Spironolactone, but not Eplerenone, Impairs Glucose Tolerance in a Rat Model of Metabolic Syndrome

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ABSTRACT. Although some clinical studies have suggested that spironolactone (SPL), a mineralocorticoid receptor (MR) antagonist, appears to increase the blood glucose levels, experimental studies have not supported this notion. Here, we investigated the effect of SPL on blood glucose levels in SHR/NDmcr-cp(cp/cp) (ND) rats, an animal model of metabolic syndrome, in comparison with that of eplerenone (EPL), another MR antagonist. At the same dose of 100 mg/kg, SPL and EPL increased the urinary sodium-to-potassium ratio to a comparable extent, indicating that both agents have similar renal MR antagonistic efficacy in ND rats. Interestingly, SPL but not EPL significantly increased the level of blood glucose. The oral glucose tolerance test revealed that treatment with SPL led to glucose intolerance. The levels of serum insulin and adiponectin, regulators of the blood glucose level, were virtually unaffected by treatment with SPL. On the other hand, SPL induced a marked increase in the blood level of aldosterone, known to be a risk factor for insulin resistance. These results indicate that both agents have similar renal MR antagonistic efficacy in ND rats. Interestingly, SPL but not EPL significantly increased the level of blood glucose. The oral glucose tolerance test revealed that treatment with SPL led to glucose intolerance. The levels of serum insulin and adiponectin, regulators of the blood glucose level, were virtually unaffected by treatment with SPL. On the other hand, SPL induced a marked increase in the blood level of aldosterone, known to be a risk factor for insulin resistance. These results demonstrate that in comparison with EPL, SPL characteristically impairs glucose tolerance in an animal model of metabolic syndrome, in association with a higher blood level of aldosterone.

KEY WORDS: blood glucose, eplerenone, metabolic syndrome, mineralocorticoid receptor, spironolactone.


Aldosterone, a physiological ligand of the mineralocorticoid receptor (MR), is classically known to regulate electrolyte homeostasis [12], and blood pressure. In addition, several basic and clinical studies have indicated that aldosterone is directly involved in tissue damage in the vasculature such as heart and kidney, through enhancement of oxidative stress, inflammation, and fibrosis.

Much attention has recently been focused on the relation between aldosterone and impairment of glucose metabolism [7]. Glucose intolerance and diabetic mellitus are often detected in patients with primary aldosteronism [11] due to adrenal adenoma or hyperplasia [1]. The impairment of glucose metabolism is known to be involved in the pathobiology in the metabolic syndrome that comprises a cluster of several abnormalities, including obesity, insulin resistance, dyslipidemia, and hypertension. Elevated plasma aldosterone levels are detected in metabolic syndrome [4], and moreover, experimental studies have produced a body of evidence for the involvement of aldosterone in the pathogenesis of certain components of metabolic syndrome, such as adipocyte dysfunction [5, 13] and insulin resistance [7].

Apart from the considerably beneficial effects of MR blockade on metabolic syndrome through improvement of glucose intolerance as well as suppression of blood pressure, no clinical trials have focused on preventing further metabolic deterioration and its complications. Spironolactone (SPL) and eplerenone (EPL), both of which are MR antagonists currently used in a clinical setting, are widely recognized to be beneficial for patients with hypertension and heart failure [24, 25, 33]. However, the two drugs are known to have distinct pharmacological/pharmacokinetic profiles; EPL shows greater MR selectivity and has non-genomic properties, and SPL has active metabolites [28]. Regarding the effect on glucose metabolism, EPL has been demonstrated to improve insulin sensitivity in obese model mice [13, 14]. On the other hand, treatment with SPL has been reported to increase the blood glucose levels in patients with resistant hypertension, which is defined as blood pressure that remains above the goal despite concurrent use of three antihypertensive agents of different classes [6]. Also, Yamaji et al. have shown that SPL increases HbA1c in patients with mild chronic heart failure. These data indicate that the results of experimental studies have not supported the clinical findings in terms of the effects of MR antagonists on blood glucose level.

