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Direct renin inhibition modulates insulin resistance in caveolin-1-deficient mice

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Abstract

Objective—To test the hypothesis that aliskiren improves the metabolic phenotype in a genetic mouse model of the metabolic syndrome (the caveolin-1 knock out (KO) mouse).

Materials/Methods—Eleven-week-old cav-1 KO and genetically matched wild-type (WT) mice were randomized to three treatment groups: placebo (n = 8/group), amlodipine (6 mg/kg/day, n = 18/group), and aliskiren (50 mg/kg/day, n = 18/group). After three weeks of treatment, all treatment groups were assessed for several measures of insulin resistance (fasting insulin and glucose, HOMA-IR, and the response to an intraperitoneal glucose tolerance test (ipGTT)) as well as for triglyceride levels and the blood pressure response to treatment.

Results—Treatment with aliskiren did not affect the ipGTT response but significantly lowered the HOMA-IR and insulin levels in cav-1 KO mice. However, treatment with amlodipine significantly degraded the ipGTT response, as well as the HOMA-IR and insulin levels in the cav-1 KO mice. Aliskiren also significantly lowered triglyceride levels in the cav-1 KO but not in the WT mice. Moreover, aliskiren treatment had a significantly greater effect on blood pressure readings in the cav-1 KO vs. WT mice, and marginally more effective than amlodipine.

Conclusions—Our results support the hypothesis that aliskiren reduces insulin resistance as indicated by improved HOMA-IR in cav-1 KO mice whereas amlodipine treatment resulted in changes consistent with increased insulin resistance. In addition, aliskiren was substantially more effective in lowering blood pressure in the cav-1 KO mouse model than in WT mice and marginally more effective than amlodipine.

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Keywords

caveolin-1; renin inhibition; insulin resistance; metabolic syndrome

INTRODUCTION

Hyperinsulinemia and insulin resistance are associated with increased cardiovascular risk [1, 2]. This risk is particularly increased if the subjects have the metabolic syndrome [2]. Although it is well documented that an activated renin-angiotensin system (RAS) is associated with insulin resistance [1, 3], there is no consensus concerning the effects of RAS inhibition on insulin resistance [4].

Recently, a new class of agents to inhibit the RAS has been developed---direct renin inhibitors (DRI) [4]. The data related to DRIs' effect on insulin sensitivity appears to be more consistent in animal models of diabetes. Iwai et al.[5] have shown that aliskiren, a DRI, improves plasma glucose levels during an oral glucose tolerance test (OGTT) and increased the response to insulin administration during an insulin tolerance test in diabetic KKAY mice. In addition, Kang et al.[6] recently demonstrated that treatment of diabetic, obese db/db mice with aliskiren improved glucose responses to an insulin tolerance test (ITT) and reduced insulin resistance as assessed by HOMA-IR. Lastra et al. [7] showed that aliskiren improved systemic insulin sensitivity, insulin metabolic signaling, and glucose transport in Ren 2 rats. However, whether aliskiren can have a beneficial effect on insulin resistance in pre-diabetic states associated with the metabolic syndrome is not known. Recent studies from our group [8] showed that caveolin-1 (cav-1) gene variants were associated with insulin resistance in hypertensive humans, thus suggesting that the cav-1 deficient (cav-1 KO) mice could be relevant to humans with hypertension that carry polymorphic variants of the cav-1 gene. This mouse model exhibits characteristics of the metabolic syndrome (insulin resistance, hyperlipidemia and mild hypertension) and has significant vascular dysfunction [9–13]. We hypothesized that aliskiren will improve the metabolic phenotype present in the cav-1 KO mouse.

