remains unclear, and alterations in drug disposition in diabetes following insulin therapy have not been investigated in detail. The possibility that the insulin treatment might have no effect on drug disposition cannot be excluded and the lowering of blood glucose level by insulin treatment might therefore not mean the amelioration of the condition.

In the present study, we aimed to clarify the effect of insulin treatment on drug metabolism and disposition. We investigated the mRNA expression and activity of CYP3A, which is involved in the metabolism of several drugs, by using rat models of type 1 diabetes and insulin-treated type 1 diabetes. We also assessed the disposition of nicardipine, which is mainly metabolised by CYP3A, using both rat models to evaluate the influence of insulin treatment on drug disposition. In addition, we investigated the mRNA expression of the nuclear receptors, which are reported to regulate the transcription of CYP3A, pregnane X receptor (PXR) and constitutive androstane receptor (CAR), to clarify the influence of insulin treatment.

Materials and Methods

Materials

STZ was obtained from Sigma Inc. (Kanagawa, Japan). Insulin (Humulin N U-100) was obtained from Eli Lilly Japan K.K. (Kobe, Japan). Sepasol RNA I Super, NADP (grade III), glucose 6-phosphate, glucose 6-phosphate dehydrogenase, testosterone, 6β-hydroxy testosterone and nitrazepam were obtained from Nacalai Tesque Inc. (Kyoto, Japan). Glycogen solution and the SuperScript III first-strand synthesis system for reverse transcriptase-polymerase chain reaction (RT-PCR) were received from Life Technologies Inc. (Tokyo, Japan) and the KOD Dash kit was obtained from Toyobo Inc. (Osaka, Japan). Sense and antisense primers for CYP3A1, CYP3A2, PXR, CAR and β-actin were purchased from Bex Inc. (Tokyo, Japan). Nicardipine and verapamil were purchased from Wako Pure Chemical Industries, Ltd (Tokyo, Japan). All the other reagents used were of molecular biology grade and the highest quality available.

Animals

Eighteen 6-week-old male Donryu rats were purchased from Japan SLC Inc. (Shizuoka, Japan). They were housed for 1 week to exclude any abnormal animals. Some of the rats (STZ group) received an intraperitoneal (i.p.) injection of STZ at a dose of 50 mg/kg body weight after an overnight fast; they were bred for 4 weeks after STZ administration. The other rats (control group) received an i.p. injection of saline; they were bred for 4 weeks after saline administration. A third group of rats (INS group) received an i.p. injection of STZ at a dose of 50 mg/kg body weight after an overnight fast; they were bred for 3 weeks after STZ administration, following which they received s.c. injections of insulin at a dose of 3 IU at 8:00 am and 8:00 pm daily for 1 week.

Liver tissues and blood samples were collected and stored at −80°C until analysis. The plasma samples were centrifuged at 12 000 rev/min for 10 min to obtain plasma samples and dispatched to FALCO Biosystems Ltd (Kyoto, Japan) to determine the concentrations of total bile acid (TBA), free fatty acid (FFA) and ketone body in the plasma. The blood glucose level (GLU) was measured using Precision Q.I.DTM (Abbott Japan Co. Ltd, Tokyo, Japan).

All rats were handled in accordance with the guidelines for the care of laboratory animals established by Kobe Gakuin University. The protocol for this animal study was approved by the Animal Experimentation Ethics Committee of Kobe Gakuin University.