We considered that further investigations would be necessary to compare the effect of SPL with that of EPL on blood glucose levels in animal models. In the present study, therefore, we examined the effect of SPL on blood glucose levels in SHR/NDmcr-cp(cp/cp) (ND) rats, an animal model of metabolic syndrome (known to spontaneously develop hypertension, obesity, and hyperlipidemia), in comparison with that of EPL, another MR antagonist.
MATERIALS AND METHODS

Experimental materials: SPL was purchased from Shanghai FWD Chemicals Co., Ltd., (Shanghai, China). EPL was extracted from Selara® tablets (purchased from Pfizer Japan Inc., Tokyo, Japan), and 0.5% (w/v) methylcellulose (MC) solution was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Experimental protocol: All experiments were carried out in accordance with the Animal Experimentation Guidelines of Daiichi-Sankyo Co., Ltd. The rats were kept in a room at 55 ± 5% relative humidity and 23 ± 1°C under a 12 hr light/dark cycle, and allowed free access to diet (FR-2, Funabashi Farm Co., Ltd., Chiba, Japan) and water.

Male SHR/NDmc-r(cp/cp) rats (ND, 15 weeks of age) and WKY/1zm rats (WKY, 15 weeks of age) as non-obese controls were purchased from Japan SLC Inc. (Shizuoka, Japan). At 18 weeks of age, the rats were divided into 6 groups: ND treated with MC solution (ND-CONT, n=6), ND treated with 30 mg/kg SPL (ND-SPL30, n=6), ND treated with 100 mg/kg SPL (ND-SPL100, n=6), ND treated with 100 mg/kg EPL (ND-EPL100, n=6), WKY treated with MC solution (WKY-CONT, n=6) and WKY treated with 100 mg/kg SPL (WKY-SPL100). We selected these doses of SPL, because 30–100 mg/kg of SPL showed antihypertensive effect in ND rats in our preliminary study. SPL and EPL were suspended in 0.5% (w/v) MC solution and administered at 2 ml/kg body weight by gastric gavage once a day for 3 weeks from the grouping day. We defined the grouping day as Day 1.

Measurement of urinary parameters and blood chemical parameters: The rats were placed individually in metabolic cages, and 24-hr urine was collected on Day 15. Urinary sodium and potassium concentrations were measured using a chemical serum analyzer (JcA-bM2250, JOEL Ltd., Tokyo, Japan) and urinary sodium-to-potassium (uNa +/K+) ratios were calculated. Water intake and food intake were also measured at the time of urine collection.

Blood was collected from the jugular vein on Day 17. Serum aldosterone (DPC Aldosterone RIA Kit, Diagnostic Products Corporation, Los Angeles, CA, U.S.A.), corticosterone (COAT-A-COUNT RAT CORTICOSTERONE, Diagnostic Products Corporation) were measured by radioimmunoassay. Insulin (REBIS Insulin Kit, Shibayagi, Gunma, Japan) and adiponectin (Rat Adiponectin ELISA Kit, CirLex Co., Ltd., Nagano, Japan) were measured by ELISA. Blood chemical parameters such as triglyceride (TG), total cholesterol (T-Cho), high density lipoprotein cholesterol (HDL-C) and blood glucose, were measured using an automated biochemical serum analyzer (JCA-BM2250, JOEL Ltd., Tokyo, Japan).

Oral glucose tolerance test: An oral glucose tolerance test (OGTT) was performed after 16 hr of fasting on Day 12. Glucose solution at 2 g/kg (OTSUKA GLUCOSE INJECTION 50%, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) was administered by gastric gavage. Blood glucose levels were measured at 0, 30, 60, 120, 180, 240 and 360 min after glucose administration using a blood glucose meter (GLUTEST®, Sanwa Kagaku Kenkyusho Co., Ltd., Nagoya, Japan). The area under the curve (AUC) of blood glucose was calculated for each group.

Statistical analysis: Data are expressed as mean ± SE. Comparisons between WKY-CONT and WKY-SPL100, and also between WKY-CONT and ND-CONT were performed by t-test. Statistical comparison between ND-CONT and the drug-treated ND groups, and WKY-CONT and the drug-treated ND groups, were performed by Dunnett’s test. Differences were considered to be statistically significant at P<0.05.

