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Liver-selective glucocorticoid receptor antagonism decreases glucose production and increases glucose disposal, ameliorating insulin resistance

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Abstract

It is unclear how hepatic glucocorticoid receptor (GR) function and hypothalamic-pituitary-adrenal axis tone contribute to the diabetic state and in particular whole-body glucose fluxes. We have previously demonstrated that long-term exposure to hepatic GR inhibition lowers glucose levels in *ob/ob* mice (J Pharmacol Exp Ther 2005;314:191). The purpose of this study was to determine the effects of a novel GR antagonist (A-348441) on whole-body glucose fluxes in a model of insulin resistance, the Zucker fatty (*fa/fa*) rat. After an overnight fast, euglycemic-hyperinsulinemic clamp studies were performed 2 hours after single oral dosing as follows: (1) A-348441 at 100 mg/kg or (2) vehicle. Furthermore, effects of 1 week of treatment with either vehicle or A-348441 (3, 10, 30, or 100 mg/kg PO, once per day) were investigated in separate groups of rats fasted overnight and given a final dose of their respective compound, followed 2 hours later by a euglycemic-hyperinsulinemic clamp. One week after catheter implantation, body weight returned to presurgery levels, with no difference between groups. A single 100-mg/kg dose of A-348441 significantly increased glucose infusion rate 4-fold ($P < .05$) and reduced endogenous glucose production by 37% ($P < .05$) but did not change glucose disposal. After 1 week of sub–long-term dosing, fasting glucose levels were reduced dose-dependently with A-348441 vs vehicle (−8%, not significant; −14%, −20%, and −25%, $P < .05$, at 3, 10, 30, and 100 mg/kg, respectively) with no observed hypoglycemia or change in fasting insulin levels. A-348441 increased the glucose infusion rates after 1-week treatment by 1.3-, 5.7-, 7.3-, and 6.4-fold ($P < .05$). Endogenous glucose production was decreased (−25%, −44%, −50%, and −61%, $P < .05$), whereas glucose disposal was increased (29% and 13%, not significant; 23% and 34%, $P < .05$), with A-348441. In summary, single-dose treatment with the liver-selective GR antagonist A-348441 decreases glucose production with no effect on glucose disposal or fasting glucose levels. After 1 week of treatment with A-348441, (1) there was no effect on body weight, (2) fasting glucose levels decreased, (3) both glucose disposal and glucose infusion rate increased during clamping, and (4) endogenous glucose production was greatly reduced. In addition, hepatic glucose production was highly correlated with fasting glucose levels ($r = 0.97$). In conclusion, these results indicate that A-348441 increases insulin sensitivity at both the liver and peripheral tissues, leading toward a normalization of the insulin resistant state. Furthermore, with 1-week vs single-dose liver-selective glucocorticoid antagonism, we have determined that the peripheral effect is secondary to the primary event of reduced hepatic glucose production. The approach of inhibiting the hepatic GR may be an advantageous treatment paradigm for individuals with type 2 diabetes mellitus.

1. Introduction

Elevated hepatic glucose production is a primary contributor to increased fasting glucose levels in individuals with type 2 diabetes mellitus, which is largely the result of excessive hepatic gluconeogenesis [1-3]. Glucocorticoids, with other counterregulatory hormones, play a significant role in regulating glucose production in the liver [4-7].

This work was performed at Abbott Laboratories, Abbott Park, IL.

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Increased glucocorticoid activity has been associated with insulin resistance in animals and humans [7]. Glucocorticoids are known to produce peripheral insulin resistance, although little is known about hepatic effects [8-14]. A decrease in insulin-stimulated glucose uptake in skeletal muscle, with no effect on the insulin receptor number or ligand affinity, is demonstrated by these studies. In total, research to date supports the hypothesis that glucocorticoids induce insulin resistance through defects in the glucose transport system in skeletal muscle and adipose tissue, disrupting insulin's capacity to assemble intracellular glucose transporters to the plasma membrane. The in vivo actions of glucocorticoids on the liver, however, are not well known, particularly in the insulin-resistant state.