Analysis of mRNA expression by reverse transcriptase-polymerase chain reaction

Total RNA was extracted from the rat liver tissues (100 mg) by using sepasol RNA I Super, chloroform and isopropanol. cDNA was synthesised by performing RT-PCR with the SuperScript III first-strand synthesis system using KOD Dash DNA polymerase. Synthetic cDNA (0.5 μl) and sense and antisense primers were added to a 24.5-μl reaction mixture containing KOD Dash DNA polymerase. Denaturation, annealing and extension were performed for 25–40 cycles by using the iCycler system. Degenerate PCR primers were designed and chemically synthesised on the basis of the amino acid sequences of rat CYP3A1 (329 base pairs (bp); sense primer, 5′-GGA AAT TCG ATG TGG AGT GC-3′; antisense primer, 5′-AGG TTT GCC TTT CTC TTG CC-3′); CYP3A2 (348 bp; sense primer, 5′-TAC TAC AAG GGC TTA GGG AG-3′; antisense primer, 5′-CTT GCC TGT CTC CGC CTC TT-3′); PXR (741 bp; sense primer, 5′-AGC TGA GAC TTC TGT ATG CAA-3′; antisense primer, 5′-TAG TTC CAG ATG CTG CCG TCT T-3′); CAR (477 bp; sense primer, 5′-TCT CAC TCA ACA CTA CGT TC-3′; antisense primer, 5′-CTG GGA AAG GAT CCA AGC CTG GG-3′); and β-actin (646 bp; sense primer, 5′-ATG TAC GTA GCC ATC CAG GC-3′; antisense primer, 5′-TCC ACA CAG AGT ACT TGC GC-3′). The PCR products were resolved by performing electrophoresis on 2% agarose gels for 50 min. The agarose gels were then stained with ethidium bromide. The bands were visualised using an ultraviolet (UV) transilluminator (Atto Corp., Tokyo, Japan) and specific bands were quantified using densitometric analysis.

Testosterone hydroxylation assay and high-performance liquid chromatography assay

Microsomes were isolated from livers by differential centrifugation. Liver tissue (200 mg) was homogenised in 1 ml of 1.15% KCl solution. Samples were then centrifuged at 10 000*g* for 30 min at 4°C. The supernatant was collected and centrifuged at 105 000*g* for 60 min at 4°C. The super- natant was discarded and the pellet was resuspended and washed in 1 ml of 100 mM potassium dihydrogenphosphate buffer (pH 7.4). The suspension was centrifuged again at 105 000*g* for 60 min at 4°C. The supernatant was discarded and the pellet was resuspended and washed in 1 ml of 100 mM

potassium dihydrogenphosphate buffer (pH 7.4), containing 1 mM EDTA and 20% glycerol. Microsomes were stored at –80°C until analysis.

For the determination of testosterone hydroxylation, a reaction mixture containing 100 mM potassium dihydrogenphosphate buffer (pH 7.4), 0.2 mg/ml microsomal protein, 60 μM testosterone in methanol, 1 mM NADP, 1 mM glucose 6-phosphate and 10 mM magnesium chloride was prepared. The total volume of the reaction mixture was 0.5 ml. The mixture was preincubated at 37°C for 3 min and reactions were initiated by the addition of glucose 6-phosphate dehydrogenase. Then, 50 μl of mixture was collected and reactions were quenched by the addition of 150 μl acetonitrile. Samples were obtained at baseline ($t = 0$) and at 5, 10 and 15 min after the addition of glucose 6-phosphate dehydrogenase. The internal standard (1.5 μg/ml nitrazepam) was added and the samples mixed. The samples were centrifuged at 10 000*g* for 15 min at 4°C and the supernatant was collected. The supernatant was evaporated at 40°C, the residue was dissolved in 200 μl of the mobile phase and 50 μl of the sample was subjected to high-performance liquid chromatography (HPLC). Testosterone, 6β-hydroxy testosterone and nitrazepam in the samples were detected using an UV-visible spectrophotometer (244 nm, SPD-10A; Shimadzu Inc., Kyoto, Japan.). The reverse-phase column (Inertsil ODS-3; GL Sciences Inc., Tokyo, Japan) was maintained at 40°C. The mobile phase was changed from 40 to 55% methanol in 20 mM phosphate buffer (pH 2.0) after 30 min measurement by a stepwise procedure. The flow rate of the mobile phase was kept constant at 1 ml/min for the total run time of 60 min.

