Effect of JTT-501 on Net Hepatic Glucose Balance and Peripheral Glucose Uptake in Alloxan-Induced Diabetic Dogs

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JTT-501, a new insulin sensitizer, improves peripheral glucose uptake in insulin-resistant animals such as KK-Ay mice and Zucker fatty rats. However, the effect of JTT-501 on hepatic glucose metabolism has not been addressed. To investigate this effect, experiments were performed on 6 alloxan-diabetic dogs. Three experiments were conducted for each dog: the treatment experiment, which followed a 10-day oral treatment with JTT-501 30 mg · kg^{-1} · d^{-1}, and 2 control experiments 2 weeks before and 2 weeks after the treatment experiment. A hyperinsulinemic-hyperglycemic clamp was performed with the tracer dilution method (intraportal insulin infusion rate, 18 pmol · kg^{-1} · min^{-1}). Arterial hyperglycemia (~10 mmol/L) was maintained by adjusting the peripheral glucose infusion rate. After a 45-minute basal period (period I), portal glucose infusion (22.2 μmol · kg^{-1}min^{-1}) was administered for 120 minutes (period II). This was followed by a 90-minutes recovery period (period III). JTT-501 increased insulin-stimulated glucose utilization ($P < .05$) and enhanced insulin-mediated suppression of glucose production ($P < .05$) in periods I and III. Net hepatic glucose balance (NHGB) determined by the arterial-venous (A-V) difference method was increased by JTT-501 in period II ($P < .01$). We conclude that JTT-501 enhances both hepatic and peripheral insulin sensitivity and therefore may have important therapeutic effects in type 2 diabetes.
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INSULIN RESISTANCE is a well-established characteristic in subjects with both type 1 and type 2 diabetes. Insulin resistance in diabetes is both peripheral and hepatic. The liver is a major organ contributing to glucose homeostasis, being capable of both glucose production and uptake. The balance between total glucose production and uptake (net hepatic glucose balance [NHGB]) is negative during fasting (net hepatic glucose production) and positive after a meal (net hepatic glucose uptake). NHGB is altered in diabetes. Many studies have demonstrated that increased hepatic glucose production is closely correlated with the degree of fasting hyperglycemia in both type 1[1] and type 2[1-3] diabetes. Our previous studies[4,5] have suggested that hepatic glucose uptake is also impaired in hyperinsulinemic-euglycemic conditions after an oral glucose load in type 2 diabetic patients. Thus, it is likely that decreased hepatic glucose uptake contributes to postprandial hyperglycemia in diabetes.

JTT-501 (an isoxazolidinedione derivative) is a newly developed compound with a chemical structure similar to that of the thiazolidinediones. JTT-501 has been shown to be effective for ameliorating hyperglycemia and hyperinsulinemia in obese insulin-resistant animal models such as KK-Ay mice,[6] Zucker fatty rats, and high-fat–fed rats,[7] without a stimulatory action on insulin secretion. In KK-Ay mice, a 4-day treatment with JTT-501 reduced the level of glucose, triglyceride, and insulin in a dose-dependent manner.[6] In Zucker fatty and high-fat–fed rats[7] and also in normal rats,[8] JTT-501 improved glucose disposal during a hyperinsulinemic-euglycemic clamp, suggesting an effect on peripheral glucose uptake. However, the effects of JTT-501 on hepatic glucose metabolism have not been addressed.

In the present study, we examined the effects of JTT-501 on insulin-stimulated peripheral glucose uptake and NHGB in the dog, an animal model in which the technique of direct measurement of hepatic glucose balance has been established.[9-11] We used the alloxan-diabetic dog,[9] a model of partial insulin deficiency and insulin resistance,[12] while simulating postprandial conditions in diabetic patients. Specifically, moderate hyperglycemia was attained and a physiological dose of insulin was delivered portally, to simulate the endogenous portal insulin secretion in type 2 diabetes.

