Diabetologia (1999) 42: 102–106

Diabetologia

Effect of masoprocol on carbohydrate and lipid metabolism in a rat model of Type II diabetes

M.J. Reed, K. Meszaros, L.J. Entes, M.D. Claypool, J.G. Pinkett, D. Brignetti, J. Luo, A. Khandwala, G.M. Reaven

Shaman Pharmaceuticals, Inc., South San Francisco, California USA

Summary Extracts of the creosote bush (*Larrea tridentata*, family Zygophyllaceae) have long been used as a folk remedy for Type II (non-insulin-dependent) diabetes by native Americans in southwestern North America. In this study we have evaluated the metabolic effects of masoprocol, a pure compound isolated from the creosote bush, in a rat model of Type II diabetes. Animals were fed a 20% fat (by weight) diet for 2 weeks prior to intravenous injection with streptozotocin (STZ, 0.19 mmol/kg). Diabetic animals (glucose 16–33 mmol/l) were treated with vehicle, metformin (0.83 mmol/kg body weight) or masoprocol (0.83 mmol/kg body weight) twice a day for 4 days. Masoprocol treatment lowered glucose concentrations an average of 35% compared with vehicle (14.2 ± 1.1 vs 21.7 ± 1.0 mmol/l, $p < 0.001$), a reduction similar to metformin treatment (12.8 ± 0.9 mmol/l), without any change in insulin concentration. Masoprocol treatment also lowered triglyceride concentrations 80% compared with vehicle (1.0 ± 0.1 vs 4.8 ± 0.3 mmol/l, $p < 0.001$), a reduction far greater than following metformin treatment (3.6 ± 0.3 mmol/l). Non-esterified fatty acid and glycerol concentration were decreased by approximately 65% by masoprocol compared with vehicle, a reduction approximately twice as great as seen with metformin ($p < 0.001$). The effect of masoprocol on in vivo insulin-mediated glucose disposal was evaluated by infusing fat-fed/STZ rats with glucose ($0.22\ \text{mmol} \cdot \text{kg} \cdot \text{min}^{-1}$) and insulin ($30\ \text{pmol} \cdot \text{kg} \cdot \text{min}^{-1}$) for 5 h. In response to the infusion, steady-state plasma glucose concentrations were reduced 30% in masoprocol-treated animals compared with vehicle controls ($p < 0.05$) with no change noted in rats treated with metformin. The effect of masoprocol treatment was also tested in primary adipocytes isolated from normal animals. Adipocytes treated with masoprocol (30 μmol/l) had higher basal and insulin-stimulated glucose clearance than did adipocytes treated with vehicle ($p < 0.05$). These data show that masoprocol decreases both plasma glucose and triglyceride concentrations in fat-fed/STZ rats, presumably as a result of its ability to both increase glucose disposal and decrease lipolysis. [Diabetologia (1999) 42: 102–106]

Keywords Masoprocol, Type II diabetes, glucose, insulin, triglyceride.

Shaman Pharmaceuticals, Inc. has initiated an ethnomedically-based research programme [1] aimed at identifying new compounds for the treatment of Type II (non-insulin-dependent) diabetes. In this context, attention was directed towards evaluation of the creosote bush [*Larrea tridentata* (DC.) Felger and Lowe, in the plant family Zygophyllaceae]. Oral decoctions of the plant have been used to treat Type II diabetes by the Pima Indians in southwestern North America [2] and by the Huichol Indians in Jalisco, Mexico (Silviano Cambreros Sanchez, personal communication). Typically, a hot water extract of dried leaves and young stems (30 g plant material

Received: 3 April 1998 and in final revised form: 21 August 1998

Corresponding author: M.J. Reed, Shaman Pharmaceuticals, Inc. 213 East Grand Avenue, South San Francisco, CA, 94080–4812, USA

Abbreviations: STZ, Streptozotocin; BSA, bovine serum albumin; TG, triglyceride; NEFA, non-esterified fatty acids.