Glucocorticoid deficiency has been shown to reduce fasting blood glucose levels and improve glucose control in type 1 diabetic patients. Phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G-6-Pase), key enzymes of hepatic gluconeogenesis, mediate these effects [15,16]. Furthermore, systemic glucocorticoid receptor (GR) antagonism by mifepristone (RU-486) has been shown to ameliorate diabetes in rodents [17,18] and specifically in patients with Cushing syndrome because of ectopic ACTH production or adrenal carcinoma [19]. In addition, mifepristone has been demonstrated to lower total glucose production in nondiabetic male subjects upon short-term oral administration [20]. Long-term antagonism of the GR, however, can lead to hypothalamic-pituitary-adrenal (HPA) axis activation and hypersecretion of cortisol.

Systemic GR antagonism would be problematic as long-term therapy for individuals with diabetes. For example, mifepristone treatment results in adrenal insufficiency [21], with its associated problems, which can include hyperglycemia and insulin resistance [9,14,22]. Thus, over time, systemic GR antagonism can become self-limiting because of increased glucocorticoid production.

Selective blockade of the liver GR by A-348441, however, has been shown to prevent HPA axis activation [17]. Through a strategy of bile acid conjugation, it has been possible to prepare molecules that are potent inhibitors of glucocorticoid binding in vitro, that demonstrate GR antagonist activity in cellular assays, and that are selective for hepatic vs systemic glucocorticoid blockade in several animal models. The optimization of these properties requires compound screening in both in vitro and in vivo models and is facilitated by information from an x-ray crystallographic study of mifepristone binding to a GR fragment. From this analysis, conjugate A-348441 was selected for in-depth evaluation as a potential therapeutic agent, acting through the reduction of hepatic glucose output, for treatment of individuals with diabetes [17]. Furthermore, we have shown that long-term treatment with A-348441 in diabetic *ob/ob* mice normalizes glucose levels, but the mechanism by which this occurred was not determined [17].

Therefore, our aim was to determine the effects of liver-selective GR blockade by A-348441 on glucose fluxes in a model of insulin resistance, the Zucker *fa/fa* rat.

2. Methods

2.1. Animals, housing, and acclimation

Zucker fatty (*fa/fa*) rats 7 to 8 weeks of age (Harlan, Madison, WI) were acclimated to the Abbott Laboratories animal research barrier facility for about 1 week. Animals were single-housed on a 12-hour light/12-hour dark cycle and maintained on water and Teklad 8640 rodent diet (Harlan) ad libitum. Animals were treated in conformity with the National Institutes of Health and US Department of Agriculture–guided Abbott Laboratories Institutional Animal Care and Use Committee guidelines.

2.2. Randomization

After acclimation, body weight was obtained and tail snip plasma glucose levels were determined by the glucose oxidase method (Precision G glucose meter, Abbott Laboratories, Bedford, MA). The animals were randomized based on glucose levels and body weight at the start of the study. Baseline plasma insulin samples were taken from each treatment group once randomized (enzyme-linked immunosorbent assay, ALPCO Diagnostics, Windham, NH). Single-dose groups were studied by oral administration of (1) A-348441 at 100 mg/kg and (2) vehicle. Sub–long-term treatment groups were as follows: (1) A-348441: 3, 10, 30, or 100 mg/kg PO, once per day for 7 days, and (2) vehicle PO, once per day for 7 days at the same dosing volume (4 mL/kg).

Note that because of the large total number of animals to be studied and to minimize any effects of age from becoming a factor in the results, 3 separate groups of rats were used for the 7-day experiments, each group with its own vehicle control (same vendor, age, diet, acclimation, and facility). The groupings were as follows: (1) vehicle vs 3 mg/kg A-348441 (n = 5 vs 8, respectively), (2) vehicle vs 10 and 30 mg/kg A-348441 (n = 6 vs 6 and 8, respectively), and (3) vehicle vs 100 mg/kg A-348441 (n = 8 vs 5, respectively). Although baseline (2 hours postdose) overnight-fasted arterial catheter glucose levels on the day of the clamp were not different between vehicle groups, overnight-fasted tail snip baseline glucose levels of 2 of the vehicle groups just narrowly achieved statistical difference ($P < .05$); therefore, for completeness, each treatment group was compared with its respective vehicle group (see "Statistics" section for further details). Results are expressed as a percentage of their respective vehicle group unless otherwise indicated.

2.3. Dosing

All rats were dosed at a dosing volume of 4 mL/kg with their respective treatments as indicated.

A-348441 was solubilized with 5% EtOH, 10% PEG 400, 1 equivalent of 1N NaOH and 85% saline.