MATERIALS AND METHODS

Animals

All experimental procedures were approved by the Animal Care Committee of the University of Toronto. Experiments were performed with 6 male mongrel dogs weighing 20 to 27 kg. The animals were injected with alloxan 65 mg/kg (Aldrich, Milwaukee, WI) dissolved at pH 4.4 in 0.1 mol/L acetate buffer to induce diabetes.[9,12] The diabetic dogs were fed once daily in the morning with a diet of 400 to 450 g dry chow (25% protein, 9% fat, and 38% carbohydrate; Ralston Purina, Mississauga, Ontario, Canada) and 670 g beef (Romar Pet Supply, Toronto, Ontario, Canada). Subcutaneous injections of a combination of intermediate-acting (NPH) and short-acting (Regular) porcine insulin (Lilly, Indianapolis, IN) were administered once daily at feeding to maintain fasting blood glucose less than 11 mmol/L. Three experiments were performed on each dog. The first was a control experiment (C1). Two weeks later after a 10-day treatment with JTT-501 (30 mg · kg^{-1} · d^{-1} as an oral preparation given at the time of the morning feeding), the second experiment was performed (JTT). JTT-501 treatment was stopped after the second experiment, and 2 weeks later, a third experiment was performed which was the second control experiment (C2). The dose of JTT-501 was chosen to produce a significant decrease in both triglyceride and cholesterol levels after 2 weeks of treatment in preliminary toxicity studies performed by the JTT-501 manufacturer in normal dogs. The duration of treatment was the minimum compatible with an effect of JTT-501 on insulin sensitivity (approximately 2 weeks

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Submitted August 2, 1999; accepted January 11, 2000.

Supported by a research grant and a postdoctoral fellowship (M.N.) from the Central Pharmaceutical Institute, Japan.

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0026-0495/00/4907-0018$10.00/0

doi:10.1053/mt.2000.6752

based on lipid levels) and the maximum allowed by the low feasibility of the experimental protocol, because of a failure of the sampling portal and hepatic vein lines over time.

Surgical Procedures

A laparotomy and vessel cannulation were performed under general anesthesia induced with sodium thiamylal and maintained with nitrous oxide, halothane, and assisted ventilation. Silastic catheters (Dow Corning, Midland, MI) were inserted into the carotid artery, portal vein, and left common hepatic vein for sampling and the jugular vein, a branch of the splenic vein, and the jejunal vein for infusion. The tip of the portal catheter was placed 3 cm from the point at which the vessel enters the liver, and the tip of the hepatic vein catheter was placed 1.5 cm inside the left common hepatic vein.[10,11] The tips of the jejunal and splenic vein catheters were placed 2 cm proximal to the point of the first vessel bifurcation. The gastroduodenal artery was ligated and Doppler flow probes (Transonic System, Ithaca, NY) were positioned around the hepatic artery and portal vein. All catheters and lines of Doppler flow probes were exteriorized at the back of the neck through a subcutaneous tunnel. The catheters were flushed with saline twice per week and filled with a heparin solution (1,000 IU/mL Hepalean; Organon Canada, Toronto, Ontario, Canada).

Experimental Protocol

At least 2.5 weeks elapsed between surgery and the first experiment. The dogs were fasted 18 hours before the experiments. The last injections of NPH and Regular insulin were given 48 and 12 hours before each experiment, respectively. The last dose of JTT-501 (30 mg/kg given orally without the morning feeding) was administered 2 hours before the experiments. A hyperinsulinemic-hyperglycemic clamp combined with an intraportal glucose load was used to determine peripheral glucose uptake and NHGB in the conscious alloxan-diabetic dogs. Before insulin infusion, blood samples were taken to determine fasting plasma glucose, insulin, and glucagon. Arterial blood pressure was determined by a pressure transducer connected to the sampling catheter of the carotid artery and recorded on a physiograph.

