Hypoglycemic effects of a novel fatty acid oxidation inhibitor in rats and monkeys

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Deems, Rhonda O., Robert C. Anderson, and James E. Foley. Hypoglycemic effects of a novel fatty acid oxidation inhibitor in rats and monkeys. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R524–R528, 1998.—Increased fatty acid oxidation contributes to hyperglycemia in patients with non-insulin-dependent diabetes mellitus. To improve glucose homeostasis in these patients, we have designed a novel, reversible inhibitor of carnitine palmitoyltransferase I (CPT I) that potently inhibits fatty acid oxidation. SDZ-CPI-975 significantly lowered glucose levels in normal 18-h-fasted nonhuman primates and rats. In rats, glucose lowering required fatty acid oxidation inhibition of ≅70%, as measured by β-hydroxybutyrate levels, the end product of β-oxidation. In cynomolgus monkeys, comparable glucose lowering was achieved with more modest lowering of β-hydroxybutyrate levels. SDZ-CPI-975 did not increase glucose utilization by heart muscle, suggesting that CPT I inhibition with SDZ-CPI-975 would not induce cardiac hypertrophy. This was in contrast to the irreversible CPT I inhibitor etomoxir. These results demonstrate that SDZ-CPI-975 effectively inhibited fatty acid oxidation and lowered blood glucose levels in two species. Thus reversible inhibitors of CPT I represent a class of novel hypoglycemic agents that inhibit fatty acid oxidation without inducing cardiac hypertrophy.

non-insulin-dependent diabetes mellitus; carnitine palmitoyltransferase I; cardiac hypertrophy

UNDER NORMAL CONDITIONS, reduced free fatty acid (FFA) levels after a meal provide a signal to the liver to decrease hepatic glucose production (16). In patients with non-insulin-dependent diabetes mellitus (NIDDM), FFA levels are elevated and contribute to the excessive fatty acid oxidation-induced glucose production in the liver (see Ref. 5 for review). One potential approach to decreasing blood glucose levels in NIDDM patients is to reduce excess fatty acid oxidation (6). Fatty acid oxidation may be inhibited indirectly by reducing the availability of fatty acids (such as with antilipolytic agents) or directly by decreasing fatty acid oxidation. The rate-limiting step regulating long-chain fatty acid oxidation is transport of FFA into the mitochondria via carnitine palmitoyltransferase I (CPT I) (11, 12, 14). Oxirane carboxylates, such as etomoxir and methyl-2-tetradecylglycidate, are irreversible inhibitors of CPT I (see Ref. 18 for review) that inhibit transport of long-chain fatty acids into the mitochondria.

As therapeutic agents, CPT I inhibitors are intended to reduce the hepatic glucose production caused by excessive oxidation of fatty acids, and as such, they may be appropriate for NIDDM patients with marked hyperglycemia. Inhibition of fatty acid oxidation leads to a transient increase in FFA levels and a marked reduction in the end products of fatty acid oxidation (i.e., ketone bodies; Ref. 20). Several studies have demonstrated that oxirane carboxylates are effective at lowering ketone and glucose levels in rodents, dogs, and humans (5–7, 9, 17, 18, 20). Unfortunately, the clinical development of these compounds was discontinued, possibly because oxirane carboxylates are active in the heart as well as the liver and are associated with cardiac hypertrophy (2).

The results with the oxirane carboxylates suggested that, although inhibition of CPT I was a useful therapeutic target, pharmacological intervention with available CPT I inhibitors was likely to be limited due to undesirable effects in cardiac muscle. One possibility for avoiding these effects in the heart would be to target CPT I inhibition to the liver. Such targeting could potentially be produced with a selective inhibitor of the hepatic isoform. However, initial results in liver and heart mitochondria suggested that sufficient isozyme selectivity was unlikely (10). An alternative approach was to design an inhibitor that was preferentially distributed (via 1st-pass effect) and metabolized by the liver. With an irreversible inhibitor, even small amounts of compound distributed to the heart could have a potential cumulative effect. Therefore a reversible CPT I inhibitor targeted to the liver appeared to be the most feasible approach.

A series of novel therapeutic agents was designed to reversibly inhibit CPT I activity by functioning as transition state mimics of the acyl transfer reaction catalyzed by CPT I (1). SDZ-CPI-975 (Fig. 1) was selected as the best candidate from this series of compounds based on its ability to reversibly and selectively inhibit fatty acid oxidation (4, 10). The present studies evaluated the acute in vivo effects of SDZ-CPI-975 on ketone and glucose metabolism in normal cynomolgus monkeys and rats.