2.4. Euglycemic-hyperinsulinemic clamp

After catheter implantation, 7 days before experimentation (left common carotid artery and right jugular vein advanced to the aortic arch and superior vena cava, respectively), conscious overnight-fasted rats were clamped by constant infusion of insulin at 4 mU/kg per minute (regular insulin, Humulin R, Lilly, Indianapolis, IN, in 0.1% bovine serum albumin) and variable glucose to maintain euglycemia (50% dextrose, Abbott Laboratories, Abbott Park, IL). After surgery and 1 week of treatment in the 7-day groups, rats had regained their presurgery body weights. A final dose (or single dose in the groups) of A-348441 or vehicle was given 2 hours before the initiation of the clamps. Approximately 20 μL arterial samples were taken every 5 minutes during the study to allow adjustment of the glucose infusion rate (GINF) for maintenance of euglycemia (130-140 mg/dL goal based on incoming overnight fasting levels).

During the final 30 minutes of the 90-minute clamp, arterial blood samples (300 μL) were taken at 60, 75, and 90 minutes for determination of glucose-specific activity (SA), insulin, free fatty acids (FFA), and triacylglycerol (TG) levels. Plasma insulin levels were measured with a rat insulin enzyme-linked immunosorbent assay kit (Alpco Diagnostics) using rat insulin standards. Plasma TG levels were measured with the Lipid LinTrol set from Sigma Diagnostics (St Louis, MO). Plasma FFA levels were determined with the NEFA C kit from Wako Chemicals (Richmond, VA). Specific activities and GINFs were averaged for the 3 samples because it was determined that specific activity was stable over the sampling period.

To determine whole-body glucose fluxes, [3-^{3}H]glucose was used (PerkinElmer, Boston, MA). [3-^{3}H]Glucose is a nonrecirculating radioisotope because the ^{3}H atom is lost to water at the glucose isomerase step. The [3-^{3}H]glucose was mixed in saline and infused at 0.01 mL/min (0.06 μCi/min) after an initial bolus of 7 μCi.

Total glucose production (R_a) and utilization (R_d) were determined by the steady-state equations for isotope dilution during a constant infusion of tracer, according to the method of DeBodo et al [23].

During a euglycemic-hyperinsulinemic clamp and steady-state conditions, endogenous R_a equals R_d minus the GINF. The endogenous R_a is representative of hepatic glucose production with a minor contribution from the kidneys.

Table 1
Body weight and plasma glucose levels during the euglycemic-hyperinsulinemic clamps

Treatment	Dose (mg/kg)	Body weight (g)	Clamp glucose levels (mg/dL)		
			60 min	75 min	90 min
Vehicle	0	362 ± 17	143 ± 5	141 ± 6	135 ± 4
A-348441	3	356 ± 10	135 ± 5	134 ± 3	134 ± 3
	10	382 ± 17	131 ± 5	138 ± 7	131 ± 4
	30	373 ± 14	136 ± 5	133 ± 7	138 ± 8
	100	383 ± 13	135 ± 5	138 ± 5	139 ± 5
Single-dose A-348441	100	409 ± 16	142 ± 4	132 ± 4	141 ± 4

All values are mean ± SE. There were no differences across time or treatments ($P > .05$).

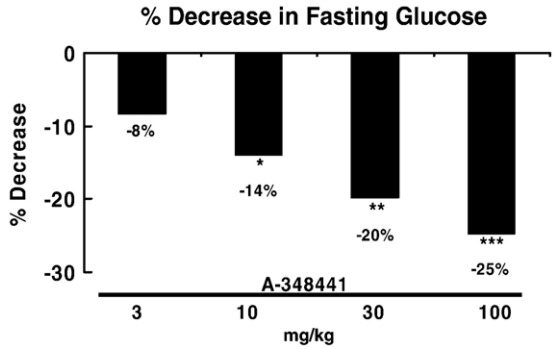

Fig. 1. Plasma glucose change after an overnight fast in the GR antagonist A-348441–treated Zucker *fa/fa* rats (as percent from vehicle after 1 week of oral, once per day, 0, 3, 10, 30, or 100 mg/kg PO dosing; see "Methods" for further details). Data are mean ± SE. *$P < .05$, **$P < .01$, ***$P < .001$.

2.5. Statistics

Statistical evaluation was performed via 1-way analysis of variance with Tukey post hoc analysis where appropriate using InStat (GraphPad Software, San Diego, CA) for each of the 3 separate study sets and the single-dose groups with their respective vehicle controls. The level of significance was $P < .05$ (2-sided test).