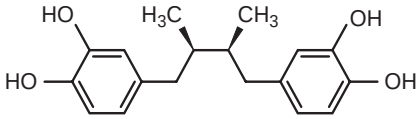

Fig. 1. Chemical structure of masoprocol (nordihydroguaiaretic acid)

in 750 ml of water) is taken orally (250 ml three times daily; Sanchez, personal communication). As a result of our interest in *Larrea tridentata*, we have recently demonstrated [3] that masoprocol (nordihydroguaiaretic acid), a pure compound isolated from *Larrea tridentata*, can lower plasma glucose concentrations in obese, diabetic (*db*/*db* and *ob*/*ob*) mice. Although masoprocol (Fig. 1) is recognized as a lipoxygenase inhibitor [4], its ability to lower plasma glucose had not been recognized prior to our recent publication [3]. The current study was initiated to see if masoprocol would also lower plasma glucose concentrations in another rodent model that closely simulates the metabolic characteristics of Type II diabetes, the fat-fed/streptozotocin (STZ) treated rat, as well as attempting to gain further insight into the physiological effects of masoprocol in this model.

Materials and methods

Male Sprague-Dawley rats (Charles River Laboratories, Hollister, Calif., USA), 6 weeks of age, and weighing approximately 140 g, were used for all studies. They were housed four per cage in a room with a 12/12-h light/dark cycle and an ambient temperature of 22–25 °C. Animals were fed a high fat diet consisting of 20% fat, 46% carbohydrate and 20% protein (w/w) (Harlan Teklad, Madison, Wis., USA). After 2 weeks on the high fat diet, animals were anaesthetized with ketamine (65 mg/kg) and xylazine (7 mg/kg), and injected with streptozotocin (STZ, 0.19 mmol/kg) into the tail vein via a temporary indwelling 24 gauge catheter. Animals had free access to food and water after the STZ injection. Experiments were begun 3 days after STZ injection. Animals were assigned to their respective treatment groups on the basis of pre-dose plasma glucose concentration. On experimental days 1–4, food was removed from the feeders at 0700 hours, animals were dosed orally twice daily with vehicle, masoprocol or metformin at 1100 hours and 1600 hours. Food was then returned to the animals at 1700 hours. Although masoprocol was originally isolated from *Larrea tridentata* [3] (botanical voucher number KR 1 determined by Amy Pool of the Missouri Botanical Garden, St. Louis, Mo., USA and housed at the herbarium of the Missouri Botanical Garden) a commercial synthetic source (Western Engineering and Research Corporation, El Paso, Tex., USA) was used for these experiments. Masoprocol (MW 302) and metformin (Sigma Chemical Co., St. Louis, Mo., USA) were formulated in 0.25% carboxymethylcellulose (CMC) and dosed at 0.83 mmol/kg by oral gavage. This dose was selected because it represented the amount of metformin (our positive control) required to consistently lower glucose concentrations in our experimental model. Consequently, the amount of masoprocol given in this study is approximately 60-fold higher than that in the decoctions of the creosote bush usually recommended to treat the symptoms of diabetes [2].

Following dosing of vehicle, metformin or masoprocol, blood was sampled daily by tail milking at 1400 hours (3 h post-dose, previously determined as optimal [3]) and serum was separated by centrifugation in serum separator tubes (Becton Dickinson, Franklin Lakes, N.J., USA) and analysed for glucose (Trinder method, Sigma Diagnostics, Sigma Chemical Co., St., Louis, Mo., USA), insulin (RIA, Linco Research, St. Charles, Mo., USA), glycerol and triglyceride (GPO-Trinder method, Sigma) and non-esterified fatty acid (ACS-ACOD method, Wako Diagnostics, Richmond Va., USA) concentrations.