METHODS

Nonhuman Primates

Normal, adult male cynomolgus monkeys (Macaca fascicularis) were maintained in a climate-controlled room with a normal 12-h light cycle. Animals were fed a standard diet (Purina monkey chow 5048) on a weight-maintaining basis (except during fasting) and received fresh fruit daily. The monkeys weighed between 3.0 and 3.3 kg during the studies and were estimated to be between 5 and 6 yr of age. Two studies were conducted: 1) time course of effect of SDZ-CPI-975; and 2) glucose and ketone lowering effects of SDZ-CPI-975. Study 1 was conducted with five primates per group, with animals receiving either vehicle [0.5% carboxymethylcellulose (CMC) and 0.2% Tween 80 in water] or SDZ-CPI-975 (12.5 mg/kg) in vehicle. Study 2 was conducted on 6 study
days with six animals, using a Latin-square design. On each study day, each animal received one of the six doses [vehicle (CMC) or 0.125, 0.625, 1.25, 6.25, and 12.5 mg/kg SDZ-CPI-975]. The order of the doses was counterbalanced so that each dose was administered on every experimental day and the order was different for each animal. Experiments were conducted at least 3 days apart to allow time for recovery for the animals.

For both studies 1 and 2, animals were fasted for 18 h before the study and remained fasted during the study. All experiments were conducted in the morning of the first day of the constant-light period. Animals were placed in restraining boxes to allow animals time to acclimate. Blood was sampled from the saphenous vein before dosing and at 1, 3, and 6 h postdose. At 0 h (0800), after a basal blood sample was obtained, animals were dosed (1 ml/kg body wt) by nasogastric intubation with either vehicle (CMC) or SDZ-CPI-975 in vehicle. Animal remained in the restraining boxes until after the second blood sample was taken (1 h postdose) and were then returned to the home cages. When blood samples were obtained at 3 and 6 h postdose, the animals were again restrained in the boxes for 15 min before each sample.

**Rats**

Adult male Sprague-Dawley rats (Hilltop Laboratories, Scottsdale, PA) weighing 250–350 g (7–10 wk of age) were housed in wire-mesh cages (3 animals/cage) under standard laboratory conditions with a reversed day-night cycle. Animals were fed ad libitum a standard laboratory diet (Purina rodent chow 5001) except when fasted overnight before the studies. Studies were conducted in the morning at the beginning of the dark cycle. Water was continuously available. Three studies were conducted: 1) time course of effect of 2.7, 4.8, and 11.0 mg/kg SDZ-CPI-975; 2) glucose and ketone lowering at 3 h postdose at doses of SDZ-CPI-975 of 0.44, 2.7, 3.3, 4.8, 5.5, 11, 22, and 88 mg/kg; and 3) muscle glucose utilization. For the studies, animals were dosed orally via gavage (1 ml/100 g body wt) with either vehicle (CMC) or compound in vehicle. For the time-course study, blood samples were collected from the tip of the tail in conscious animals before dosing and at 1, 3, and 6 h postdose. For the dose-response study, animals were anesthetized with CO2 at 3 h postdose, and blood samples were taken via cardiac puncture. All blood samples were analyzed for serum β-hydroxybutyrate (β-HBA) and glucose levels.

Glucose utilization in individual tissues was determined in normal Sprague-Dawley rats bearing indwelling carotid artery and jugular vein cannulas (19). Animals were allowed 2–3 days to recover from surgery. After an 18-h fast, animals were dosed as described above. Three treatment groups were included: vehicle, SDZ-CPI-975, and the standard CPT 1 inhibitor etomoxir. Compounds were tested at 10 times their approximate half-maximal effective dose (ED_{50}) values for acute (3 h postdose) lowering of serum levels of β-HBA in normal rats: SDZ-CPI-975 at 48 mg/kg or etomoxir at 3.6 mg/kg (20). Compounds or vehicle were administered orally 3 h before the administration of a bolus of 2-deoxy-[2-3H]glucose (2-DG). Animals were killed 45 min later, and individual muscles (heart, soleus, gastrocnemius, and diaphragm) were frozen for later analysis of 2-DG uptake. Glucose metabolic rate was estimated according to the procedures of Kraegen et al. (8). This technique is based on tissue accumulation of phosphorylated 2-DG, which is not metabolized in most tissues. The tissue glucose metabolic index is calculated from the measurement of phosphorylated 2-DG, plasma glucose levels, and the time course of plasma disappearance of 2-DG.

All procedures involving animals received prior approval by the Institutional Animal Care and Use Committee.

**Compound Preparation**

Details of compound preparation for SDZ-CPI-975 (1) and etomoxir (18) have been described previously.

**Metabolic Analyses**

Glucose levels were measured with a glucose oxidase method using an automated analyzer (Yellow Springs Instruments model YSI 27, Yellow Springs, OH). Insulin levels were analyzed using a radioimmunoassay (Linco Research, St. Louis, MO). FFA and ketone levels were measured enzymatically with kits (Sigma Chemical, St. Louis, MO).