CYP3A activity, i.e. testosterone hydroxylase activity, in the hepatic microsome was calculated as the slope of the 6β-hydroxy testosterone generation time profiles and was expressed as nanomoles per minute per milligram of protein.

Disposition of nicardipine

Three male rats from each group were continuously injected intravenously with nicardipine at a dose of 1 mg/kg body weight via the lower-limb arteries for 5 min, and 0.5 ml blood samples were collected from the arteries of the contralateral limb under isoflurane anaesthesia. After collecting the sample, 0.5 ml of saline was injected to maintain the blood flow. Samples were obtained at baseline ($t = 0$) and at 5, 10, 15, 25, 45, 65, 95 and 125 min after nicardipine administration, and were centrifuged at 12 000 rev/min for 10 min to obtain plasma. The plasma samples were stored at –80°C until analysis.

Next, 50 μl of the internal standard (30 μg/ml verapamil) and 10 μl of 2 M sodium hydroxide were added to 100 μl of the plasma samples. A quantity of 600 μl of a solution of t-butylmethylether and hexane (50 : 50 by volume) was then added and the samples mixed. The supernatant was collected and evaporated at 40°C. The residue was dissolved in 150 μl of the mobile phase and 50 μl of the sample was injected for the HPLC assay. Nicardipine was detected using a UV-visible spectrophotometer (235 nm, SPD-2A; Shimadzu Inc., Kyoto, Japan). The reverse-phase column (Mightysil RP 18 GP250–4.6; Kanto Chemical Co. Inc., Tokyo, Japan) was maintained at 40°C. The mobile phase used was a solution of 25 mM phosphate buffer (pH 7.0) and acetonitrile (50 : 50 by volume). The flow rate of the mobile phase was kept constant at 1 ml/min for the total run time of 25 min. The pharmacokinetic parameters of nicardipine in plasma were calculated on the basis of the plasma concentration–time profiles by two-compartment model fitting.

Statistical analyses

The data are presented as mean ± standard deviation (SD). Non-parametric comparisons were performed using the Kruskal–Wallis test. Significant differences were evaluated using Dunnett's test if the Kruskal–Wallis test was significant. Values of $P < 0.05$ were considered statistically significant.

Results

A rat model of diabetes

The blood glucose levels (GLU) and the concentrations of total bile acid (TBA), free fatty acid (FFA) and ketone bodies in the rats of each group are shown in Table 1. GLU was significantly higher in the STZ group than in the control group. The volume of drinking water and food intake was significantly higher in the STZ group than in the control group. In contrast, the body weight was significantly lower in the STZ group (329.0 ± 13.5 g) than in the control group (412.3 ± 28.0 g). These results indicate that an STZ-administered rat can be used as a rat model of diabetes type 1.

GLU was significantly lower in the INS group than in the STZ group and that of the INS group was similar to the control group. Figure 1 shows the circadian GLU of the STZ and INS groups during the day, when the insulin administration in the INS group was begun. Although GLU was high at ~400 mg/dl in the STZ group during the day, in the rats of the INS group it was maintained at ~150 mg/dl by the subcutaneous administration of insulin every 8 h. The body weight in the INS group (334.3 ± 24.6 g) was lower than that in the control group and was similar to that in the STZ group. TBA and ketone body levels were higher in the STZ group than in the control group, but lower in the INS group. However, these differences were not statistically significant. FFA levels were not significantly different between control, STZ and INS groups (Table 1).