3. Results

3.1. Basal parameters

A-348441 had no effect on body weight after 1 week of dosing and an overnight fast (Table 1). Liver (14 ± 1 vs 13 ± 1 g, vehicle vs A-348441, respectively), and brain-liver weight ratios (13.2% ± 0.7% vs 13.7% ± 0.9%) were not different from vehicle with 100 mg/kg A-348441. Single-dose treatment of A-348441 had no effect on these parameters (Table 1).

3.2. Fasting glucose and insulin levels

Overnight fasting plasma glucose levels (105 ± 7 vs 115 ± 7, 126 ± 8 vs 147 ± 7, 118 ± 6 vs 147 ± 7, 98 ± 3 vs 130 ± 8 mg/dL; 3, 10, 30, and 100 mg/kg vs their respective vehicle group, see "Methods" section) were reduced compared with vehicle in a dose-dependent manner in this mildly hyperglycemic model with 1 week of A-348441 treatment. The percent change from vehicle is shown in Fig. 1. Fasting plasma insulin was not different from vehicle at any dose (5.7 ± 0.5 vs 5.3 ± 0.4, 6.2 ± 0.8, 5.2 ± 0.8, 4.6 ± 0.7 ng/mL, vehicle vs A-348441 at 3, 10, 30, 100 mg/kg, respectively), although at 100 mg/kg there was a

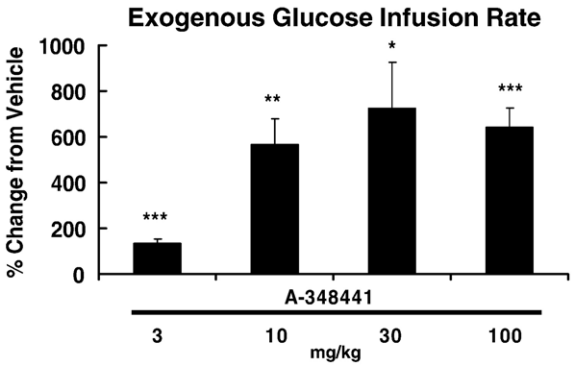

Fig. 2. Glucose infusion rate required to maintain euglycemia during the euglycemic-hyperinsulinemic clamp after 1-week treatment with vehicle or the GR antagonist A-348441 in Zucker *fa/fa* rats, expressed as percent change from vehicle (see Fig. 1 legend for details).

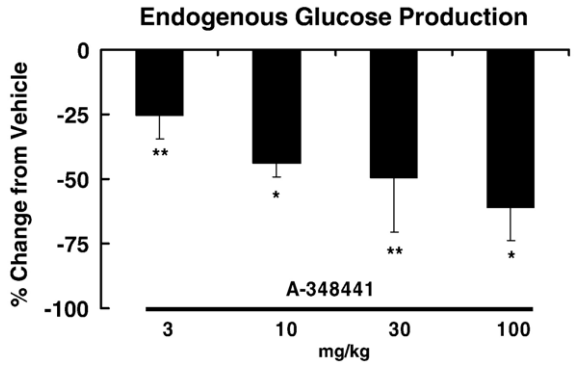

Fig. 4. Endogenous glucose production during the euglycemic-hyperinsulinemic clamp after - week treatment with vehicle or the GR antagonist A-348441 in Zucker *fa/fa* rats, expressed as percent change from vehicle (see Fig. 1 legend for further details).

nonsignificant 13% decrease vs its respective vehicle control (P = .25, 1-tailed t test). In addition, after 1 week of dosing, overnight fasting plasma TG levels were reduced with 3 (−40%) and 100 (−27%) mg/kg A-348441 but were unchanged with 10 (8%) and 30 (−10%) mg/kg vs vehicle. There was clearly no dose dependency in TG response with A-348441. Free fatty acid levels were not changed by A-348441 treatment during this period (data not shown).

Single-dose treatment did not change fasting glucose (123 ± 7 vs 135 ± 6 mg/dL; 100 mg/kg vs vehicle), insulin, TG, or FFA levels 2 hours postdose.