Each experiment consisted of a 120-minute tracer equilibration period (from −165 to −45 minutes), a 45-minute basal sampling period (from −45 to 0 minutes, period I), a 120-minute portal glucose infusion period (from 0 to 120 minutes, period II), and a 90-minute recovery period (from 120 to 210 minutes, period III) (Fig 1). Throughout the experiments, insulin was infused portally at a constant rate (18.0 $pmol \cdot kg^{-1} \cdot min^{-1}$) via the jejunal and splenic veins, while exogenous glucose (25% dextrose) was administered peripherally via the jugular vein to maintain moderate hyperglycemia (~10 mmol/L). KCl was mixed with the peripheral glucose infusate (20 μEq/mL) to prevent insulin-induced hypokalemia. To determine the glucose turnover rate, [3-^{3}H]-glucose (Du Pont-New England Nuclear, Lachine, Quebec, Canada) was given as a primed constant infusion (140,666 Bq + 6,233 Bq/min) throughout the experiment. A constant infusion of indocyanine green ([ICG] 0.1 $mg/m^2 \cdot min^{-1}$) was started at −120 minutes to measure hepatic blood flow. After a 45-minute basal period, portal glucose infusion (25% dextrose, 22.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) was administered for 120 minutes. During portal glucose infusion, a fraction of the portal glucose load that is not extracted by the liver enters the systemic circulation and reduces the peripheral glucose infusion requirement to maintain the hyperglycemic clamp. *p*-Aminohippuric acid (PAH) was given intraportally with glucose to estimate glucose mixing with portal blood.[11] During period III, peripheral glucose infusion alone was given to maintain the hyperglycemic clamp. Both the peripheral and portal glucose infusates were radiolabeled. The use of labeled glucose infusates allowed us to minimize the changes in plasma glucose specific activity induced by variations in the glucose infusion rate. Blood samples were taken every 5 minutes from the carotid artery cannula for the determination of plasma glucose. The peripheral glucose infusion rate was adjusted according to the plasma glucose level to maintain steady arterial hyperglycemia throughout the experiments.[13] Blood samples from the carotid artery and portal and hepatic vein cannulae were drawn at 15-minute intervals for the determination of glucose turnover, other metabolites, and hormones. Arterial and portal vein blood samples were collected simultaneously, whereas hepatic vein samples were taken after a 30-second delay to compensate for transit time through the liver.[10] The amount of blood withdrawn per experiment was approximately 150 mL. Hepatic blood flow was estimated using ICG[14] in all experiments. To verify this estimation and to determine the proportion of hepatic flow contributed by the hepatic artery and portal vein, portal and hepatic artery blood flow were determined every 15 minutes using Doppler flow meters.[15]

Laboratory Methods

The plasma glucose concentration was measured by a glucose oxidase method using Glucose Analyzer II (Beckman, Fullerton, CA). Insulin and glucagon were analyzed by radioimmunoassay (coefficient of variation, 12% and 15%, respectively). Plasma nonesterified fatty acids (NEFAs) were determined by a microfluorometric method.[16] Arterial and venous concentrations of ICG (Becton Dickinson, Cockeysville, MD) were measured spectrophotometrically at 810 nm.[14] Plasma PAH levels were determined on a spectrophotometer at 465 nm.[17] To measure radioactivity from [3-^{3}H]-glucose, samples were deproteinized with zinc sulfate and barium hydroxide. The supernatant was evaporated at 50°C, redissolved in water, and counted in Ready Safe scintillation fluid (Beckman).

Calculations

To assess the completeness of glucose mixing in the portal blood, PAH (Sigma, St Louis, MO) was mixed with intraportal glucose infusate and the recovery of PAH in both the portal and hepatic vein was determined according to the method of Myers et al.[11] Experiments were considered valid only when the recovery of PAH was within the acceptable range (100% ± 40%).

The rates of glucose appearance (Ra) and disappearance (Rd) were calculated with [3-^{3}H]-glucose as tracer[18] after the data were smoothed by the optimal-segments method.[19] Endogenous glucose production (EGP) was calculated as the difference between the Ra and exogenous glucose infusion in periods I and III. Using only one tracer, EGP could not be assessed in period II, since during this period the difference between the total Ra and peripheral G_{inf} corresponds to the amount of glucose leaving the liver, which is the sum of EGP plus the peripheral appearance of portally infused glucose. Furthermore, since the portal glucose infusate was radiolabeled and therefore the amount of radioactivity reaching the peripheral circulation was unknown in period II, also the Rd (= total Ra) could not be reliably assessed during this period.

In 4 dogs, the arterial-venous (A-V) difference method was applied to calculate NHGB. In 2 other dogs, we could not use the A-V difference method because either the hepatic or portal venous catheter was not patent. The load of glucose reaching the liver (influx) was calculated using the formula, influx = ([A] × 0.25 + [P] × 0.75) × HPF, where [A] and [P] are the arterial and portal vein plasma concentration of glucose and HPF is ICG-determined hepatic plasma flow. In agreement with previous studies,[20] the average distribution of hepatic blood flow determined by a Doppler flow meter between the hepatic artery and portal vein was 25% and 75%, respectively. The amount of glucose leaving the liver (efflux) was calculated by the formula, efflux = [H] × HPF, where [H] is the hepatic vein plasma concentration of glucose. NHGB was determined by the formula, NHGB = influx − efflux, where positive balance indicates uptake.