Table 1 Blood chemical properties observed at 4 weeks after streptozotocin treatment

	Control	STZ	INS
GLU (mg/dl)	140 ± 7	450 ± 45*	81 ± 27[#]
TBA (μmol/l)	22.8 ± 8.4	46.7 ± 26.1	10.6 ± 9.7
FFA (mEq/l)	0.41 ± 0.07	0.31 ± 0.04	0.50 ± 0.51
Ketone body (μmol/l)	567 ± 24	1320 ± 789	187 ± 105

Control, streptozotocin-treated and insulin-treated rats are represented as Control, STZ and INS, respectively. GLU, blood glucose level; TBA, total bile acid concentration; FFA, free fatty acid. Data are expressed as the mean ± SD, $n = 3$. *$P < 0.05$ compared with the control rats. [#]$P < 0.05$, compared with the STZ rats.

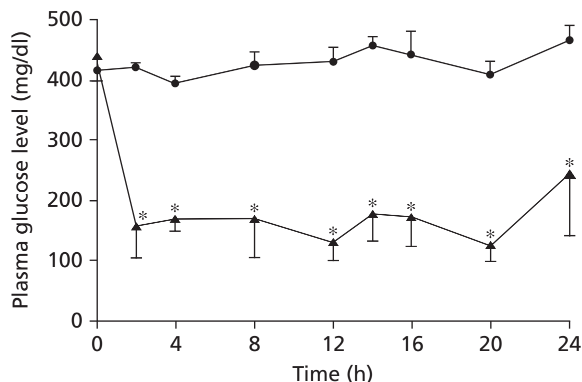

Figure 1 Circadian blood glucose levels of the streptozotocin- and insulin-treated rats observed 3 weeks after streptozotocin treatment. The value of 0 min was that before the first insulin treatment. Streptozotocin-treated and insulin-treated rats are represented as STZ and INS, respectively. ●, STZ group; ▲, INS group. Data are expressed as mean ± SD of results obtained for three animals. $*P < 0.05$, compared with the STZ rats.

Analysis of mRNA expression by reverse transcriptase-polymerase chain reaction

The mRNA expressions of CYP3A1 and CYP3A2 were investigated in the liver of the rats in the control, STZ and INS groups. The corresponding electrophoretic profiles and band strengths are shown in Figure 2. The mRNA expressions of CYP3A1 in the STZ and INS groups increased significantly by approximately 250 and 190%, respectively, as compared to the expressions in the control group (Figure 2b). Furthermore, the mRNA expression of CYP3A1 was significantly lower in the INS group than in the STZ group. Similarly, the mRNA expressions of CYP3A2 in the STZ and INS groups increased significantly, by approximately 320 and 240%, respectively, as compared to the expressions in the control group (Figure 2b). Moreover, the mRNA expression of CYP3A2 was significantly lower in the INS group than in the STZ group.

We also assessed the changes in the mRNA expression of the nuclear receptors PXR and CAR in the liver of the rats in the control, STZ and INS groups. The corresponding electrophoretic profiles and band strengths are shown in Figure 3. The mRNA expressions of PXR in the STZ and INS groups increased by approximately 130 and 200%, respectively, as compared to the expressions in the control group (Figure 3b). Although the mRNA expression of CAR was approximately two-fold higher in the STZ groups than in the control group, it was significantly lower in the INS group; the value in the INS group was 0.8 times that in the control group (Figure 3b).

Testosterone hydroxylation assay and HPLC assay

The activity of CYP3A is defined as the generation rate of 6β-hydroxy testosterone from testosterone catalysed by microsomes in the liver. It is shown in Figure 4. Although the activity of CYP3A was significantly higher in the STZ group (0.90 ± 0.24 nmol/min per mg protein) than in the control group (0.46 ± 0.18 nmol/min per mg protein), the activity of CYP3A was significantly lower in the INS group (0.21 ± 0.04 nmol/min per mg protein) than in the STZ group. On the other hand, no significant difference was observed between the activity of CYP3A in the control group and in the INS group.