On the fifth day of dosing, insulin-mediated glucose disposal was estimated. Food was removed at 0700 hours and animals were given a dose of vehicle, metformin or masoprocol at 0930 hours. At 1000 hours, animals were anaesthetized with sodium pentobarbital (65 mg/kg i.p.) and a microenathane cannula (0.064 cm internal diameter, Braintree Scientific, Braintree Mass., USA) was placed in the jugular vein. Animals were infused with epinephrine ($0.08\ mg \cdot kg \cdot min^{-1}$) and propranolol ($1.7\ mg \cdot kg \cdot min^{-1}$) for 1 h and then were additionally infused with glucose ($0.22\ mmol \cdot kg \cdot min^{-1}$) and insulin ($30\ pmol \cdot kg \cdot min^{-1}$) for 4 h. Sodium pentobarbital was reinjected i.p. as necessary to maintain anaesthesia. Blood samples were taken every 30 min during the final hour of the infusion. Under these conditions, our pilot work showed that glucose and insulin concentrations were in a steady state during the last hour of the infusion. Using these conditions, the steady state plasma glucose concentration achieved by each animal was a measure of its whole body insulin-mediated glucose disposal [5].

Basal and insulin-stimulated glucose clearance by isolated adipocytes was determined by methods described previously [6–8]. Epididymal fat pads from normal rats were removed, minced with scissors, and placed in plastic flasks in Krebs bicarbonate buffer with 2% bovine serum albumin (BSA), 3 mmol/l glucose, and 1 mg collagenase/ml. Collagenase digestion was carried out at 37 °C in a gyratory shaker for 1 h. Cells were washed three times in fresh Krebs buffer with 2% BSA and allowed to separate from the infranatant by flotation. Isolated adipocytes (2% lipocrit) were incubated for 90 min in 200 μl Krebs buffer with 2% BSA, metformin or masoprocol (30 μmol/l), and tracer (300 nmol/l) amounts of D-[U-14C]-glucose in the absence and presence of insulin (8 nmol/l). The cell suspension was incubated in a water bath at 37 °C for 1 h with continuous shaking at 40 rpm, followed by addition of 200 μl of cell suspension to a microcentrifuge tube containing 200 μl of silicone oil. The assay was terminated by centrifugation of the microcentrifuge tube. The supernatant layer of the tube, containing the fat cells and incorporated glucose, was placed in a scintillation vial, and the amount of radioactivity associated with the adipocytes (and the total radioactivity in the incubation medium) was determined by liquid scintillation counting.

Isolated adipocytes were fixed with 2% osmium tetroxide, washed and diluted in saline. Cells in a 100 μl aliquot were counted using a Coulter electronic cell counter (model ZB; Coulter Electronics. Inc., Hialeah, Fla., USA) with a 400 μm aperture equipped with logarithmic range-expanded channelyser. No clumping of adipocytes or partial digestion of the fat tissue was observed by viewing the fixed adipocytes under a microscope [9].

Results are presented as means ± SEM. One way analysis of variance with Fisher's protected least significant difference post-hoc test was used to compare treatments.

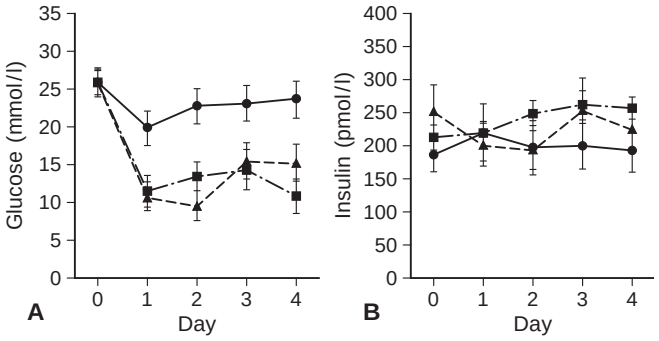

Fig. 2. Serum glucose (**A**) and insulin (**B**) concentrations in rats receiving vehicle (●), metformin (■), or masoprocol (▲). There were 8 animals in each group. Serum glucose levels were lower ($p < 0.001$) in rats treated with either metformin or masoprocol compared with vehicle