3.3. Glucose fluxes during the euglycemic-hyperinsulinemic clamps

Glucose levels were clamped at overnight fasting baseline levels (130 to 140 mg/dL) and were not different over time or across treatments (Table 1). Insulin levels during the clamp were not different from vehicle at any dose of A-348441 (5.6 ± 0.4 vs 6.0 ± 0.7, 5.6 ± 0.5, 6.6 ± 0.8, and 5.5 ± 0.6 ng/mL; vehicle vs 3, 10, 30, and 100 mg/kg A-348441) or among vehicle groups (6.2 ± 0.7 vs 5.4 ± 0.3 vs 4.5 ± 1.2 ng/mL; mean of all vehicles, 5.6 ± 0.4 ng/mL). Glucose-specific activities were in steady state during the 30-minute experimental sampling period for all treatments (data not shown).

A-348441 increased GINFs dose-dependently (Fig. 2). These rates were increased 1.3, 5.7, 7.3, and 6.4 times their respective vehicles at 3, 10, 30, and 100 mg/kg of A-348441, respectively. Tracer-determined glucose disposal rates were increased 29%, 13%, 23%, and 34% vs vehicle (Fig. 3). Endogenous glucose production rates were significantly decreased at all doses and dose-dependently (−25%, −44%, −50%, and −61% vs vehicle; Fig. 4). Average vehicle GINF, glucose disposal, and endogenous glucose production were 1.0 ± 0.2, 7.0 ± 0.5, and 5.8 ± 0.6 mg/kg per minute, respectively.

The percent change in endogenous glucose production was highly correlated to the percent change in fasting glucose level, r = 0.97 (Fig. 5). The ED_{50} for suppression of endogenous glucose production was determined to be 30 mg/kg.

Single-dose A-348441 treatment (100 mg/kg PO) increased GINF 4-fold vs vehicle control (Fig. 6). Whole-body glucose disposal was not changed with single-dose dosing (Fig. 7), yet endogenous glucose production was significantly decreased by 37% (Fig. 8). Clamp insulin

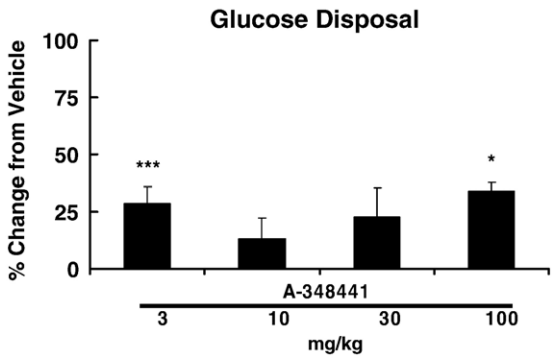

Fig. 3. Whole-body glucose disappearance (R_d) during the euglycemic-hyperinsulinemic clamp after 1-week treatment with vehicle or the GR antagonist A-348441 in Zucker *fa/fa* rats, expressed as percent change from vehicle (see Fig. 1 legend for further details).

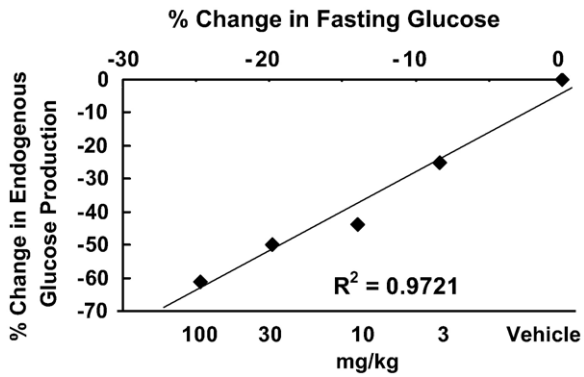

Fig. 5. Percent change in fasting glucose vs endogenous glucose production after 1-week treatment with each group's respective vehicle (see "Methods") or the GR antagonist A-348441 in Zucker *fa/fa* rats (see Fig. 1 legend for further details). Data are means for the indicated dose of A-348441.

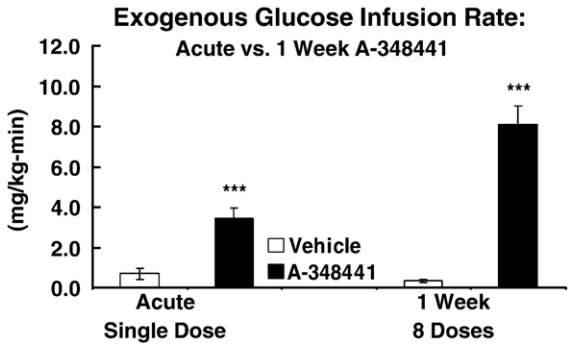

Fig. 6. Glucose infusion rate required to maintain euglycemia during the euglycemic-hyperinsulinemic clamp after single-dose or 1-week treatment with vehicle or the GR antagonist A-348441 at 100 mg/kg in Zucker *fa/fa* rats (see Fig. 1 legend for details).