**Statistical Analyses**

In nonhuman primates, Latin-square analysis of variance was used for dose-response comparisons and two-way analysis of variance for time course of effects of a single dose: paired and nonpaired Student’s t-tests were used for individual comparisons, respectively. Statistical comparisons in rodents were conducted using analysis of variance with nonpaired Student’s t-tests for individual comparisons.

**RESULTS**

**Nonhuman Primates**

Study 1. Time course of effect. SDZ-CPI-975 treatment (12.5 mg/kg) significantly decreased glucose levels at 6 h postdose (2.6 ± 0.6 mM for SDZ-CPI-975 and 4.1 ± 0.2 mM for vehicle, P < 0.05). There was no effect of SDZ-CPI-975 on glucose levels 1 and 3 h postdose. β-HBA levels were significantly lower in animals treated with SDZ-CPI-975 compared with levels in vehicle-treated control animals at both 3 and 6 h postdose (Fig. 2). The largest relative difference in β-HBA levels between control and SDZ-CPI-975-treated animals occurred at 6 h postdose. β-HBA levels continued to increase with the extended fasting in vehicle-treated animals, whereas levels tended to decrease in SDZ-CPI-975-treated animals. There were no significant effects on FFA levels.

Study 2. Glucose and ketone lowering. There were no significant effects at 1 or 3 h postdose on glucose, insulin, ketone, or FFA levels. As determined by Latin-square analysis, there was a significant effect of SDZ-CPI-975 on glucose levels at 6 h postdose (Fig. 3) at doses of SDZ-CPI-975 between 1.25 and 12.5 mg/kg.

Although β-HBA levels were lowered at the highest dose of SDZ-CPI-975 tested when evaluated with a Student’s t-test, there was no main effect of compound as determined by Latin-square analysis (Fig. 3). Unlike the control levels in study 1, β-HBA levels of vehicle-
treated animals did not increase between baseline and 6 h postdose (400 ± 60 vs. 410 ± 60 µM, respectively). There was a slight, but significant, increase in FFA levels, with levels being increased at the highest dose tested (1.0 ± 0.1 vs. 1.4 ± 0.2 meq/l for vehicle and 12.5 mg/kg SDZ-CPI-975, respectively). Insulin levels were also significantly decreased (maximal reduction of 50% relative to vehicle treatment) at the same doses of SDZ-CPI-975 that resulted in glucose lowering (Fig. 3).

Rats

Study 1. Time course of effect. Compared with levels in vehicle-treated control animals, β-HBA levels were significantly reduced (n = 10) at 3 and 6 h postdose by all doses tested (2.7–11 mg/kg) of SDZ-CPI-975 (data not shown). Because reduction of β-HBA levels was maximal at 3 h postdose, dose-response evaluations were conducted at this time. There were no significant reductions in glucose at these doses.

Study 2. Glucose and ketone lowering. Three hours after oral administration to normal fasted rats, SDZ-CPI-975 significantly reduced serum glucose levels at a dose of 22 mg/kg SDZ-CPI-975 and by a maximum of 20% at a dose of 88 mg/kg SDZ-CPI-975 (Fig. 4). At least a 70% reduction in ketone levels was required for significant glucose-lowering activity at a dose of 22 mg/kg. The maximal decrease in serum β-HBA levels achieved at a dose of 88 mg/kg was ~77%.

Study 3. Muscle glucose utilization. At 10 times the approximate ED50 value for acute (3 h postdose) lowering of serum levels of β-HBA in normal rats, there was no significant effect of SDZ-CPI-975 on glucose utilization in heart, gastrocnemius, soleus, or diaphragm muscles. Alternatively, glucose utilization was significantly increased in heart muscle from normal rats treated acutely with etomoxir (Fig. 5).

DISCUSSION

SDZ-CPI-975 was an effective hypoglycemic agent in both nonhuman primates and rats. In these studies with normoglycemic animals, glucose levels were lowered by a maximum of 20% with acute SDZ-CPI-975 treatment. In normal animals, glucose homeostasis is tightly regulated; even modest glucose lowering would be expected to elicit counterregulatory processes. More marked hypoglycemic activity would be anticipated under hyperglycemic conditions. This has been observed with CPT I inhibitors in both humans and rodents. In streptozotocin-treated diabetic rats, we previously demonstrated that SDZ-CPI-975 decreased glucose levels by 17% at the lowest dose tested (22 mg·kg⁻¹·day⁻¹) and practically normalized levels after 11 days of treatment with 66 mg·kg⁻¹·day⁻¹ SDZ-CPI-975 (56% decrease; Ref. 1). Similarly, administration of etomoxir to humans after an overnight fast had no effect on glucose levels in normal individuals, but significantly lowered glucose levels in patients with NIDDM (see Ref. 18).
vehicle-treated controls.