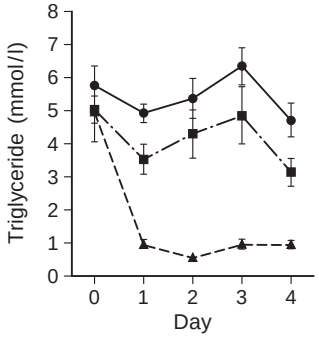

Fig. 3. Serum triglyceride (TG) concentrations in rats receiving vehicle (●), metformin (■), or masoprocol (▲). There were 8 animals in each group. Serum TG concentrations were lower ($p < 0.001$) in masoprocol-treated rats compared with the other two groups

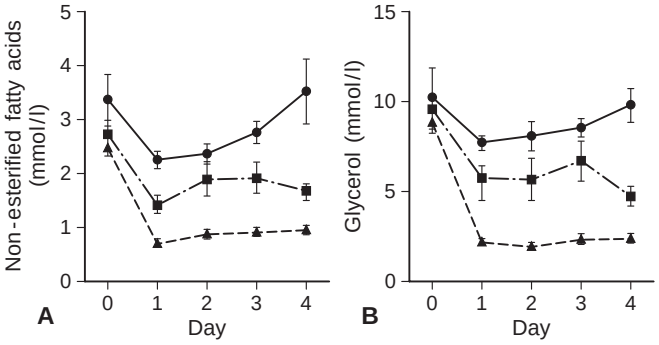

Fig. 4 A, B. Serum non-esterified fatty acid (NEFA) (**A**) and glycerol (**B**) concentrations in rats receiving vehicle (●), metformin (■), or masoprocol (▲). There were 8 animals in each group. NEFA and glycerol concentrations were lower ($p < 0.001$) in masoprocol-treated rats compared with the other two groups

Results

Serum glucose concentrations were lower in rats after equimolar doses of either masoprocol or metformin, compared with vehicle-treated rats, with an approximate 35% decrease seen throughout the treatment period ($p < 0.001$) and the fall in serum glucose concentration was seen without any increase in serum insulin concentration (Fig. 2).

Treatment with masoprocol led to a dramatic fall in serum triglyceride (TG) concentration and the effect persisted throughout the study (Fig. 3). Since there was little, if any, effect of metformin treatment on serum TG concentration, the masoprocol-treated rats had lower values than in either the vehicle or metformin-treated groups ($p < 0.001$).

Masoprocol-treated rats had serum concentrations of non-esterified fatty acids (NEFA) and glycerol that fell to approximately one-third of the value seen in rats receiving vehicle (Fig. 4). In contrast, serum NEFA and glycerol concentrations were only marginally lower in metformin-treated rats. Thus, serum NEFA and glycerol concentrations were lower ($p < 0.001$) in rats receiving masoprocol compared with the other two groups.

Although food consumption was somewhat lower following initial dosing with metformin or masoprocol, it increased in both groups as the study continued and was essentially identical during the second half of the study (Fig. 5). Body weights in the two groups were similar throughout the study, and were slightly higher than in vehicle-treated rats.

Despite similar steady state plasma insulin concentrations, the steady state plasma glucose concentrations were 30% lower in the masoprocol-treated animals compared with the other two groups ($p < 0.05$) (Fig. 6).

Masoprocol increased adipocyte glucose clearance in the basal state (no insulin) and across a wide range of insulin concentrations (Fig. 7). This difference was statistically significant ($p < 0.05$).