RESULTS

Increase of uNa+/K+ ratio in ND-SPL100 is similar in extent to that in ND-EPL100: Once aldosterone binds to MR on renal epithelial cells, sodium Na+ reabsorption and K+ excretion occur, and therefore the uNa+/K+ ratio becomes lower [16, 27]. Table 1 summarizes the uNa+/K+ ratio as well as body weight, urinary volume, food intake, and water intake in the experimental groups on Day 15. In comparison with the WKY-CONT group, the uNa+/K+ ratio in the ND-CONT group was somewhat higher. Although the reason for this is still unclear, sodium diuresis might occur in ND rats because of their hypertensive state. In comparison with the ND-CONT group, a significantly higher uNa+/K+ ratio was observed in both the ND-SPL100 and ND-EPL100 groups, the degree of elevation being comparable, suggesting that both drugs have similar inhibitory potency on MR signaling in renal epithelia.

SPL, but not EPL, increases the blood glucose level and improves lipid profiles in ND rats: On Day 17, blood glucose and lipid parameters were measured under normal feeding conditions, and the results have been summarized in Fig. 1. In comparison with WKY-CONT, the ND-CONT group showed a significantly higher blood glucose level (Fig. 1A). Although SPL had no effect on the blood glucose level in WKY rats, administration of SPL to ND rats dose-dependently and significantly increased the levels. In contrast, EPL had no effect on the blood glucose level (Fig. 1A) in ND rats. TG level in ND rats also increased, and this increase was significantly reduced by treatment with SPL, and tended to be reduced in the ND-EPL100 group (Fig. 1B). In comparison with WKY rats, the T-Cholesterol level was significantly higher and HDL-C was lower in ND rats, respectively, although the values were within the normal ranges (Fig. 1C and 1D). Treatment of ND rats with SPL decreased T-Cholesterol to the level in WKY rats (Fig. 1C), but EPL had no significant effect on T-Cholesterol. Neither SPL nor EPL had a significant effect on HDL-C (Fig. 1D). These data indicate that SPL characteristically increases blood glucose and ND rats.

SPL, but not EPL, induces impairment of glucose tolerance in ND rats: Next, we performed the OGTT on Day 12. The fasting blood glucose levels (at 0 min after administration) were comparable among all experimental groups. The OGTT showed that the blood glucose concentration increased quickly, peaked at around 60 min, and then returned to the basal level within 300 min after glucose administration in each experimental group, except for the ND-SPL100 group.
Table 1: Body weight, urinary parameters, food and water intake on Day 15

<table>
<thead>
<tr>
<th></th>
<th>WKY-CONT (n=6)</th>
<th>WKY-SPL100 (n=6)</th>
<th>ND-CONT (n=6)</th>
<th>ND-SPL30 (n=6)</th>
<th>ND-SPL100 (n=6)</th>
<th>ND-EPL100 (n=6)</th>
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<tr>
<td>Body weight (g)</td>
<td>418 ± 4</td>
<td>420 ± 3</td>
<td>550 ± 5 b)</td>
<td>529 ± 10 c)</td>
<td>509 ± 7 c)</td>
<td>548 ± 5 c)</td>
</tr>
<tr>
<td>Urinary volume (g)</td>
<td>12.2 ± 1.5</td>
<td>18.0 ± 2.6</td>
<td>26.9 ± 2.0 b)</td>
<td>27.9 ± 3.9 c)</td>
<td>28.1 ± 5.6 c)</td>
<td>32.0 ± 4.0 c)</td>
</tr>
<tr>
<td>uNa+/K+ ratio</td>
<td>0.55 ± 0.01</td>
<td>0.80 ± 0.08 a)</td>
<td>0.65 ± 0.03 b)</td>
<td>0.78 ± 0.08 c)</td>
<td>0.88 ± 0.08 c, d)</td>
<td>0.92 ± 0.03 c, d)</td>
</tr>
<tr>
<td>Food intake (g/24 hr)</td>
<td>24.5 ± 1.3</td>
<td>20.3 ± 1.8</td>
<td>26.3 ± 2.6</td>
<td>25.2 ± 0.9</td>
<td>20.9 ± 1.0 c, d)</td>
<td>26.1 ± 0.7</td>
</tr>
<tr>
<td>Water intake (g/24 hr)</td>
<td>35.9 ± 0.8</td>
<td>41.2 ± 3.3</td>
<td>37.9 ± 2.9</td>
<td>40.6 ± 3.2</td>
<td>42.0 ± 5.4</td>
<td>43.6 ± 2.7</td>
</tr>
</tbody>
</table>

uNa+/K+ ratio: urinary sodium-to-potassium ratio. Data are expressed as mean ± SE. a): $P<0.05$ in comparison between WKY-CONT and WKY-SPL100 by t-test. b): $P<0.05$ in comparison between WKY-CONT and ND-CONT by t-test. c): $P<0.05$ vs. WKY-CONT by Dunnett’s test. d): $P<0.05$ vs. ND-CONT by Dunnett’s test.