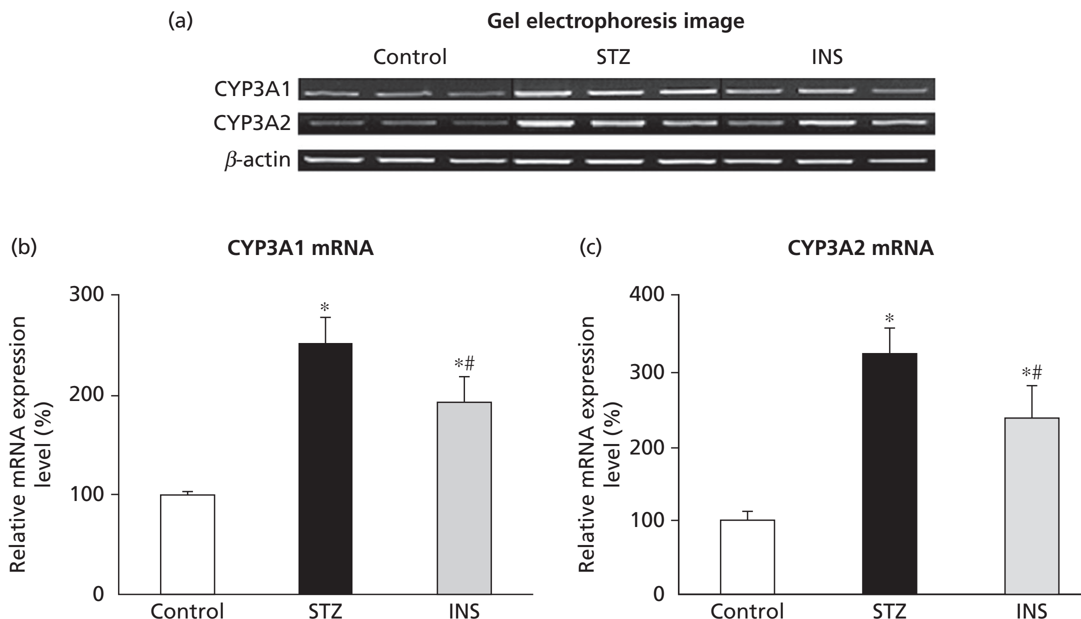

Figure 2 The mRNA expression of cytochrome P450 in the liver. Control, streptozotocin-treated and insulin-treated rats are represented as Control, STZ and INS, respectively. Electrophoretic profiles of the mRNA expression of cytochrome P450 (CYP) in the liver of STZ and INS rats (a) and the band strengths of CYP3A1 (b) and CYP3A2 (c) are expressed as a percentage of the control value. β-actin was used as the quantitative reference mRNA. Data are expressed as mean ± SD of results obtained for three animals for experiments performed in triplicate. $*P < 0.05$, compared with the control rats. $^{\#}P < 0.05$, compared with the STZ rats.

