Masoprocol Decreases Serum Triglyceride Concentrations in Rats With Fructose-Induced Hypertriglyceridemia

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Historically, extracts of the creosote bush have been used by native healers of the Southwest region of North America to treat symptoms of type 2 diabetes. More recently, we have shown that masoprocol (nordihydroguaiaretic acid), a pure compound isolated from the creosote bush (*Larrea tridentata*), decreases serum glucose and triglyceride (TG) levels when administered orally in rodent models of type 2 diabetes. The present studies were undertaken to determine if masoprocol also decreases TG concentrations in rats with fructose-induced hypertriglyceridemia (HTG), a nondiabetic model of HTG associated with insulin resistance and hyperinsulinemia. Serum TG levels, which were significantly higher after rats ate a fructose-enriched (60% by weight) diet for 14 days as compared with chow-fed controls (411 *v* 155 mg/dL, $P < .01$), decreased in a stepwise fashion in fructose-fed rats treated orally with masoprocol for 4 to 8 days over a dose range of 10 to 80 mg/kg twice daily. Using the nonionic detergent Triton WR 1339 to compare TG secretion rates in masoprocol- and vehicle-treated rats, masoprocol at a dose of 40 or 80 mg/kg twice daily, significantly reduced hepatic TG secretion ($P < .01$) and liver TG content ($P < .001$), whereas lower doses of masoprocol decreased serum TG without an apparent reduction in hepatic TG secretion. Administration of Intralipid (a fat emulsion) showed that the half-time for removal of TG from serum was also shorter in masoprocol-treated rats versus vehicle-treated controls (31 *v* 64 minutes, $P < .05$). In addition adipose tissue lipoprotein lipase (LPL) activity was increased in masoprocol-treated rats and adipose tissue hormone-sensitive lipase (HSL) activity was decreased. We conclude that masoprocol administration to rats with fructose-induced HTG results in lower serum TG levels associated with reduced hepatic TG secretion and increased peripheral TG clearance.

MASOPROCOL (nordihydroguaiaretic acid) is a pure compound isolated from the creosote bush (*Larrea tridentata*), which has been recently shown to decrease serum glucose and triglyceride (TG) concentrations in rodent models of type 2 diabetes.[1,2] The rodent models used in these studies are insulin-resistant and hyperinsulinemic, features characteristic[3-5] of rats with fructose-induced hypertriglyceridemia (HTG). Based on these similarities, we initiated the present study to determine if masoprocol also decreases plasma TG concentrations in a nondiabetic rat model with dietary-induced HTG.

MATERIALS AND METHODS

Six-week-old male Sprague-Dawley rats from Charles River Laboratories (Hollister, CA) were used for all experiments. The rats weighed 125 to 175 g upon arrival and were housed 3 to 4 per cage in a light- and temperature-controlled animal facility with ad libitum tap water and either standard chow or a diet containing 60% (by weight) fructose (TD 78463) from Harlan Teklad (Madison, WI). After approximately 2 weeks on the fructose-enriched diet, rats were screened for HTG (TG > 250 mg/dL) by obtaining blood from the tail vein 2 to 5 hours after food was removed. Based on the results, the animals were divided into groups with comparable plasma TG concentrations and used to assess the effects of masoprocol administration in the subsequent experimental protocol.

Following the creation of 2 or more experimental groups with similar plasma TG concentrations, the rats were treated with vehicle or masoprocol at a dose of 10 to 80 mg/kg twice daily for 8 days by oral gavage at a vol of 2.5 mL/kg body weight. Masoprocol was obtained from Sigma (St Louis, MO) or from Western Engineering and Research (El Paso, TX) and was formulated in Gelucire 44/14 (Gattefosse, Westwood, NJ). Gelucire 44/14 is a waxy solid at room temperature, and it therefore required heating to a liquid state before the addition of masoprocol. The mixture was vortexed and then sonicated in a bath sonicator at 50° to 55°C until the masoprocol dissolved completely. The control solution and the one containing masoprocol were maintained in a warm water bath while gavaging the animals, to keep the solution in a liquid state.