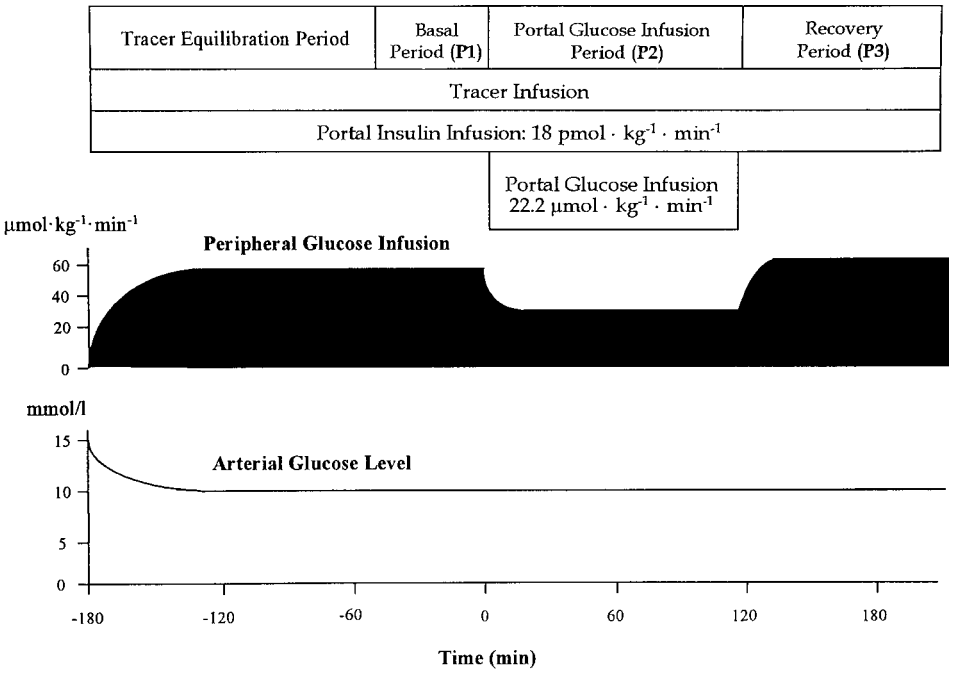

Fig 1. Experimental protocol. Three paired experiments were performed in 6 overnight-fasted and conscious alloxan-diabetic dogs.

Statistical Analysis

The data are expressed as the mean ± SEM and represent the average values for the period. Two-way ANOVA for repeated measurements followed by Tukey's *t* test was used for comparisons between and within experimental groups.

RESULTS

Arterial Glucose, Insulin, Glucagon, NEFA, Total Cholesterol, and Triglyceride and Arterial Blood Pressure in the Postabsorptive State

There were no significant differences in the mean fasting blood glucose and the requirement for intermediate-acting (NPH) and short-acting (Regular) insulin for 7 days before the experiments between JTT and C1 and C2 (Table 1). JTT-501 did not affect plasma insulin, glucagon, and NEFA in the postabsorptive state. There were significant differences in total cholesterol between JTT and C1 and C2 and in triglyceride between JTT and C1. Systolic, diastolic, and mean blood pressure were not affected by JTT-501.

Plasma Glucose, Insulin, Glucagon, and NEFA

The hyperinsulinemic-hyperglycemic clamp achieved stable and comparable arterial plasma glucose levels in both JTT (10.0 ± 0.1 mmol/L) and C1 and C2 (9.8 ± 0.1 and 9.8 ± 0.1 mmol/L). During period II, portal plasma glucose levels were approximately the same in each group (JTT, 10.4 ± 0.1 mmol/L; C1, 10.5 ± 0.1; C2, 10.4 ± 0.1), but the mean plasma glucose in the hepatic vein was 9.9 ± 0.1 mmol/L with JTT, which was less than the 10.2 ± 0.1 mmol/L in C1 and C2 ($P < .05$). Peripheral and portal insulin levels were stable and similar throughout the experiments in both JTT (peripheral, 417.6 ± 11.4 pmol/L; portal, 1,134.4 ± 87.7) and the controls (C1: peripheral, 422.4 ± 13.8 pmol/L; portal, 1,018.7 ± 101.0; C2: peripheral, 407.4 ± 13.8 pmol/L; portal, 1,135.5 ± 83.3). Peripheral glucagon levels were significantly higher in C1, but not C2, versus JTT (C1, 64.6 ± 2.5 pg/mL; JTT, 50.9 ± 2.6; $P < .01$; C2, 54.8 ± 2.3). Peripheral NEFA levels were lower in JTT (0.17 ± 0.01 mmol/L) versus C1 (0.35 ± 0.04, $P < .001$) and C2 (0.31 ± 0.03, $P < .001$).