MATERIALS AND METHODS

Animals

Eleven-week-old cav-1 knockout (KO) and genetically matched wild-type (WT) male mice (stock no: 004585 and 101045, respectively; n = 44 per genotype) were purchased from Jackson Laboratories, Bar Harbor, ME. The genotypes were confirmed by PCR, as previously described [13]. Animals were housed in the animal facility in 12-h light, 12-h dark cycle at an ambient temperature of 22 ± 1 °C and were maintained on *ad libitum* Purina rodent normal chow (catalog number: 5053, 0.3% NaCl; Purina, St.Louis, MO) and tap water. After 8 days of acclimatization and before randomization, systolic blood pressure (SBP) was measured; animals were then fasted over night, and the next morning an intra-peritoneal glucose tolerance test (ipGTT) was performed as described below. On day 14, fasting blood samples were collected from the submandibular plexus. Animals from each genotype were then randomized to three treatment groups: placebo (n = 8/group), amlodipine (6 mg/kg/day, n = 18/group), and aliskiren (50 mg/kg/day, n = 18/group). Amlodipine was delivered in food, while aliskiren was administered via Alzet osmotic sc micropumps (model 1007D; Durect Corp., Cupertino, CA). After two weeks of treatment, SBP measurements and an ipGTT were performed. Three days later, blood was collected by cardiac puncture under deep isoflurane anesthesia, after which mice were sacrificed. All experimental procedures followed the guidelines of and were approved by the Institutional Animal Care and Use Committee at Harvard Medical School.

Intraperitoneal glucose tolerance test (ipGTT) and glucose measurements

The procedure was performed as previously described by us [8]. Briefly, mice were fasted for 15–18 hours (overnight), and the next morning the baseline body weights were recorded. Mice were injected with 1.5 mg glucose/g body weight (intraperitoneal). At 0, 15, 30, 60, 90 and 120 min, blood was sampled (via the tail vein) for glucose determinations. At the end of the glucose tolerance test, mice were returned to cages and allowed free access to food. Blood glucose levels were determined using a Freestyle glucometer (Abbott Labs).

Blood pressure (BP) measurements

SBP was measured in conscious mice by tail-cuff plethysmography (BP analyzer, CODA, Kent Scientific) as previously described [12]. Conscious mice were warmed at 30°C for 10 min and allowed to rest before BP measurements. BP measurements were taken in the morning in a quiet room, and the mice were kept calm and handled by the same person. No sedation was used. Mice were acclimatized to the tail cuff BP measurement procedure for at least one week prior to the final measurements. We previously demonstrated very strong correlation between tail cuff and telemetry BP measurements [12].

Insulin measurements and HOMA-IR calculation

Sera from blood samples collected before and after treatment were stored at –80°C until assayed. Measurements were made in duplicates by enzyme-immunolinked assay, using the Mouse Ultrasensitive Insulin kit (Alpco Diagnostics), as previously described [8]. Homeostasis model assessment-insulin resistance (HOMA-IR) was calculated to assess changes in insulin resistance [14].

Plasma renin activity measurements

Blood samples were collected in purple-top BD microtainer tubes (EDTA) before and after treatment and plasma were kept at –80°C. Measurements were performed in duplicates by using a solid-phase RIA kit (DiaSorin Inc., Northwestern Avenue Stillwater, MN), as previously described [15].

Triglycerides measurements

Triglycerides are measured by an enzymatic colorimetric test (Roche Integra 400, Indianapolis, IN). This method is based on the work by Wahlefeld using a lipoprotein lipase from microorganisms for the rapid and complete hydrolysis of triglycerides to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced reacts with 4-aminophenazone and 4-chlorophenol under the catalytic action of peroxidase to form a red dyestuff (Trinder endpoint reaction). The color intensity of the red dyestuff formed is directly proportional to the triglyceride concentration and can be measured photometrically. The detectable range is 8.85–885mg/dL. The analytical sensitivity is 8.85mg/dL and precision is <0.7%.

Statistical analysis

Data were presented as means $\pm$ SE. Student's *t*-test for paired data was used for comparison of two means before and after treatment. Student's *t*-test for unpaired data was used for comparison between treatment groups. One-way ANOVA (with a Bonferroni correction for multiple comparisons) was used for comparisons between placebo and the treatment groups. Differences were considered statistically significant if $p < 0.05$. All studies were completed with the individual performing the study blinded as to the treatment group and genotype of the animals.

RESULTS

Effect of Aliskiren on Glucose Homeostasis

To test the hypothesis that aliskiren treatment modifies insulin resistance, we used two approaches. First, we measured fasting glucose and insulin levels and calculated HOMA-IR levels (Fig. 1A–C). Consistent with our previous findings [8], fasting glucose, insulin and HOMA-IR levels before treatment were significantly higher in the cav-1 KO than in the WT mice (Fig. 1A–C). In the cav-1 KO, treatment with aliskiren did not change glucose levels, but significantly decreased both fasting insulin and the calculated HOMA-IR levels ($p < 0.05$, Fig. 1). In contrast, treatment with amlodipine induced a significant increase in fasting insulin and HOMA-IR levels in these animals ($p < 0.05$, (Fig. 1B–C). In the WT mice, neither treatment induced significant changes in glucose, insulin or HOMA-IR.