After several days (4 to 8) of treatment with masoprocol or vehicle, blood was collected by tail snip once per day 3 hours after the first dose of vehicle or masoprocol, and the serum was assayed for TG, glucose, insulin, and free fatty acid (FFA). Blood samples were collected in Microtainer (Becton Dickinson, Franklin Lakes, NJ) serum separator tubes and centrifuged at 12,000 rpm for 10 minutes. The serum was removed and TG and glucose concentrations were measured immediately by enzymatic colorimetric methods[6,7] using Sigma diagnostic kits. Insulin concentrations were measured in previously frozen and thawed serum samples by radioimmunoassay using Linco Rat Insulin Radioimmunoassay kits (St. Charles, MO). FFA concentrations were measured by an enzymatic colorimetric method using Wako NEFA C test kits (Richmond, VA).

Triton WR 1339, a nonionic detergent that prevents TG removal, was obtained from Sigma to measure the hepatic secretion rate of TG[8,9] 3 hours after the final dose of vehicle or masoprocol. Triton WR 1339 (600 to 800 mg/kg) was diluted with saline to a concentration of 300 mg/mL, vortexed, and sonicated with heat (60° to 70°C) until the solution was homogeneous. Blood samples were collected from conscious animals before and at 60 and 120 minutes after injection of Triton WR 1339 (2.0 mL/kg) into a catheterized tail vein. The accumulation of TG in the serum following injection with Triton WR 1339 was used as a measure of the hepatic secretion rate (milligrams per deciliter per hour).

The TG removal rate was estimated, using Intralipid (Vitrum, Stockholm, Sweden) to quantify the half-time ($t_{1/2}$) for TG removal,[10,11] 3 hours after the final dose of vehicle or masoprocol. Blood samples were collected from conscious animals before and at 1, 10, 30, 60, and 90 minutes after injection of Intralipid (2.0 mL/kg or 400 mg/kg lipid) into a catheterized tail vein. The WinNonlin pharmacokinetic modeling program (Scientific Consulting, Cary, NC) was used to determine the $t_{1/2}$ of TG removal using a single-compartment, 1st-order model of elimination.

Lipoprotein lipase (LPL) activity was measured in tissue extracts of

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Submitted August 18, 1998; accepted March 15, 2000.

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0026-0495/00/4909-0006$10.00/0

doi:10.1053/meta.2000.8604

soleus and epididymal fat tissue collected from rats treated with vehicle or masoprocol (80 mg/kg twice daily) for 5 days. The animals were anesthetized with pentobarbital 3 hours after the morning dose of vehicle or masoprocol. Soleus and adipose tissue samples were collected, rapidly frozen in liquid nitrogen, and stored at −80°C until assay for LPL activity. The assay method described previously by Nilsson-Ehle and Schotz[12] was used. Briefly, a stable emulsion of ^{14}C-triolein (in toluene) was obtained by sonication in glycerol to produce the concentrated substrate. Extracts of soleus (60 to 80 mg) and epididymal fat (100 to 150 mg) tissue were obtained by homogenizing tissue samples in a solution of Tris hydrochloride containing 1% bovine serum albumin (BSA), heparin (10 U/mL), 0.5% deoxycholate, and 0.02% Nonidet P-40. The tissue homogenates were centrifuged (12,000 rpm for 30 minutes) and the supernatant was removed and incubated for 1 hour in concentrated substrate diluted 1:10 with Tris hydrochloride, dH_2O, and 15% BSA. Incubations were performed in the presence and absence of serum from fasted rats, and specific LPL activity was determined as the difference in activity between these 2 conditions.