Hepatic Blood Flow and Hepatic Glucose Load

The estimated hepatic blood flow determined by ICG was constant in each experiment and unaffected by JTT-501 treat-

Table 1. Mean Fasting Plasma Glucose and Daily Requirement of Regular and NPH Insulin During Seven Days Before the Experiments and Fasting Arterial Insulin, Glucagon, Total Cholesterol, Triglyceride, NEFA, and Arterial Pressure on the Day of Experiment in Alloxan-Diabetic Dogs With and Without JTT-501 Treatment

Parameter	C1	JTT-501	C2
Fasting plasma glucose (mmol/L)	11.6 ± 0.7	10.5 ± 0.6	11.3 ± 0.7
Regular insulin (U)	9.4 ± 0.5	9.3 ± 0.6	9.1 ± 0.6
NPH insulin (U)	19.5 ± 1.0	18.7 ± 1.3	18.7 ± 1.3
Insulin (pmol/L)	38.4 ± 6	36.0 ± 4.8	40.2 ± 5.4
Glucagon (pg/mL)	117 ± 25	104 ± 29	103 ± 11
NEFA (mmol/L)	1.85 ± 0.1	1.82 ± 0.2	1.84 ± 0.1
Total cholesterol (mmol/L)	5.90 ± 0.5*	4.62 ± 0.2	6.08 ± 0.4†
Triglyceride (mmol/L)	7.65 ± 0.9†	4.44 ± 0.4	5.52 ± 1.1
SBP (mm Hg)	127 ± 10.4	132 ± 6.9	127 ± 10.4
DBP (mm Hg)	100 ± 8.2	103 ± 5.4	100 ± 8.2
MBP (mm Hg)	109 ± 8.9	113 ± 5.8	109 ± 8.9

Data are expressed as the mean ± SEM; n = 6 in each group.

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean arterial blood pressure.

*$P < .05$ *v* JTT-501.

†$P < .01$ *v* JTT-501.

Table 2. Estimated Hepatic Blood Flow Determined by ICG, Hepatic Blood Flow Determined by Doppler Flow Probes, Hepatic Artery and Portal Vein Contribution to Hepatic Blood Flow, and Hepatic Glucose Load During Basal Period (period I), Portal Glucose Infusion Period (period II), and Recovery Period (period III) in Alloxan-Diabetic Dogs With and Without JTT-501 Treatment

Parameter	Period I	Period II	Period III
Estimated hepatic blood flow ($mL \cdot kg^{-1} \cdot min^{-1}$)			
C1	47.4 ± 2.8	44.8 ± 3.6	45.7 ± 2.3
JTT-501	47.9 ± 0.9	49.7 ± 1.0	46.0 ± 1.4
C2	44.7 ± 1.7	47.0 ± 1.3	48.8 ± 1.2
Hepatic blood flow ($mL \cdot kg^{-1} \cdot min^{-1}$)			
C1	45.6 ± 0.7	45.4 ± 0.8	47.7 ± 0.5
JTT-501	46.7 ± 0.9	46.5 ± 1.0	47.5 ± 1.6
C2	44.5 ± 0.7	44.3 ± 0.6	42.3 ± 0.7
Hepatic artery (%)			
C1	24.9 ± 1.1	25.2 ± 0.9	23.8 ± 0.7
JTT-501	26.2 ± 1.5	25.1 ± 1.6	25.1 ± 0.9
C2	25.3 ± 0.4	26.4 ± 0.4	26.7 ± 0.6
Portal vein (%)			
C1	75.1 ± 1.1	74.8 ± 0.9	76.2 ± 0.7
JTT-501	73.8 ± 1.6	74.9 ± 1.6	74.9 ± 0.9
C2	74.7 ± 0.4	73.6 ± 0.4	73.3 ± 0.6
Hepatic glucose load ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)			
C1	271.6 ± 24.3	304.8 ± 22.3	275.1 ± 11.6
JTT-501	302.4 ± 9.8	336.0 ± 12.0	300.5 ± 11.8
C2	300.1 ± 14.3	342.3 ± 8.8	309.0 ± 11.7