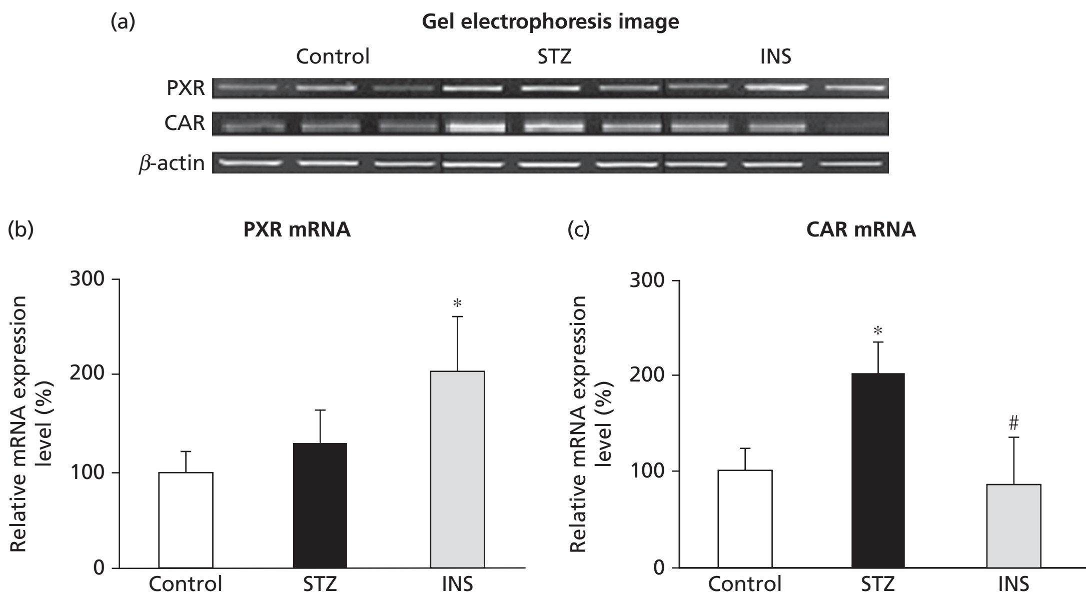

Figure 3 The mRNA expression of nuclear receptors in the liver. Control, streptozotocin-treated and insulin-treated rats are represented as Control, STZ and INS, respectively. Electrophoretic profiles of the nuclear mRNA of receptors in the nuclear extract obtained from the liver of STZ and INS rats (a) and the band strengths of pregnane X receptor (PXP) (b) and constitutive androstane receptor (CAR) (c) are expressed as a percentage of the control value. β-actin was used as the quantitative reference mRNA. Data are expressed as mean ± SD of results obtained for three animals for experiments performed in triplicate. *$P < 0.05$, compared with the control rats. $^{\#}P < 0.05$, compared with the STZ rats.

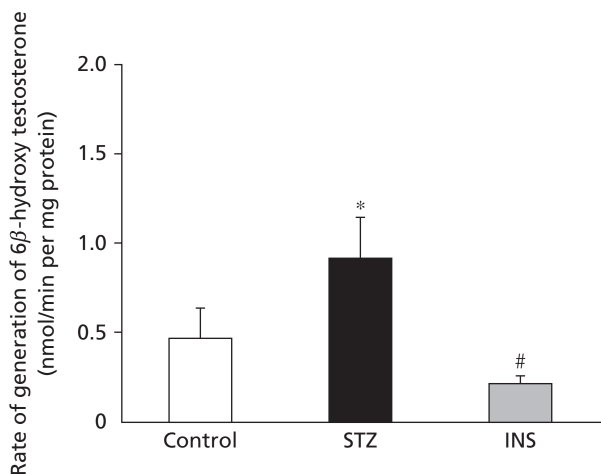

Figure 4 Testosterone hydroxylase activity in hepatic microsomes. Hepatic microsomes were incubated with testosterone for 15 min and the main metabolite, 6β-hydroxy testosterone, was separated by high-performance liquid chromatography as described in the Methods section. Control, streptozotocin-treated and insulin-treated rats are represented as Control, STZ and INS, respectively. CYP3A activity is expressed as nanomoles minutes milligrams of protein. Data are expressed as mean ± SD of results obtained for three animals for experiments performed in duplicate. *$P < 0.05$, compared with the control rats. $^{\#}P < 0.05$, compared with the STZ rats.