Second, we measured the glucose responses during an intra-peritoneal glucose tolerance test (ipGTT) (Fig. 2). As compared to the pre-treatment responses, aliskiren treatment had no effect on the glucose tolerance curves in either WT or cav-1 KO mice. In contrast, amlodipine impaired the glucose tolerance response in both WT and cav-1 KO mice, but reached significance only in the cav-1 KO ($p = 0.057$ in WT, and $p < 0.001$ in cav-1 KO mice).

As previously reported, pre-treatment body weights were significantly lower in cav-1 KO versus WT mice (24.4 ± 0.4 vs. 26.8 ± 0.6 g, $p < 0.05$). After treatment with aliskiren, both animal groups showed a non-significant weight gain. In contrast, following amlodipine treatment, body weights increased significantly in both the WT (28.6 ± 0.9 , $p < 0.05$) and the cav-1 KO (26.8 ± 0.6 , $p < 0.05$).

Effect of Aliskiren on Triglycerides

Triglyceride levels are an important indicator of the metabolic profile; therefore we measured fasting triglycerides at sacrifice following treatment with placebo, aliskiren or amlodipine (Fig. 3). As compared to placebo, treatment with either aliskiren or amlodipine induced a significant decrease in triglyceride levels in the cav-1 KO, but not in the WT mice.

Effect of Aliskiren on Blood Pressure

Before treatment, SBP levels were significantly higher in cav-1 KO vs. WT mice, similar to what we have previously described [11–13]. Two weeks of treatment with either aliskiren or amlodipine significantly lowered SBP in the cav-1 KO, but the effect was significantly greater in response to aliskiren (Fig. 4). In contrast, neither agent significantly modified SBP in WT mice. Plasma renin activity (PRA) before treatment was similar in the WT and cav-1 KO, at levels anticipated in mice on this sodium intake (Fig. 5).

DISCUSSION

The present study was designed to test the hypothesis that the anti-hypertensive agent, aliskiren improves the metabolic phenotype of the cav-1 KO mouse, when compared to another commonly used anti-hypertensive---amlodipine. Our results showed that, in the cav-1 KO mice, both triglyceride levels and insulin resistance (as assessed by insulin levels and HOMA-IR) were improved by treatment with aliskiren. In addition, SBP in the cav-1 KO was significantly reduced by aliskiren and to a greater extent than with amlodipine treatment. Although the glucose response to a parenteral glucose tolerance test was not modified by aliskiren, amlodipine significantly compromised this response in cav-1 KO animals. Importantly, the beneficial effects of aliskiren on fasting insulin and HOMA-IR

levels seemed to also apply to the WT mice; however, these protective effects did not reach significance in the WT group.

It has been recently shown that chronic RAS inhibition by telmisartan significantly improved BP, insulin and HOMA levels in the Cohen-Rosenthal non-obese animal model of diabetes and hypertension [16, 17]. These results are in agreement with the data presented herein and implicate the RAS in the etiology of the metabolic syndrome; however, the dual role of telmisartan as an angiotensin II receptor antagonist and a peroxisome proliferator activator receptor (PPAR) γ agonist [18] makes it difficult to assign causality of these results just to the RAS.

Two previous studies [7, 19], used the transgenic Ren 2 rat model (Ren-2) to show that aliskiren treatment significantly lowered the glucose response to ipGTT and insulin resistance index, when compared to untreated rats. However, these studies seem to show opposite results in the control Sprague Dawley (SD) littermates. Thus, the SD rats treated with aliskiren had higher insulin levels and insulin resistance index when compared to control treatment of the same model. This may indicate that the insulin resistance in Ren-2 rats is not specific enough, therefore the efficacy of aliskiren on insulin sensitivity cannot be assessed in all conditions. Moreover, in a recent study, Manrique et al., [20] showed that beta blockade by nebivolol improves insulin sensitivity in the transgenic Ren 2 rats, thus strongly supporting the concept above regarding the specificity of the animal model for insulin resistance.