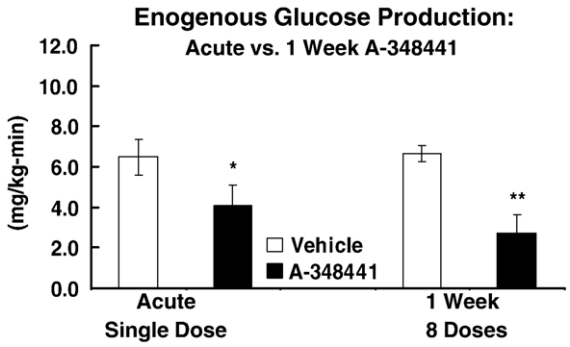

Fig. 8. Endogenous glucose production during the euglycemic-hyperinsulinemic clamp after single-dose or 1-week treatment with vehicle or the GR antagonist A-348441 at 100 mg/kg in Zucker *fa/fa* rats (see Fig. 1 legend for further details).

levels were not different between single-dose vehicle vs A-348441 100 mg/kg treatment (5.7 ± 0.4 vs 7.0 ± 0.6 ng/mL). Comparison of the single-dose–treated animals to those dosed at 100 mg/kg of A-348441 for 1 week before clamping clearly demonstrates the additional benefit of sub–long-term dosing on glucose disposal vs the single-dose effects on hepatic glucose production alone (Figs. 6-8).

4. Discussion

We have determined that liver-selective GR antagonism alleviates the insulin-resistant state by significantly reducing hepatic glucose production in an animal model of insulin resistance. Acute single dosing of A-348441 decreased hepatic glucose production with no change in glucose disposal. Whole-body glucose disposal was increased, in addition to a decrease in hepatic glucose production, with 1 week of sub–long-term dosing. Fasting glucose levels were reduced in a dose-dependent manner. These results were observed in the severely insulin resistant, mildly hyperglycemic Zucker *fa/fa* rat, a model in which there is only a small window to detect a decrease in fasting glucose levels vs other models (eg, *ob/ob*, *db/db* mice). It has been determined that the primary mechanism by which A-348441

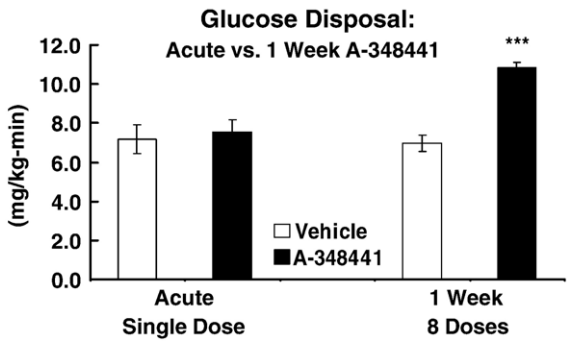

Fig. 7. Whole-body glucose disappearance (R_d) during the euglycemic-hyperinsulinemic clamp after single-dose or 1-week treatment with vehicle or the GR antagonist A-348441 at 100 mg/kg in Zucker *fa/fa* rats (see Fig. 1 legend for further details).

ameliorates elevated systemic glucose levels is by a reduction in hepatic glucose production with a secondary benefit of enhanced whole-body insulin sensitivity, as indicated by the increased glucose disposal.

Hepatic glucose production, as determined by the euglycemic-hyperinsulinemic clamp, was significantly reduced in the present study both after a single dose and 1 week of sub–long-term dosing. These findings are similar to our results in the nondiabetic single-dose–treated dog [24]. The sub–long-term results of the current study are consistent with a 3-week antisense oligonucleotide treatment in the diabetic *db/db* mouse [25]. Furthermore, we observed that hepatic glucose production was inhibited in a dose-dependent manner with increasing dose of A-348441 upon sub–long-term dosing, independent of a change in fasting insulin levels. At 3 mg/kg once per day, the lowest dose investigated, hepatic glucose production was significantly decreased by 25%, clearly demonstrating that the lowest effect level had not been achieved. The ED_{50}, mathematically determined to be 30 mg/kg, may be lower. It is also possible that with a longer dosing period or a twice-daily schedule a reduced ED_{50} may be obtained, depending on the pharmacokinetics and pharmacodynamics in individuals with diabetes vs rodents.