The TG content of the liver was measured in animals treated with vehicle or masoprocol (80 mg/kg twice daily) for 4 days. Measurement methods for the lipids were described by Brown et al.[13] Briefly, tissues were homogenized in homogenization buffer (0.3 mol/L sucrose, 25 nmol/L 2-mercaptoethanol, and 10 mmol/L EDTA, pH 7.0). An aliquot was transferred to stoppered tubes for extraction of the lipids. Methanol containing 30,000 dpm [^{3}H]cholesterol was added (to monitor recovery) and the tubes were vortexed vigorously. Chloroform was added, mixed, and allowed to stand for 1 hour at room temperature. Then, equal volumes of chloroform and 0.15 mol/L NaCl were added to separate the phases, and the tubes were allowed to stand overnight at 4°C. The tubes were centrifuged lightly to ensure that separation of the phases was complete.[14] The lower organic phase was removed as completely as possible by aspiration, and the aqueous phase was washed once with chloroform. The combined lower phase was dried under nitrogen. The lipids were resuspended in 95% ethanol and 100 μL was removed for liquid scintillation counting of [^{3}H]cholesterol (to correct for recovery). TG content in the lipid extract was measured by the method of Pinter et al[15] using a kit from Sigma Diagnostics and is expressed as micromoles of TG per gram of tissue.

Hormone-sensitive lipase (HSL) activity was measured in adipose tissue collected from rats treated with vehicle or masoprocol (80 mg/kg twice daily) for 4 days. The adipose tissue was homogenized using a Polytron (Brinkman Instruments, Westbury, NY) on setting 3 in 50 mmol/L Tris hydrochloride buffer (pH 7) containing 250 mmol/L sucrose and 5 μmol/L EDTA. The homogenate was centrifuged at 1,500× *g* for 10 minutes at 4°C, followed by ultracentrifugation at 43,000× *g* for 15 minutes at 4°C.[16] The protein concentration was determined on the clear supernatant (43,000× *g*) by the modified Lowry technique,[17] and aliquots were used for measurement of HSL activity. HSL activity was measured as neutral cholesteryl esterase by determining the release of [1-^{14}C]oleic acid from cholesteryl [1-^{14}C]oleate as described previously by Nakamura et al,[16] with minor modifications. Briefly, the supernatant of adipocytes (10 μg) and the substrate (approximately 3×10^4 dpm/1.22 nmol cholesteryl oleate/4 μL acetone) were incubated at 37°C for 10 minutes in 100 mmol/L potassium phosphate buffer (pH 7.0) containing 0.025% BSA (total vol, 200 μL).[16] The reaction was terminated by the addition of 1 mL borate/carbonate buffer (0.1 mol/L, pH 10.5) followed by 3 mL chloroform:methanol: heptane (1.39:1.28:1).[18] The reaction tubes were vortexed vigorously for 1 minute and centrifuged (1,500× *g* for 20 minutes at 10°C). The amount of [1-^{14}C]oleate released in the aqueous phase was determined by scintillation counting using a Beckman (Fullerton, CA) LS3800 scintillation counter and is expressed as picomoles of [1-^{14}C]oleate released per minute per milligram of protein.

Statistical Analysis

Results are presented as the mean ± SEM. Statistical comparisons were made using Student's *t* test for unpaired samples or a 1-way ANOVA as appropriate, with post hoc comparisons using Fisher's protected least-significant difference test, except for LPL activity, which was analyzed using the Mann-Whitney test for nonparametric unpaired samples.

RESULTS

The effects of the fructose-enriched diet for 2 weeks on postabsorptive serum concentrations of glucose, insulin, TG, and FFA are shown in Table 1. The results show that body weight and serum glucose were lower in fructose-fed versus chow-fed rats. In contrast, serum insulin ($P < .02$), TG ($P < .001$), and FFAs ($P < .001$) were higher in fructose-fed rats.

Serum TG concentrations in fructose-fed rats treated with vehicle or masoprocol (10 to 80 mg/kg twice daily) for 4 to 8 days are shown in Fig 1. Masoprocol was administered twice per day at a dose of 10, 20, and 40 mg/kg (for 8 days) and 80 mg/kg (for 4 days). These results show that masoprocol produced a significant decrease ($P < .01$) in serum TGs compared with vehicle treatment and the decrease was dose-dependent: the greater the dose of masoprocol, the greater the decrease in TG concentration. The masoprocol-induced decrease in TGs was also associated with a significant decline in the FFA concentration (2.4 ± 0.2 *v* 1.4 ± 0.08 mEq/L, $P < .01$) but a comparable insulin concentration (49 ± 3 *v* 49 ± 4 μU/mL).