In agreement with our results, a previous study using type 2 diabetic KK-A^Y mice (KK-A^Y) [5] has shown that aliskiren improved the post-prandial circulating lipids. In addition, the same study suggested that aliskiren improves the glucose responses during either an oral glucose tolerance test (OGTT) or an insulin tolerance test (ITT). However, this study did not assess insulin resistance by indices such as HOMA-IR, which is based on the dynamic interaction between glucose and insulin output and is a well documented clinical representation of insulin resistance when compared to the gold standard reference, the euglycemic-hyperinsulinemic clamp [21]. The insulin levels in the study by Iwai et al [5] were significantly higher in the aliskiren-treated as compared to the control group. The discrepancy between this study and our present results could be due to the fact that insulin levels were assessed in a fed state [5], while our results include fasting insulin levels. In addition, the KK-A^Y mouse model is fundamentally different from the cav-1 KO model, not only relative to the presence/absence of a diabetic phenotype, but also relative to the fact that the KK-A^Y mice owe their phenotype to multiple (and yet unidentified genes).

A recent study by Kang and Dong et al. [6, 22] has demonstrated that aliskiren treatment in diabetic db/db mice led to lower HOMA-IR and improved glucose responses during ipGTT or ITT, when compared to db/db mice with placebo treatment. Similarly, aliskiren significantly while modestly improved SBP levels, glucose tolerance, HOMA-IR, fasting glucose but not insulin levels in obese Zucker rats [23]. However, the db/db mice as well as the Zucker rats in these experiments have defects in the leptin receptor whereas humans with leptin receptor defects do not develop insulin resistance and diabetes [24]. Therefore, the generalizability of these results to humans is unclear. In contrast our findings may extend to humans that have the polymorphic variant in the cav-1 gene, given the similar phenotype displayed in humans and cav-1 KO mice [8–13].

Previous studies in humans [25, 26] have shown that amlodipine treatment did not modify insulin sensitivity. Paradoxically in our study, amlodipine treatment further impaired glucose tolerance during ipGTT in both WT and particularly in cav-1 KO mice. Indeed, in the cav-1 KO the insulin and HOMA-IR levels were also increased by amlodipine treatment.

These findings suggest an adverse interaction between amlodipine and the glucose homeostasis systems particularly in the absence of cav-1. Potentially this effect is secondary to amlodipine blocking calcium ion influx via the L-type calcium channel, a critical pathway for pancreatic insulin release. This channel also has been reported to be regulated by cav-1 [27, 28].

Aliskiren treatment significantly reduced SBP in the cav-1 KO to a greater extent than in the WT. Moreover, the change in SBP in response to aliskiren was greater than in response to amlodipine treatment. Our BP results are in good agreement with previous animal studies [5–7, 19, 20, 22, 23, 29] and human studies [30–34].

The strength of the study presented herein lies in its key conclusions. First, treatment with aliskiren significantly lowered while amlodipine significantly degraded the HOMA-IR and insulin levels in cav-1 KO mice. Second, treatment with amlodipine significantly degraded the ipGTT response, as well as the HOMA-IR and insulin levels in the cav-1 KO but not WT mice. Moreover, aliskiren treatment induced a significantly greater improvement in SBP in the cav-1 KO vs. WT mice, while amlodipine did not. Thus, it can be speculated that the antagonistic responses yielded by the two drugs in the cav-1 KO (but not in the WT) mice may point to a specific subtype of metabolic phenotype, susceptible to beneficial effects from renin inhibition with aliskiren, but to deleterious effects from a calcium channel blocker like amlodipine.

Our study has several limitations. First, we do not have data on insulin levels during ipGTT; thus, we could not explain the real causes for the lack of effect of aliskiren on glucose levels in the cav-1 KO model. We speculate that this result may be due to aliskiren inhibiting insulin secretion during ipGTT [35, 36]; alternatively, poor absorption and distribution after glucose injection could also explain our results in this model. However, our HOMA-IR results, showing a significant reduction in response to aliskiren in the cav-1 KO mice, may represent a better interpretation of insulin resistance than a glucose tolerance test [35, 36]. Thus, we cannot distinguish between a delay in insulin secretion versus a difference in glucose absorption. Second, we only studied male mice, and thus do not know what happens in female mice. Third, the current study did not investigate the mechanism by which aliskiren modulates insulin sensitivity in cav-1 deficient mice.