The contribution of cortisol to the increase in hepatic glucose output is unclear in individuals with diabetes. A recent investigation, however, has determined that total body cortisol production and that from the splanchnic bed did not differ among lean, obese, and obese subjects with type 2 diabetes mellitus, although endogenous glucose production was elevated in subjects with diabetes [26]. It was observed that splanchnic cortisol uptake was increased in obese subjects with type 2 diabetes mellitus. Further investigation into the potential contributions of the HPA axis, the function of the bidirectional enzyme 11β-hydroxysteroid dehydrogenase type 1, in conjunction with additional sites of cortisol production and uptake, and glucose metabolism will be required to examine this complex pathway. It is clear, however, that glucose production decreased with liver-selective glucocorticoid blockade in the present study.

Glucocorticoids, with other counterregulatory hormones, contribute significantly to the regulation of glucose production at the liver [7]. It is possible that increased glucocorticoid levels can worsen the diabetic condition or trigger overt diabetes in individuals with asymptomatic latent diabetes. Deficiency of glucocorticoids, however, reduces fasting blood glucose levels and improves glucose control in individuals with type 1 diabetes mellitus. PEPCK and G-6-Pase [15,16], key enzymes responsible for hepatic gluconeogenesis, mediate these effects.

Glucose production is composed of contributions from both gluconeogenesis and glycogenolysis and, depending on the metabolic state (eg, diabetes, fasting, sepsis, exercise), different or equal contributions of each. We have observed that hepatic messenger RNA expression of G-6-Pase is prevented from increasing (~50% of control) in single-dose–treated A-348441 plus prednisolone–challenged normal Sprague-Dawley rats (100 mg/kg) [17]. We have also observed similar effects on hepatic PEPCK messenger RNA (unpublished observations). This effect supports the current results where endogenous glucose production was inhibited and strongly suggests that the decrease in hepatic glucose production is, at least in part, determined by an inhibition of gluconeogenesis, although effects on glycogenolysis cannot be ruled out.

Glucocorticoid receptor antagonism, such as that due to systemic mifepristone (RU-486), can improve diabetes in humans, particularly in patients with Cushing syndrome due to ectopic corticotropin production or adrenal carcinoma [19]. Glucose production has been shown to be reduced with mifepristone treatment in nondiabetic male subjects upon single-dose oral administration [20]. In addition, we have shown that 1 week of mifepristone treatment (once per day, 100 mg/kg PO) reduces endogenous glucose output and increases glucose disposal in the Zucker *fa/fa* rat [27].

Systemic GR antagonists, however, cannot be used as generalized long-term therapy for type 2 diabetes mellitus. Systemic exposure to mifepristone results in adrenal insufficiency, with its associated problems [21]. Chronic exposure to a systemic GR antagonist leads to activation of the HPA axis and hypersecretion of cortisol. This can lead to hyperglycemia and insulin resistance [9,14,22]. Over time, systemic GR antagonism, therefore, can become problematic due to increased glucocorticoid production.

We observed a significant dose-dependent decrease in fasting glucose levels with liver-selective glucocorticoid blockade. The relationship between fasting glucose levels and its dependence on hepatic glucose output in humans has been previously demonstrated [2,3]. In the present experiment this relationship is again confirmed (R^2 = 0.97). This correlation strongly suggests that our fasting glucose reduction is related to the glucose output at the liver. By selectively blocking the transcriptional effects of corticosterone at the hepatic GR, we have been able to reduce glucose production. Furthermore, these results suggest that the reduction in glucose production led to a secondary increase in peripheral insulin sensitivity and whole-body glucose disposal and a further decrease in the systemic glycemic burden.