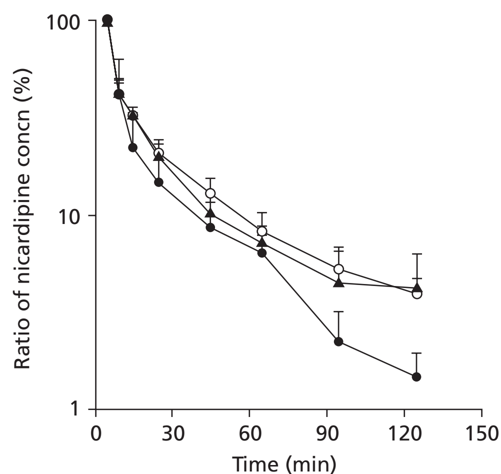

Figure 5 Pharmacokinetics of nicardipine in the control, streptozotocin- and insulin-treated groups. Control, streptozotocin-treated and insulin-treated rats are represented as Control, STZ and INS, respectively. Pharmacokinetics of nicardipine in the control, STZ and INS groups is expressed as a percentage relative to the initial ($t = 5$ min) plasma concentration of nicardipine. Groups of rats continuously received intravenous morphine at a dose of 1 mg/kg body weight for 5 min. ○, Control ($n = 3$); ●, STZ ($n = 3$); ▲, INS ($n = 3$). Data are expressed as mean ± SD.

Disposition of nicardipine

The plasma concentration–time profile of nicardipine is shown in Figure 5 as a percentage of the initial concentration. The initial concentration was defined as the plasma concentration of nicardipine at the end of the infusion for 5 min. The slope of the plasma nicardipine concentration expressed as a percentage of the initial concentration was similar in the INS group and in the control group. In contrast, this slope was greater in the STZ group than in the control and INS groups. Although the initial concentration of the control group (2.9 ± 0.53 μg/ml) was similar to the STZ group (2.7 ± 1.4 μg/ml), the initial

Table 2 Pharmacokinetic parameters in the two-compartment model as calculated from the plasma concentration of nicardipine

	Control	STZ	INS
$T_{1/2\alpha}$ (min)	2.8 ± 0.5	2.8 ± 0.5	4.4 ± 1.3
$T_{1/2\beta}$ (min)	43.1 ± 1.6	31.4 ± 2.0*	53.5 ± 4.1*,#
$AUC_{0\rightarrow\infty}$ (μg/min per ml)	63.4 ± 6.7	39.5 ± 14.7	42.4 ± 5.7
CL_{tot} (ml/min)	79.5 ± 8.3	137.5 ± 43.9	119.3 ± 15.8
Vd_{ss} (l/kg)	0.51 ± 0.07	0.48 ± 0.35	0.96 ± 0.14

Control, streptozotocin-treated and insulin-treated rats are represented as Control, STZ and INS, respectively. $T_{1/2\alpha}$, distributional half-time; $T_{1/2\beta}$, elimination half-time; CL_{tot}, total body clearance; AUC, area under the blood concentration–time curve; Vd_{ss}, steady-state volume of distribution. Data are expressed as mean ± SD of results obtained for four animals. *$P < 0.05$ compared with the control rats. #$P < 0.05$, compared with the STZ rats.

concentration of the INS group (1.9 ± 0.20 μg/ml) was lower than that of the other groups.

The pharmacokinetic parameters calculated as a two-compartment model are shown in Table 2. There were no significant differences between groups in distributional half-time ($T_{1/2\alpha}$). Elimination half-time ($T_{1/2\beta}$) was significantly lower in the STZ group than in the control group. In contrast, it was significantly higher in the INS group than in other two groups. The area under the blood concentration–time curve (AUC) in the STZ group was similar to that in the INS group and the values were lower than that in the control group. Systemic clearance (CL_{tot}) in the STZ group was similar to that in the INS group and the values were higher than that in the control group. Steady-state volume of distribution (Vd_{ss}) in the STZ group was similar to that in the control group and the values were lower than that in the INS group.