The effect of masoprocol treatment on the rate of hepatic TG secretion as estimated by intravenous administration of Triton WR 1339 is shown in Fig 2. These studies were performed in the same animals as shown in Fig 1, 3 to 4 hours after the final dose of vehicle or masoprocol. Treatment with masoprocol 40 or 80 mg/kg twice daily led to a significant decrease ($P < .01$) in the rate of hepatic TG secretion as compared with vehicle. However, lower doses of masoprocol (10 and 20 mg/kg twice daily) did not affect TG secretion. To determine if the masoprocol-induced decrease in hepatic TG secretion was associated with accumulation of fat in the liver, the TG content was determined in liver tissue obtained from rats (n = 12) treated with either masoprocol (80 mg/kg twice daily) or vehicle. These measurements showed that, if anything, hepatic TG content was lower in masoprocol-treated rats (7.6 ± 0.3 *v* 9.6 ± 0.7 μmol/g, $P < .05$).

The effect of masoprocol treatment on the $t_{1/2}$ for TG removal is shown in Fig 3. Animals were treated with vehicle or

Table 1. Effect of a Fructose-Enriched Diet on Serum Glucose, Insulin, TG, and FFA Concentrations

Variable	Fructose-Fed (n = 12)	Chow-Fed (n = 12)	*P*
Weight (g)	253 ± 5	304 ± 5	<.001
Glucose (mg/dL)	124 ± 2	131 ± 2	<.05
Insulin (μU/mL)	49 ± 4	36 ± 2	<.02
TG (mg/dL)	411 ± 53	155 ± 10	<.001
FFA (mEq/L)	1.6 ± 0.09	0.8 ± 0.04	<.001

NOTE. Data are the mean ± SEM. Animals were fed a fructose-enriched diet or regular chow for 20 days.

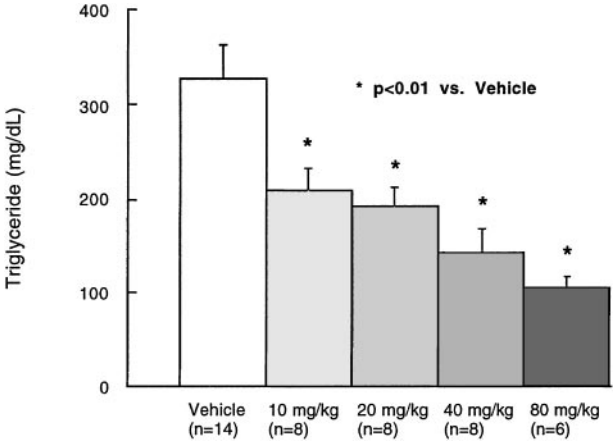


Fig 1. Postabsorptive serum TG concentration 8 days after the twice-daily oral administration of vehicle or varying amounts of masoprocol. All 4 doses of masoprocol significantly decreased plasma TG ($P < .01$). The number of animals in each group is given in parentheses.

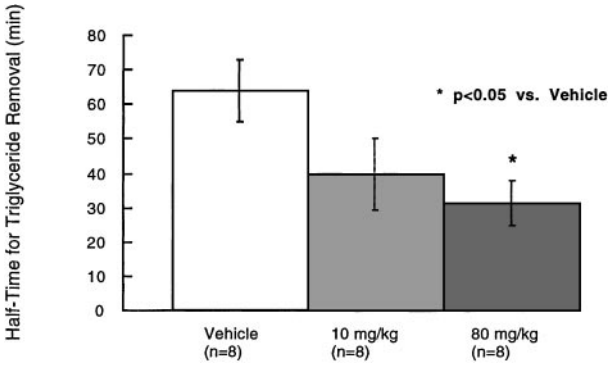


Fig 3. Estimates of the rate of TG removal from the plasma in fructose-fed rats treated for 8 days with a twice-daily dose of vehicle or masoprocol (10 and 80 mg/kg). The half-time for TG removal was determined by measuring the plasma TG concentration at frequent intervals following injection of a lipid emulsion (Intralipid). There were 6 to 8 rats in each group, and the half-time was significantly lower ($P < .05$) for fructose-fed rats with the higher dose of masoprocol.

masoprocol (10 or 80 mg/kg twice daily) for 8 days prior to determination of peripheral TG removal following the injection of Intralipid. The mean $t_{1/2}$ for TG removal was reduced by masoprocol treatment (10 mg/kg, 40 minutes; 80 mg/kg, 31 minutes) compared with vehicle treatment (64 minutes), although it was significant ($P < .05$) only at the higher dose.