Discussion

In this study we have extended our earlier observation [3] that treatment with masoprocol lowered glucose concentrations in *db*/*db* and *ob*/*ob* mice to a non-genetic rodent model of Type II diabetes, the fat-fed/STZ rat. In addition, we have shown in this animal model that the potency of masoprocol was similar to that of metformin in terms of lowering serum glucose, with the added benefit of considerably reducing serum TG concentrations. Since masoprocol has been shown previously to inhibit glucose-stimulated insulin secretion in vitro [10], its ability to lower plasma glucose concentrations in fat-fed/STZ rats was surprising. On the other hand, there is no a priori reason to assume that the in vivo effect of masoprocol in fat-fed/STZ rats will mirror its in vitro effect on isolated pancreatic islets. In any event, as the results showed that masoprocol was as potent as metformin in lowering glucose concentrations in fat-

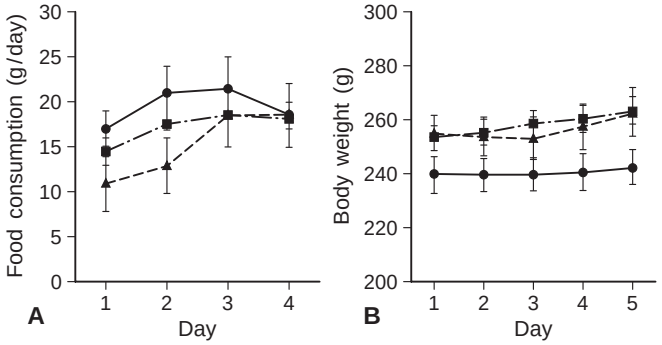

Fig. 5 A, B. Food consumption (**A**) and body weight (**B**) in rats receiving vehicle (●), metformin (■), or masoprocol (▲). There were eight animals in each group

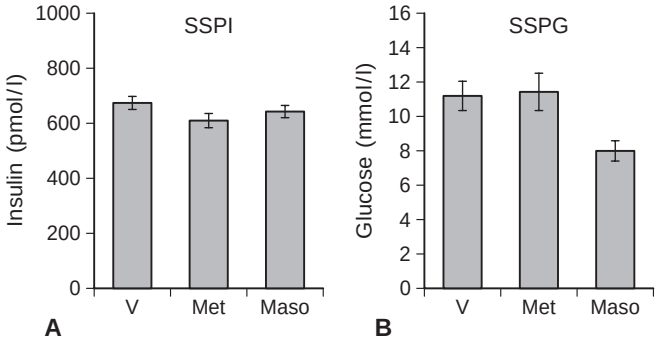

Fig. 6 A, B. Steady-state plasma insulin (SSPI) (**A**) and steady-state plasma glucose (SSPG) (**B**) concentrations in rats receiving vehicle (V), metformin (Met), or masoprocol (Maso). There were 8 animals in each group. SSPG was lower ($p < 0.05$) in masoprocol-treated rats compared with the other two groups

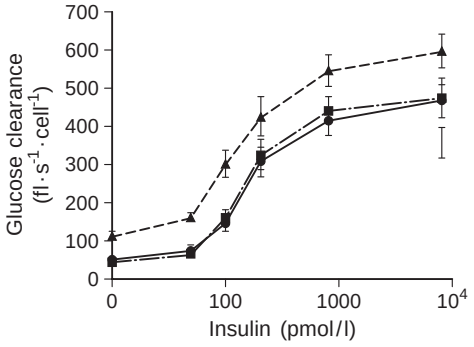

Fig. 7. Glucose clearance rates by isolated rat adipocytes after incubation with vehicle (●), metformin (■), or masoprocol (▲). Results of three incubation experiments. Glucose clearance was greater ($p < 0.05$) after addition of masoprocol compared with the other two groups

fed/STZ rats, and was also capable of lowering TG concentrations, this suggests that masoprocol is a potentially useful compound for the treatment of Type II diabetes.