starvation in the rat, would be expected to have less
human primates. An 18-h fast, which is equivalent to
determinant of fatty acid oxidation in 18-h-fasted non-
mal reduction to 20% of control levels).
SDZ-CPI-975 effectively decreased ketone levels (maxi-
lized to ketones (13). Under these conditions in rodents,
fatty acids are almost exclusively metabo-
ized to CO2. In liver from 18-h-
was available in these studies, inhibition was apparent
with SDZ-CPI-975 treatment was a result of the com-
ound's ability to inhibit fatty acid oxidation. Although
no direct measure of inhibition of fatty acid oxidation
was available in these studies, inhibition was apparent
from the reduction in ketone levels. In liver from 18-h-
fasted rats, fatty acids are almost exclusively metabo-
lized to ketones (13). Under these conditions in rodents,
SDZ-CPI-975 effectively decreased ketone levels (maxi-
mal reduction to 20% of control levels).
In contrast, ketone levels would not be an exclusive
determinant of fatty acid oxidation in 18-h-fasted non-
human primates. An 18-h fast, which is equivalent to
starvation in the rat, would be expected to have less
severe metabolic effects in the nonhuman primate. Under normal metabolic conditions, the acetyl-CoA
formed during fatty acid oxidation is oxidized to CO2.
With fasting, acetyl-CoA is diverted toward ketone
body formation; this process progresses until ketone
production predominates during starvation. During an
18-h fast in the nonhuman primate, significant oxida-
tion to CO2 would still be anticipated and total assess-
ment of fatty acid oxidation would therefore require
measurement of both CO2 and ketone levels. Nonethe-
less, inhibition of fatty acid oxidation was apparently
sufficient to produce significant glucose lowering in the
overnight fasted monkeys. Under these experimental
conditions, it may be speculated that, in the cynomol-
gus monkey, hepatic glucose production is more depen-
dent on fatty acid oxidation than is the case in the rodent.

It is likely that the reduced insulin levels observed in
the nonhuman primates were secondary to the reduced
glycemia. Alternatively, it has been speculated that the
CPT system is involved in fuel sensing in the β-cell (15).
However, such a direct effect to reduce insulin secretion
would be predicted to result in elevated glucose levels
rather than in diminished levels, as was observed.

There was no acute effect of SDZ-CPI-975 on 2-DG
uptake in muscle, including cardiac tissue, whereas
treatment with the irreversible inhibitor etomoxir led
to a significant increase in glucose utilization by the
heart. The data predict that SDZ-CPI-975 will not lead
to the cardiac hypertrophy observed previously with
other CPT inhibitors. This has recently been confirmed
in a long-term (26 wk) evaluation in normal rats (M.
Rudin, N. Beckmann, M. Baumgartner, and K. Bruttel,
personal communication). The lack of an effect on
glucose utilization in the muscle suggests the preferen-
tial inhibition of hepatic CPT I activity. This selective
inhibition is probably due to a combination of the
 reversible nature of the compound and the result of
preferential distribution to the liver. It is also possible
that the selectivity reflects differences in intrinsic
inhibitory activity of SDZ-CPI-975 for the tissue-
specific isoforms of CPT I (3). However, CPT I activity
in liver mitochondria was only slightly (2- to 4-fold)
more sensitive to inhibition by SDZ-CPI-975 than heart
mitochondria (10). It is unlikely that this relative
difference in specificity would be sufficient to account
for the metabolic effects.

In summary, SDZ-CPI-975 is a novel, reversible, and
selective inhibitor of CPT I, which inhibits fatty acid
oxidation by restricting the transport of long-chain
fatty acids into the mitochondria (1). We have demon-
strated that treatment with SDZ-CPI-975 significantly
reduced glucose levels in several animal models. The
data are supportive of the hypoglycemic activity of
SDZ-CPI-975 being related to effective inhibition of
fatty acid oxidation. Furthermore, blood glucose lower-
ing is accomplished without promoting increased glu-
cose utilization in cardiac muscle.

Perspectives
The implications of these results are that reversible,
selective inhibitors of CPT I, such as SDZ-CPI-975,
may provide effective therapy for patients with NIDDM. Previous studies with etomoxir have already shown that CPT inhibitors lower glucose levels in NIDDM patients (5, 6, 18). The current study predicts that reversible CPT inhibitors will also be effective in lowering glucose levels in NIDDM patients without the cardiac problems associated with irreversible CPT inhibitors such as etomoxir. Of course, whether other problems associated with chronic and potentially excessive inhibition of fatty acid oxidation in liver, such as peroxisomal proliferation and accumulation of triglyceride in the liver (5, 6), will appear remains to be determined.

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