Figure 4 compares the activity of LPL in soleus and epididymal fat tissue and adipose tissue HSL in tissue extracts from animals treated with vehicle or masoprocol (80 mg/kg twice daily) for 8 days. LPL activity was increased in both muscle and adipose tissue, but the difference was significant only in adipose tissue ($P < .05$). HSL activity in adipose tissue was lower ($P < .001$) in masoprocol-treated animals.

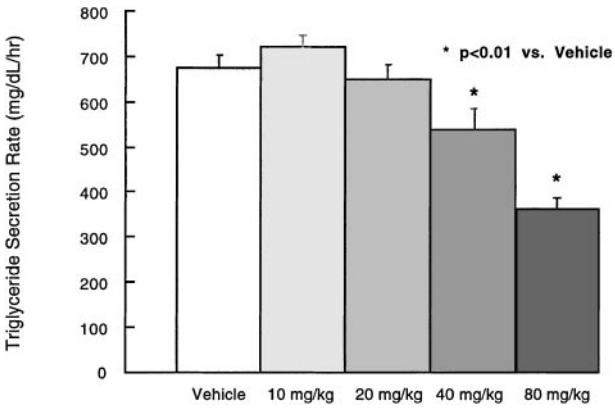
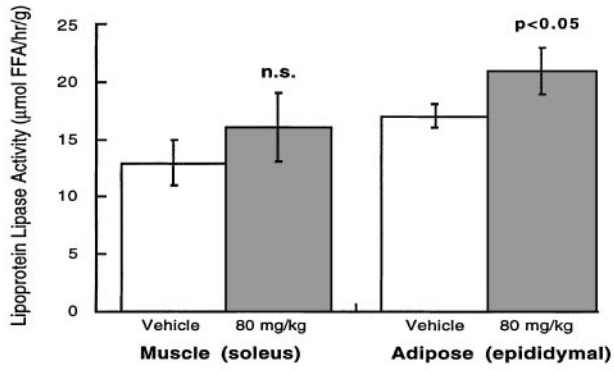


Fig 2. Estimates of TG secretion rates in fructose-fed or vehicle-treated rats after 4 days (80 mg/kg) or 8 days (vehicle and 10, 20, and 40 mg/kg) of twice-daily administration of test substance. TG secretion was estimated by determining the difference in plasma TG secretion before and 2 hours after administration of Triton WR 1339. There were 6 to 8 rats in each group, and the TG secretion rate was significantly lower *v* vehicle ($P < .01$) in rats treated with either 40 or 80 mg/kg masoprocol.



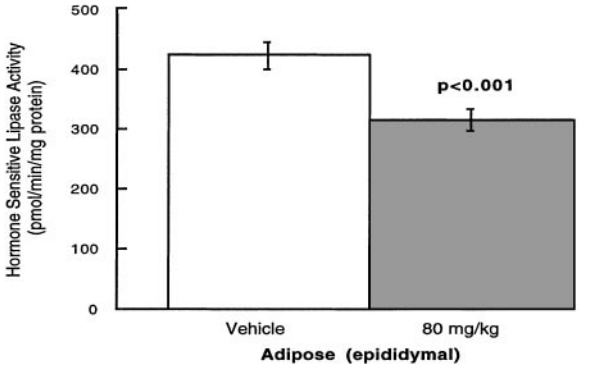


Fig 4. LPL activity in soleus muscle and epididymal adipose tissue and HSL activity in adipose tissue of fructose-fed rats 8 days after twice-daily oral administration of either vehicle or 80 mg/kg masoprocol. There were 6 to 8 rats in each group.