NOTE. Data are expressed as the mean ± SEM; n = 4 in each group.

ment (Table 2). Hepatic blood flow determined by Doppler probes was comparable to the estimated hepatic blood flow. The proportion of hepatic flow contributed by the hepatic artery and portal vein was constant throughout the experiment. There was no significant difference in the hepatic glucose load between the 3 groups in each period.

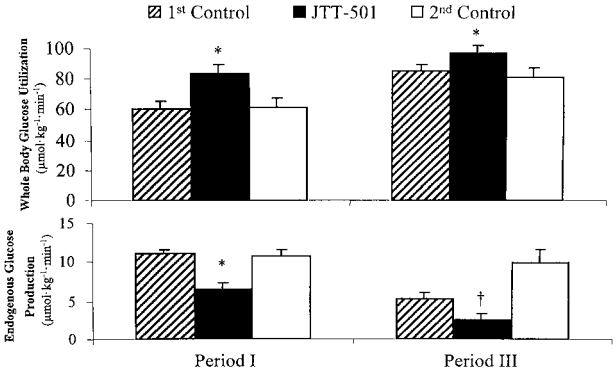

Fig 3. Whole-body glucose utilization and EGP during the basal (period I) and recovery (period III) periods in the 3 groups. Values are expressed as the mean ± SEM. *$P < .05$ *v* 1st and 2nd control groups; †$P < 0.5$ *v* 2nd control (n = 6 in each group).

Peripheral Glucose Infusion Rate, Glucose Utilization, and EGP

The mean peripheral glucose infusion rate (G_{inf}) was higher ($P < .001$) in JTT versus both controls in all periods (Fig 2). The G_{inf} was higher in period III versus period I in all groups ($P < .001$). As already described, neither glucose utilization (Rd) or EGP could be reliably assessed in period II. During periods I and III, the Rd was higher in JTT-501 versus both controls (Fig 3). During period III, the Rd was higher versus period I in all groups ($P < .001$). EGP was not fully suppressed in any protocol during any period. It was lower in period III versus period I in C1 and JTT ($P < .001$). In period I, EGP in JTT was significantly lower versus C1 and C2; in period III, EGP was significantly lower versus C2 (Fig 3). EGP was not correlated with NEFA or glucagon.

NHGB Determined by A-V Difference Method and Hepatic Glucose Uptake

NHGB determined by the A-V difference method was positive in all periods, indicating net uptake. NHGB was greater