Discussion

It is well known that the mRNA expression of CYPs, conjugation enzymes and transporters is regulated by nuclear receptors.[13] Urquhart *et al*. reported that the mRNA expression of CYP3A is regulated by several nuclear receptors such as PXR and CAR.[14] PXR and CAR are known to influence the transcriptional activation of each target gene.[15] It is known that the network between nuclear receptors and target genes is extremely complex. Moreover, it has been reported that the mRNA expression of MRP2, which is a target gene of PXR, is increased by the activation of CAR in the PXR-deficient mouse.[16]

In the present study, the tendency to alterations in the mRNA expression of CYP3A1 and 3A2 differed from that in PXR (Figures 2, 3b). Most PXR ligands are xenobiotic substances.[17] The levels of several substances increase in the blood with diabetes and not all of them will decrease with insulin treatment, therefore these substances may have influenced the expression of PXR in the STZ and the INS groups. It is difficult to accurately mimic this phenomenon *in vitro* because many xenobiotic substances complicate the expression of PXR *in vivo*. Thus, the expression of PXR shown in our study has complex regulating mechanisms and the alterations in the expression of nuclear receptors in disease states needs to be clarified using animal models and compared with in-vitro evidence.

Teng *et al*. reported that the concentration of serum bile acid is associated with the expression of CYP3A.[16] In this study, the tendency to alterations in the mRNA expressions of CYP3A1 and 3A2 is similar to that in CAR (Figures 2, 3c). This result indicates that the increase in serum bile acid, one of the ligands of CAR,[17] enhances the mRNA expression of CAR and the increase in the mRNA expression of CAR enhances the mRNA expressions of CYP3A1 and 3A2 in the STZ group. On the other hand, the decrease in serum bile acid reduces the mRNA expression of CAR and the decrease in the mRNA expression of CAR reduces the mRNA expression of CYP3A1 and 3A2 in the INS group (Figures 2 and 3, Table 1).

The tendency to alterations in the activity of CYP3A is similar to the tendency of the alterations in the mRNA expressions of CYP 3A1 and 3A2 (Figure 4). This phenomenon is also shown in the alterations of blood glucose levels and the concentration of serum bile acid (Table 1). Although the detailed mechanism underlying these differences remains unclear, the hypoglycaemic state in the INS group was likely to have influenced the decrease in the serum bile acid concentration and CYP3A activity.

Nicardipine, a dihydropyridine calcium channel antagonist, is also mainly metabolised by CYP3A. Although the plasma concentration of nicardipine and the $T_{1/2\beta}$ were significantly lower in the STZ group than in the control group, $T_{1/2\alpha}$ and Vd_{ss} did not differ (Figure 5, Table 2). These results illustrate that the nicardipine disposition of the type 1 diabetic rat was changed in the elimination phase. The reason for the change in the elimination phase of drug disposition is mainly the alteration in the metabolic process. Thus, these results indicate that the increase in the activity of CYP3A in the STZ group accelerates the elimination of nicardipine. The elimination rate of nicardipine, i.e. the slope of the time–concentration profile, did not differ between the INS group and the control group. However, the initial concentration was lower in the INS group than in the control group. As a result, the plasma nicardipine concentrations at all points were lower in the INS group than in the control group (Figure 5). $T_{1/2\beta}$ and Vd_{ss} were higher in the INS group than in the control group (Table 2). From these results, we consider that the activity of CYP3A, which is enhanced in diabetes, was decreased in insulin-treated rats and that subsequently the elimination phase of nicardipine disposition became similar between the diabetes and non-diabetes groups.

Additionally, the main reason for the decrease in the initial nicardipine concentration in the INS group might be the increased Vd_{ss}, although the reason for increased Vd_{ss} was not clarified in this study.

Conclusions

In this study, we showed that the enhanced CYP3A activity in type 1 diabetic rats was decreased to a level identical to that in the control rats. However, the plasma nicardipine concentrations were lower in insulin-treated rats than in the control rats; Vd_{ss} was higher in the insulin-treated rats than in the control rats. These results suggest that the insulin treatment quickly affects CYP3A activity and mRNA

expression. Otherwise, it might take a long period to reach the same drug disposition in the insulin-treated and the control rats. From this study, we suggest that the same alterations in drug disposition might occur in humans and that alterations in drug disposition in type 1 diabetes patients and insulin-treated type 1 diabetes patients should be taken into consideration in order to provide safe and effective drug therapy.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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