Normal rats on a high fat diet become insulin resistant but are able to maintain normoglycaemia by virtue of an increase in insulin secretion [11]. As such, fat-fed rats resemble the pre-diabetic stage of Type II diabetes [12, 13]. It is apparent from prospective studies that Type II diabetes develops when insulin resistant subjects are no longer able to maintain the magnitude of compensatory hyperinsulinaemia necessary to maintain glucose homeostasis [12, 13]. We have attempted to simulate the natural history of Type II diabetes by injecting fat-fed, insulin resistant rats with just enough STZ to decrease their insulin response to the point that hyperglycaemia develops, but absolute hypoinsulinaemia does not. The data in Figure 2 indicate that we were able to accomplish this goal; specifically, significant hyperglycaemia was present in fat-fed, STZ-injected rats, with mean serum insulin concentrations about 250 pmol/l; values equal in absolute terms to those seen in normal rats. Based upon these considerations, we believe that the rat model of Type II diabetes used in these studies mimics both the natural history and the pathophysiologic characteristics of patients with this syndrome [14].

The metabolic abnormalities in patients with Type II diabetes include a decrease in glucose uptake and an increase in lipolysis [14]. The physiological consequences of these cellular defects are an increase in ambient glucose and NEFA concentrations. It is apparent from the results presented that our rat model of Type II diabetes displayed both of these changes, and that treatment with masoprocol lowered both serum glucose and NEFA concentrations. Serum insulin concentrations were not higher in masoprocol-treated animals, whereas in vivo insulin-mediated glucose disposal was enhanced. Thus, masoprocol does not appear to be acting as an insulin secretagogue and its ability to lower serum glucose concentration is perhaps, at least partly, due to stimulation of peripheral tissue glucose uptake. This possibility receives additional support from the in vitro data showing that masoprocol enhanced glucose uptake by isolated adipocytes. Note that this effect of masoprocol was seen in both the absence and in the presence of physiological concentrations of insulin. As such, these data suggest that masoprocol could be acting as an insulin mimetic, rather than as an insulin sensitizer, as regards its ability to stimulate glucose disposal. On the other hand, as described below, the ability of masoprocol to enhance glucose disposal by isolated adipocytes does not necessarily mean that this action is responsible for its anti-hyperglycaemic effect.

The observation that plasma NEFA and glycerol concentrations were lower in masoprocol-treated rats is consistent with the view that it also decreases the accelerated adipose tissue lipolysis associated with Type II diabetes [14]. This finding is of relevance in that suppression of adipose tissue lipolysis and reduction in serum NEFA concentrations provides an alternative mechanism to account for the glucose lowering action of masoprocol. More specifically, in-

creases in NEFA concentrations in vivo are associated with inhibition of muscle glucose disposal, the so-called "glucose-fatty acid cycle" [15] and it is reasonable to assume that the masoprocol-induced decrease in NEFA concentrations would increase muscle glucose disposal, thus contributing to its anti-hyperglycaemic effect. Direct support for the view that masoprocol could be exerting its antihyperglycaemic effect via adipose tissue comes from preliminary data showing inhibition by the compound of isoproterenol-induced lipolysis in isolated adipocytes [16].

Although it is likely that the fall in NEFA concentration in masoprocol-treated rats could have enhanced its glucose-lowering effect, this change undoubtedly played a more important part in the associated fall in serum TG concentrations observed in fat-fed/STZ rats. This conclusion is based upon abundant evidence from both animal and human studies that hepatic TG secretion and hypertriglyceridaemia in Type II diabetes is a function of the NEFA flux to the liver [14, 17–19]. Given the fact that hypertriglyceridaemia is the lipoprotein abnormality characteristic of Type II diabetes [20] and the importance of raised TG concentrations as a risk factor for coronary heart disease in this population [21, 22], the dramatic effect of masoprocol on serum TG concentrations in fat-fed/STZ rats is of potentially enormous clinical relevance.

In conclusion, treating fat-fed/STZ rats orally with masoprocol was as potent in lowering serum glucose concentration as metformin, and had the added value of considerably lowering serum TG concentrations. Masoprocol appears to both stimulate glucose disposal and decrease lipolysis and these actions provide an explanation for the effect of this compound on serum glucose and TG concentrations. Although masoprocol is a well-known lipoxygenase inhibitor, its beneficial metabolic effects have not been described previously. As such, the results of these experiments have possibly identified a new class of chemical compounds of use in the treatment of Type II diabetes.

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