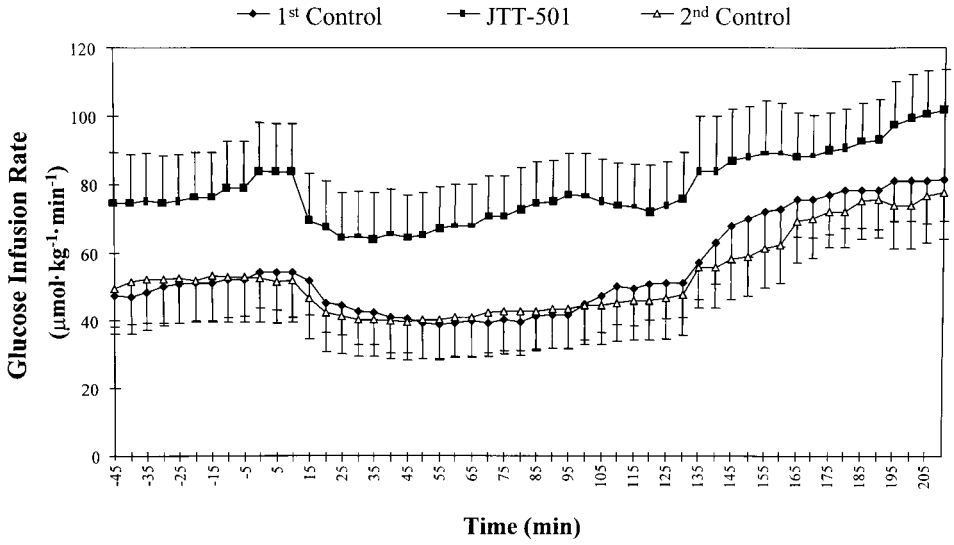

Fig 2. Glucose infusion rate during the experimental protocol. Values are expressed as the mean ± SEM. The rates were higher ($P < .001$) after 10 days of treatment with JTT-501 (30 $mg \cdot kg^{-1} \cdot d^{-1}$) than in the control experiments performed before treatment and 2 weeks after discontinuation of treatment (n = 6 in each group).

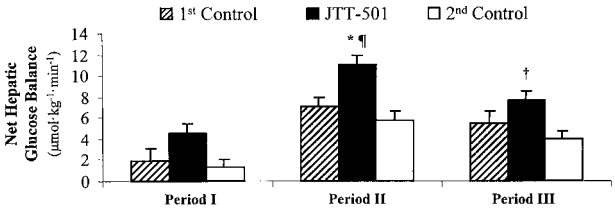

Fig 4. NHGB during the basal (period I), portal glucose infusion (period II), and recovery periods (period III). Values are expressed as the mean ± SEM. *$P < .01$ *v* 1st control; ¶$P < .001$ *v* 2nd control; †$P < 0.5$ *v* 2nd control (n = 4 in each group).

in JTT versus C1 ($P < .01$) and C2 ($P < .001$) in period II. NHGB tended to be greater in JTT versus C1 and C2 also in periods I and III; in period III, NHGB was significantly greater in JTT versus C2 (Fig 4). In periods I and III, total hepatic glucose uptake (sum of EGP and NHGB) was similar between treatments.

DISCUSSION

In the present study, JTT-501 increased NHGB (uptake) and peripheral glucose uptake during a hyperinsulinemic-hyperglycemic clamp in alloxan-induced diabetic dogs. During the experiments, arterial insulin was approximately 400 pmol/L and portal insulin was approximately 1,200 pmol/L and there were no significant differences between the JTT group and the 2 controls. The insulin concentrations achieved were not far from those measured under physiological postprandial conditions. Hence, these results show that JTT-501 indeed ameliorates impaired peripheral glucose uptake and NHGB in alloxan-induced diabetic dogs, at least under postprandial-like conditions.

In this study, JTT-501 increased NHGB (uptake) and suppressed hepatic glucose production, which suggests that this compound may increase hepatic insulin sensitivity. JTT-501 also decreased both NEFA and glucagon levels, which may have indirectly contributed to the improvement in hepatic glucose metabolism induced by JTT-501. Although there was no correlation between EGP and NEFA or glucagon levels, this may be due to the low range of variability for these parameters in the present study.

In periods I and III, net hepatic glucose uptake occurred in JTT, C1, and C2, contrary to our previous study using the same experimental protocol during euglycemia,[9] where NHGB was close to zero in all groups. The difference between the 2 studies is presumably due to the different glucose levels, since the portal insulin levels were similar. JTT-501 tended to improve NHGB in all periods. In periods I and III, this was entirely accounted for by decreased glucose production, as JTT-501 did not enhance total hepatic glucose uptake (sum of EGP and NHGB). JTT-501 increased NHGB in period II, and we cannot exclude the possibility that in this period an enhancement of total hepatic glucose uptake was induced. We tried to use an indirect method to derive total hepatic glucose uptake in period II, as in our previous study using the same experimental protocol during euglycemia[9]; however, in the present study, the indirectly derived values for total hepatic glucose uptake were markedly lower than the NHGB, which indicates that these values were likely underestimated. We believe that the indirect method is not reliable under conditions of hyperglycemia as in the present study where hepatic glucose uptake in periods I and III is substantial.

Regardless of the effect of JTT-501 on total hepatic glucose uptake, the improvement in NHGB during portal glucose delivery (period II) appears to be the most important effect of JTT-501 from a physiological point of view, since NHGB is the amount of glucose that is metabolized by the liver and can be stored as glycogen during portal glucose absorption.