DISCUSSION

The results of the current study have again shown that HTG develops when rats consume a fructose-enriched diet. As in previous studies,[3-5] the fructose-induced increase in the plasma TG concentration was associated with hyperinsulinemia without a substantial change in the serum glucose concentration. In addition, the results demonstrate that masoprocol dramatically decreases serum TG concentrations in rats with fructose-induced HTG, extending prior observations that TGs decreased when rats with an experimental form of type 2 diabetes were treated with masoprocol.[2] Thus, the TG-lowering effect of masoprocol can be discerned irrespective of the initial serum glucose concentration.

In addition to showing that masoprocol decreases the serum TG concentration in rats with fructose-induced HTG, the data provide insight as to how this is accomplished. More specifically, evidence has been presented that masoprocol both inhibits hepatic TG secretion and increases the TG removal rate. If attention is first directed to the conclusion that masoprocol decreases hepatic TG secretion, the experimental data are most consistent with the view that this is secondary to the antilipolytic effect of masoprocol. In the first place, the fact that hepatic TG secretion was lower in masoprocol-treated rats suggests that the associated decrease in serum TGs was due to decreased TG secretion. Furthermore, based on the results of hepatic perfusion studies,[19] the decrease in FFA concentrations in masoprocol-treated rats in the presence of similar insulin concentrations would result in a decrease of hepatic TG secretion. Finally, the decrease in HSL activity in fructose-fed rats treated with masoprocol provides an explanation at the cellular level for the lower FFA concentrations. Thus, it is possible to propose the following coherent formulation to account for the dramatic ability of masoprocol to reduce serum TG concentrations in fructose-fed rats: masoprocol decreases adipose tissue HSL activity, resulting in lower circulating FFA concentrations, leaving less FFA available for hepatic TG synthesis, leading to a decrease in hepatic TG secretion and lower serum TG concentrations.

Although the evidence is indirect, it appears that the ability of masoprocol to reduce serum TG concentrations is not entirely due to a decrease in hepatic TG secretion. For example, plasma TGs decreased significantly in fructose-fed rats treated with masoprocol 10 and 20 mg/kg twice daily, doses that were not associated with a significant decrease in hepatic TG secretion. By inference, it could be argued that masoprocol also has the ability to enhance the rate of removal of TG-rich lipoproteins from the plasma. Providing some support for this possibility is the observation that the removal rate of plasma TG was increased following injection of Intralipid in masoprocol-treated rats. In addition, the increases in LPL activity were consistent with an effect of masoprocol on TG catabolism. Although only the increase in adipose tissue LPL was statistically significant, the increase in soleus muscle activity was of the same order of magnitude, and the combined effect on both tissues might well be viewed as offering support for the view that masoprocol enhances the rate of TG removal from plasma.

In conclusion, administration of masoprocol, a well-known lipoxygenase inhibitor, can profoundly decrease serum TG concentrations in fructose-fed rats in a dose-dependent manner. At least some of this effect appeared to be due to a decrease in hepatic TG secretion, secondary to an increase in adipose tissue HSL and a decrease in serum FFAs. However, masoprocol may also enhance TG catabolism, and this effect may contribute to its ability to reduce serum TG concentrations. On the other hand, evidence in support of this alternative is not as strong, and other possibilities have not been excluded, ie, a decrease in intestinal fat absorption. A definitive answer as to the mechanism by which masoprocol decreases serum TGs in fructose-fed rats will depend on the results of further experiements. Finally, masoprocol is a well-known lipoxygenase inhibitor, and it is not clear whether its effect on TG metabolism is related to this action. In that context, there is evidence that various amine-carboxyboranes can both decrease plasma TGs in rodents and inhibit lipoxygenase activity.[20] Although it is possible that lipoxygenase inhibitors might offer a useful approach for the treatment of HTG in humans, it may be that this effect is masoprocol-specific and unrelated to the inhibition of lipoxygenase pathways. Irrespective of the answer to this question, the extreme potency of masoprocol to reduce serum TG concentrations makes it worthy of further study.

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