Fig. 1. SPL improves lipid parameters, but increases blood glucose in ND rats. SPL, EPL or vehicle was administered orally to ND or WKY rats once a day during the experimental period. On Day 17, blood was collected from the jugular vein and blood glucose (A), triglyceride (B), total cholesterol (C) and HDL-cholesterol (D) were measured. The values are given as mean ± SE. n=6 for each group. **, $P<0.01$, *, $P<0.05$ compared with ND-CONT. ††, $P<0.01$, †, $P<0.05$ compared with WKY-CONT. NS, not significant.
On the other hand, in the ND-SPL100 group, the blood glucose level peaked at around 120 min and then gradually returned toward the original level, although the level was still higher than that at the baseline at 360 min after administration (Fig. 2A). The AUc value calculated from Fig. 2A and 2b, an index of glucose tolerance, was significantly increased by SPL in both WKY and ND rats (Fig. 2C). In contrast, EPL had no significant effect on the AUc value in ND rats (Fig. 2C).

SPL does not affect serum insulin or adiponectin concentration in ND rats: On Day 17, we measured serum insulin in the experimental groups. As shown in Fig. 3A, ND rats showed marked hyperinsulinemia, compared with that of WKY rats. Administration of neither SPL nor EPL exerted any significant effects on the serum insulin levels in ND rats (Fig. 3A).

The serum level of adiponectin, a reduction of which is known to cause insulin resistance [23], was also measured. The level in the ND-CONT group was higher than that in the WKY-CONT group (Fig. 2C). Although a slight increase was evident in the ND-SPL30 group, the adiponectin levels in both the ND-SPL100 and ND-EPL100 groups did not differ from that in the ND-CONT group (Fig. 3B).

SPL increases the serum aldosterone concentration and reduces the serum corticosterone concentration in ND rats: On Day 17, the serum aldosterone and corticosterone concentrations were also measured. The level of aldosterone in the ND-CONT group was significantly higher than that in
the WKY-CONT group (Fig. 4A). The serum aldosterone concentration in the ND-SPL100 group was significantly increased relative to that in the ND-CONT group (Fig. 4A). On the other hand, EPL had no effect on the serum aldosterone level even at the same dosage as that of SPL. SPL also increased the serum aldosterone level in WKY rats, but the extent of the increase was smaller than that in ND rats (Fig. 4A).

The serum concentrations of corticosterone, a major functional glucocorticoid in rodents, in the experimental groups have been summarized in Fig. 4B. In comparison with the WKY-CONT group, the level of corticosterone in the ND-CONT group was significantly higher (Fig. 4B). In the ND-SPL100 group, the level was significantly lower than that in the ND-CONT group (Fig. 4B). On the other hand, EPL did not significantly affect the serum corticosterone level (Fig. 4B).
DISCUSSION

ND rats are congenic animals harboring a nonsense mutation introduced into the leptin receptor [29]. ND rats are known to spontaneously develop symptoms of metabolic syndrome, including obesity, hypertension, hyperlipidemia, and diabetes [3, 20], accompanied by age-related renal injury [21]. In comparison with non-obese genetic control animals, aldosterone excess has also been reported in ND rats [21]. These symptoms are known to be frequently observed in patients with metabolic syndrome, and therefore we considered ND rats to be an appropriate animal model of metabolic syndrome.

In the present study, administration of SPL significantly elevated the levels of blood glucose with impairment of glucose tolerance in ND rats, whereas EPL at a similar renally effective dosage did not. On the other hand, serum insulin levels in rats treated with SPL were not significantly different from those in rats treated with EPL, suggesting that SPL impaired insulin signaling. Previously, the CHARM study has indicated that treatment of patients with SPL is associated with the development of diabetes mellitus [26]. Furthermore, SPL has been reported to increase blood glucose levels in patients with type 2 diabetes mellitus [8]. Also, Yamaji et al. have shown that SPL increases the level of HbA1c in patients with chronic heart failure, whereas EPL does not [32]. Taken together, these data suggest that SPL, but not EPL, increases the blood glucose level in association with glucose intolerance.