In conclusion, our results support the hypothesis that aliskiren reduces insulin resistance as indicated by improved HOMA-IR in a genetic mouse model of the metabolic syndrome. Moreover, two further conclusions are of interest. In cav-1 KO mice, aliskiren was substantially more effective at lowering blood pressure than in WT mice and marginally more effective than amlodipine. Furthermore, amlodipine worsened the glucose response to ipGTT in the cav-1 KO mice. Thus our results provide a potential entrée to a unique role for aliskiren in the treatment of hypertension. In the subset of hypertensive subjects who carry the polymorphic variant of cav-1 that is associated with the phenotypic characteristics of the cav-1 KO mouse [8], treatment with aliskiren may be particularly beneficial, specifically relative to amlodipine. These individuals may have a better blood pressure response to aliskiren than to amlodipine while amlodipine may increase insulin resistance in this subgroup. Thus, genotypic identification of these individuals may allow for specific, personalized medicine particularly in relation to the more commonly prescribed anti-hypertensive, amlodipine.

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Abbreviations

cav-1	caveolin-1
DRI	direct renin inhibitor
HOMA	homeostasis model assessment
ipGTT	intraperitoneal glucose tolerance test
IR	insulin resistance
KO	knockout
RIA	radioimmunoassay
SBP	systolic blood pressure
WT	wild type

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Fig.1.

Fasting glucose (A), insulin (B) and HOMA-IR (C) levels were measured pre-and post-treatment in WT and cav-1 KO mice.

A: At baseline, fasting glucose levels in cav-1 KO were significantly higher than in WT ($p < 0.001$)

B: At baseline insulin levels in cav-1 KO were significantly higher ($p < 0.001$) than in the WT mice.

* $p < 0.01$ in cav-1 KO when comparing pre- vs. post-aliskiren treatment

$p < 0.05$ in cav-1 KO when comparing pre- vs. post-amlodipine treatment

C: At baseline HOMA-IR in cav-1 KO was significantly higher than in WT ($p < 0.001$).

* $p < 0.05$ in cav-1 KO when comparing values pre- vs. post-treatment

Fig.2. Glucose levels during an intra-peritoneal glucose tolerance test (ipGTT) were compared in WT and cav-1 KO mice, pre-and post-treatment with either amlodipine or aliskiren. A: Area under the curve (AUC) of ipGTT in WT post-amlodipine reached borderline significance vs. pre-amlodipine treatment ($p = 0.057$). B: Area under the curve (AUC) of ipGTT in cav-1 KO post-amlodipine was significantly greater vs. pre-amlodipine treatment ($p < 0.0001$).

Fig.3.

Fasting triglyceride levels in WT and cav-1 KO mice after treatment with placebo, aliskiren or amlodipine.

* $p < 0.05$ in cav-1 KO group when comparing post aliskiren vs. placebo

$p < 0.01$ in cav-1 KO group when comparing pre-amlodipine vs. placebo

Fig.4.

Pretreatment, SBP levels in cav-1 KO (126.1 ± 2.91 mmHg (SEM); $n = 36$) were significantly higher ($p < 0.001$) than in WT (110.0 ± 2.17 mmHg; $n=34$). Following treatment with aliskiren the SBP did not change significantly in the WT (0.10 ± 2.59 mm Hg; $n=16$), but aliskiren caused a highly significant ($p=0.00004$) change in the cav-1 KO mice (-13.1 ± 2.34 mm Hg; $n=16$). Thus, the effect of aliskiren on SBP was highly significantly different depending on genotype ($p=0.0005$). Similarly, in response to amlodipine only the cav-1 KO mice significantly ($p=0.025$, Wilcoxon Sum of Ranks Test) reduced their SBP. In the WT no change occurred. However, there was no significant difference in the SBP change between the two drug treatment groups of WT mice. Finally, the SBP response in the cav-1 KO to aliskiren was significantly greater than to amlodipine (* $p=0.05$ Mann-Whitney test).

Fig.5. Plasma renin activity (PRA) pre-treatment did not differ between WT group cav-1 KO groups.