JTT-501 markedly improved peripheral glucose uptake in the present study. However, despite the fact that JTT-501 showed peripheral and liver effects in these experiments and decreased plasma triglyceride and cholesterol levels, it did not induce significant improvements in fasting blood glucose or the daily insulin requirement. One of the possible reasons for the lack of effect of JTT-501 on these parameters is that 10 days of treatment may be an insufficient period to produce an effect on the insulin requirement and fasting glucose level. Specifically, the improvements in peripheral and hepatic glucose metabolism were observed at high physiological insulin levels under controlled conditions simulating the postprandial condition. It is possible that JTT-501 improved postprandial glucose control in our dogs. However, postprandial glucose levels were not determined, because they are highly variable in dogs due to unpredictable eating behavior, eg, the timing of meal consumption.

Our results in alloxan-diabetic dogs are consistent with the preliminary toxicity studies performed by the JTT-501 manufacturer in normal dogs. The latter studies showed that 2 weeks of treatment with JTT-501 dose-dependently decreased plasma triglyceride and cholesterol but had no effect on fasting plasma glucose in normal dogs. Four weeks of treatment with JTT-501 induced a further decline in plasma triglyceride and cholesterol and decreased fasting glucose levels in some dogs, although the decrease in glucose was quite variable. As explained in the Methods, the duration of the treatment period of our alloxan-diabetic dogs had to be the minimum compatible with an effect of JTT-501 on insulin sensitivity (approximately 2 weeks based on lipid levels in normal dogs) and the maximum allowed by the low feasibility of the experimental protocol, because of a failure in sampling of the portal and hepatic vein lines over time. The duration of the washout period appeared adequate, as the results in C1 were very similar to those in C2.

Another possible reason for the negative results on fasting blood glucose or insulin requirements with short-term JTT-501 treatment may be the experimental model, as short-term treatment with JTT-501 reduced glucose and insulin levels in rodent models of hyperinsulinemia-associated insulin resistance. We used a dog model because the triple-catheter technique for direct measurement of hepatic glucose uptake has been established only in the dog. Alloxan-induced diabetic dogs represent a model of partial insulin deficiency and insulin resistance,[9,12] presumably secondary to suboptimal control. Unfortunately, there is no good model of hyperinsulinemia-associated insulin resistance in the dog.

As mentioned before, we have previously used a similar experimental protocol (portal glucose infusion during a euglyce-

mic clamp with high-dose peripheral insulin infusion) to investigate the effects of pioglitazone, a thiazolidinedione, on NHGB and peripheral glucose uptake. In that study in the same animal model, the effects of pioglitazone on both glucose production and NHGB in periods I and III were not significant; however, in period II (portal glucose infusion) pioglitazone increased NHGB and total hepatic glucose uptake calculated using an indirect method. Therefore, despite some dissimilar results that may be explained by the different glucose levels, both JTT-501 and pioglitazone improved hepatic glucose metabolism at high insulin levels. However, neither JTT-501 nor pioglitazone led to a decrease in fasting plasma glucose or the insulin requirements.

In contrast to the present study with JTT-501, pioglitazone did not increase peripheral glucose uptake in our previous study. The differential effects of JTT-501 and pioglitazone on peripheral glucose uptake in the 2 studies may be related to the experimental protocol, since glucose clearance was markedly lower in the present study than with pioglitazone, in accordance with the lower peripheral insulin levels, whereas in the previous study, glucose clearance was already high and might not have been further stimulated by pioglitazone. However, we cannot exclude that JTT-501 is more potent than pioglitazone in peripheral tissues. The different structure of JTT-501, which is an isoxazolidinedione derivative, might account for a certain degree of disparity in its action compared with thiazolidinediones. The precise mechanism of the action of JTT-501 is not fully understood. However, it is known that JTT-501, similar to thiazolidinediones, binds to the nuclear receptor PPAR γ.[7]

In conclusion, JTT-501 treatment for 10 days in alloxan-induced diabetic dogs in the presence of insulin significantly reduces glucose production and enhances peripheral glucose utilization and NHGB. JTT-501 appears to be an effective insulin sensitizer and may have therapeutic significance in the treatment of type 2 diabetes.

ACKNOWLEDGMENT

The authors thank Debra Bilinski, Mayliza Vandelangeryt, and Loretta Lam for excellent technical assistance, and Drs Munehide Matsuhisa and Z. Qing Shi for advice on implementation of the surgical and experimental procedures.

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