In the present sub–long-term studies, not only did a clear reduction in hepatic glucose output occur, but whole-body glucose disappearance also increased. A strong correlation between fasting glucose levels and glucose disposal was observed (R^2 = 0.59), but this was not as robust as that with endogenous glucose production (R^2 = 0.97), suggesting that both are related to glucose lowering but that reduced glucose production is a greater contributor by which glucose levels were reduced. This paradoxical increase in peripheral glucose disposal would not be expected with a hepatically targeted GR antagonist. Indeed, A-348441 is a stable, cholic acid conjugate of RU-486 that was designed to specifically target the liver [17,24]. It remains, however, that we did obtain an increase in peripheral glucose disposal. We believe that the beneficial increase in glucose disposal is due to a decrease in systemic glucose levels alone, leading to an increase in whole-body insulin sensitivity [28]. If this hypothesis were true, a single dose of the inhibitor would decrease endogenous glucose production by its direct actions and not lead to an increase in glucose disposal, as whole-body insulin sensitivity would not change short-term because glucose levels would only transiently be decreased. As determined in our single-dose treatment groups, this hypothesis was supported; a single dose of A-348441 led to a significant decrease in glucose production (−37%) with no change in peripheral glucose disposal. The possibility does exist that there could be a direct effect of the drug on glucose disposal and that the beneficial increase in glucose disposal occurred before the observed 1-week effect. That A-348441, however, is chemically designed to target the liver via a cholic acid conjugate would strongly argue against a direct peripheral effect [17], particularly in combination with the short-term responses. The precise timing of the enhanced glucose disposal is unknown, but it is clear that the reduction in glucose production occurs before any benefit on peripheral glucose disposal.

As a result of this mechanism, we believe it is possible to further control and reduce glucose and insulin levels and hepatic glucose production and increase glucose disposal as well as whole-body insulin sensitivity. Although insulin levels were not different, they were slightly, but not significantly, reduced 13% at the highest dose of 100 mg/kg. In addition, we hypothesize that parallel beneficial effects on lipids in individuals with diabetes and or insulin resistance could be achieved through longer dosing as shown in the *ob/ob* mouse where we have observed, with 6 weeks of dosing, significant reductions in glucose, insulin, FFA, and TG levels, food intake, and body weight gain [17,24]. An improvement in glucose control was also observed, as indicated by a reduction in percent HbA_{1c} [17]. These results are consistent with previous results after 3-week treatment of the systemic glucocorticoid antagonist RU-486, also in the *ob/ob* mouse [18].

It is possible that combination therapy for liver-selective GR antagonism, in conjunction with a peripheral insulin sensitizer acting primarily at the skeletal muscle (eg, thiazolidinedione), could provide an additive and potentially synergistic response. Glucocorticoid receptor blockade with A-348441 plus rosiglitazone treatment has been assessed in the diabetic *ob/ob* mouse over a 2-week period [17]. There was a clear synergistic benefit on glucose control by combining subefficacious dosing of both treatments in these diabetic mice. The combination-treated mice had normalized glucose levels compared with their lean littermates. In addition, the well-known increase in body weight with rosiglitazone was prevented with combination treatment.

Potential adverse effects of inhibiting the liver GR may include hypoglycemia as a primary risk. It should be noted that during no time in this study was hypoglycemia observed, even at 2 hours postdose after an overnight fast. Thus, liver-selective GR blockade by A-348441, at least in the Zucker *fa/fa* rat, did not increase the risk of hypoglycemic events. No other adverse effects were observed in rodents with A-348441 treatment from single-dose or sub–long-term dosing in the present investigation to 6 weeks of dosing in the *ob/ob* mouse [17].

In summary, single-dose oral treatment with the novel liver-selective GR antagonist A-348441 at 100 mg/kg (1) significantly reduces hepatic glucose output, (2) increases glucose infusion rate, and (3) was without effect on glucose disposal. One week of treatment with A-348441 (1) does not affect body weight, (2) significantly decreases fasting plasma glucose, with no effect on insulin or FFA, (3) is without any observed hypoglycemia, (4) increases glucose disposal and exogenous glucose infusion rate, and (5) reduces endogenous glucose production (hepatic glucose output) with near-equal but opposite effect to the disposal increase, in a dose-dependent manner. These results indicate that 1-week administration of A-348441 increases insulin sensitivity at both the liver and peripheral tissues, leading toward a normalization of insulin resistance.

In combination with the single-dose study results, liver-selective glucocorticoid antagonism substantially decreases hepatic glucose output as its primary mechanism of action, with secondary effects on improving insulin sensitivity in the periphery with prolonged treatment. Studies of greater length are needed to further examine this mechanism and its actions and to more specifically determine the therapeutic potential of liver-selective GR inhibition. In conclusion, the approach of selectively inhibiting the hepatic GR may be an advantageous therapy for individuals with type 2 diabetes mellitus.

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