There are some plausible reasons for the differences in the effects of SPL and EPL on the blood glucose level. Since MR activation by aldosterone plays a well-recognized role in insulin resistance [7], increased aldosterone levels in response to treatment with SPL would be a notable effect. If higher aldosterone levels resulted in insufficient blockade of MR, then insulin resistance would be expected to occur. Alternatively, non-specific binding of SPL and its metabolites to the sex steroid hormone [17] and glucocorticoid receptors could exacerbate insulin resistance. EPL was originally identified during the screening of SPL analogues for more selective binding to MR with minimal binding to other steroid receptors [9]. Therefore, in comparison with EPL, SPL shows 100- to 1,000-fold higher binding affinity for glucocorticoid, androgen, and progesterone receptors [9]. Since glucocorticoid receptor activation is widely known to increase blood glucose levels and it has also been reported that progesterone contributed to insulin resistance [17, 30], the lower selectivity of SPL to MR might be attributed to the difference. The decrease in the serum corticosterone concentration resulting from treatment with SPL, as observed in this study, may reflect a feedback mechanism between the serum glucocorticoid level and activation of its receptor.

In the present study, we found that SPL increased blood aldosterone levels, whereas EPL did not. Although the reason for this is still unclear, a difference in the resulting level of aldosterone might be explained by the view of the differences in the physicochemical and pharmacokinetic properties of SPL and EPL. EPL does not produce active metabolites, whereas SPL is metabolized quickly, generating various active metabolites. Many pharmacological effects of SPL are thought to be mediated by these metabolites. Higher concentrations of canrenone, a metabolite of SPL, were observed in liver, adrenals, kidney and testis in comparison with that in plasma. Furthermore, the retention of radioactive materials was observed in the adrenals after oral administration of 14C-labeled SPL [15], indicating that active metabolites were practically distributed in the adrenal glands [15]. If these active metabolites affect MR in the periphery, aldosterone release may occur as a compensated action. Further studies are required to clarify the mechanisms underlying the differential actions of MR antagonists on the blood level of aldosterone.

The increased blood glucose levels resulting from treatment with SPL in ND rats in our present study contrasted with results obtained from mice with high-energy diet-induced obesity [31], in which SPL reduced blood glucose levels and improved glucose tolerance. However, the two models are quite different in that mice with high-energy diet-induced obesity do not have an increased serum aldosterone level, whereas the serum aldosterone level in our present model rats was four times higher than in control WKY rats, reflecting results that have been obtained in humans. Moreover, methods used for oral administration of SPL differed between the two studies. In the mouse study, SPL was administered by a subcutaneously implanted drug release tablet, whereas in our study the drug was administered orally by gastric gavage, thus resembling the clinical situation.

MR blockade not only lowers blood pressure but also ameliorates cardiac morbidity and mortality in patients with heart failure [24, 25, 33]. Furthermore, accumulated evidence indicates that MR antagonists reduce albuminuria in patients with chronic kidney disease or diabetic nephropathy [2, 18]. These results suggest that treatment of metabolic syndrome with MR antagonists would have various beneficial effects, reducing the complications and risks of cardiovascular disease. However, our present findings indicate that SPL has the potential to increase the blood glucose level associated with glucose intolerance in metabolic syndrome. Although it remains unclear the influence of blood glucose elevation by SPL on clinical outcome, SPL has been reported to increase blood glucose levels in humans with type 2 diabetes and to impair endothelial function due to failure of glycemic control [8]. In terms of hyperglycemia, EPL is considered to have a superior profile. However, administration of EPL is contraindicated in diabetic patients with albuminuria, because of the risk of hyperkalemia. Recently, some novel non-steroidal MR antagonists with minimized unfavorable effects have been reported [10, 19, 22]. Such a new class of MR antagonist may be a novel therapeutic option for patients with metabolic syndrome.

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of the aldosterone-blocking agents eplerenone and spironolactone. Clin. Cardiol. 31: 153–158. [Medline] [CrossRef]


