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Effect of Azilsartan, Aliskiren or Their Combination on Body Weight and Adipogenesis of High-fat Diet Induced Non-alcoholic Fatty Liver Disease in Rats

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Abstract The renin-angiotensin system (RAS) plays an important role in the pathophysiology of many diseases including non-alcoholic fatty liver disease (NAFLD). This study aims to evaluate the effects of blocking RAS with azilsartan, aliskiren or their combination on the body weight and adipogenesis in rat's model of NAFLD. Thirty-two rats were allocated into four groups and treated with either vehicle, azilsartan, aliskiren or their combination. All groups were maintained on high-fat diet for 60 days, and their body weights were monitored each 3 days. At the end of treatment all animals were authenticated and visceral adipose tissues obtained and weighed. Azilsartan attenuates both the increase in total body and adipose tissue weight compared with control, while azilsartan alone affects adipogenesis only. In conclusion, Administration of azilsartan attenuates adipogenesis and obesity in rat's model of high-fat diet induced NAFLD, while aliskiren affects adipogenesis only.

Keywords: *high-fat diet, NAFLD, azilsartan, aliskiren, adipogenesis*

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1. Introduction

Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of liver disorders, ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), and mostly associates with metabolic syndrome, obesity, insulin resistance, hypertension, and dyslipidemia [1]. It is characterized by inflammation, hepatocellular injury, cirrhosis, hepatic failure and liver cancer [2]. It has been reported that RAS plays important role in the pathophysiology of many disorders related to the dysregulation of carbohydrate and lipid metabolism including metabolic syndrome, diabetes mellitus, and fatty liver diseases [3-5]. Accumulating evidence from in vitro and animal studies indicated that excessive stimulation of RAS predispose to hepatic steatosis, impaired mitochondrial functions, and accelerate progression to liver cirrhosis [6]. Although many in vitro and in vivo studies supported the idea that interference with RAS activity with angiotensin converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) might be of value in the management of hepatic steatosis [7,8], the outcome of these studies is conflicting [9,10], and the effects of these agents in NAFLD is not conclusive [11]. Marked increase in plasma renin angiotensin II (Ang II) levels was reported in rodents fed high-fat diet [12,13]; meanwhile, depletion of the RAS through gene targeting attenuates body weight gain in animal models of diet-induced obesity [14]. Moreover, similar outcome was observed due to the use of ARBs and ACEIs [15,16]. Our idea was that blockade of the RAS by means of long-term treatment with the renin antagonist, aliskiren, alone or its combination with the ARB azilsartan may attenuate the increase in total body and adipose tissue weights during NAFLD. The aim of the present work was to evaluate the effects of aliskiren, azilsartan and their combination on the body weight and adiposity in rat's model of NAFLD.

2. Materials and Methods

2.1. Animals and Experimental Design

The research protocol and animal care procedures was approved by the local research ethics committee, College of Pharmacy, University of Baghdad, and in accordance with the standard requirements for the care and use of experimental animal reported elsewhere. Thirty-six, four-week-old male Wistar rats weighing 160-200 g were obtained from the local bred of the animal house, department of pharmacology and toxicology, University of Baghdad and housed there under light/dark cycle 912/12 hr) and controlled room temperature (24°C±2) with standard rat chaw and drinking water *ad libitum*. After 1 week acclimatization, the animals were allocated randomly into four groups (each contains 8 rats), housed 1 per cage and treated as follow: 1st group (control) was treated with the 5% carboxymethyl cellulose (CMC), and fed during 60 days a high fat diet (standard chaw contains

30% lard and 5% cholesterol) especially prepared for this purpose. The other three groups were administered either 0.5mg/kg azilsartan (AZ) (Takeda, Japan), 25mg/kg aliskiren (Novartis, Switzerland) or their combination (formulated as suspension in 5% CMC), one week before starting fed with high fat diet formula as mentioned previously for the control group (zero time) for 60 days. Body weights of the animals were measured at zero time and each 3 days during the treatment period. After 60 days, rats were euthanized by intraperitoneal injection of 100mg/kg sodium thiopental (Health Biotech, India). In addition to the liver, kidney and hearts, lumbar, mesenteric, epididymal and periaortic adipose tissues were rapidly dissected and weighed. Weight and volume of adipose tissues were normalized to tibia length, and the organs and adipose tissue/ total body weight were calculated.

2.2. Statistical Analysis

Using Graph Pad software for statistics, the increase in total body weight, organ and adipose tissue weight, and their ratio to body weight were analyzed by repeated measures ANOVA. One-way ANOVA, followed by Bonferroni's *post hoc* test, was performed when appropriate. Statistical significance was set at $P<0.05$.

3. Results

Figure 1 demonstrates the increase in body weights of the rats during treatments. This increase was apparently more pronounced in control group and those treated with aliskiren alone. Treatment with azilsartan alone or its combination with aliskiren seems to decrease the rate of increase in body weight, and the influence of diet effect was excluded since all groups are maintained on high-fat diet that induce NAFLD. In Figure 2, the area under the curve (AUC) that represent the increase in body weight with time was compared for all groups. Treatment with azilsartan or its combination results in significant decrease ($P<0.05$) in AUC compared with control and aliskiren groups, while they are not significantly different when compared with each other. However, treatment with aliskiren did not show significant difference ($P>0.05$) in AUC compared with control group. Evaluation of total body weight in all groups of rats after 60 days indicates that in all treatment approaches total body weights were significantly increased compared with their respective values at zero time ($P<0.05$). Meanwhile, when post-treatment body weights were compared using ANOVA, azilsartan and its combination with aliskiren resulted in significantly lower body weights compared with control and aliskiren alone groups ($P<0.05$). Moreover, aliskiren produces comparable increase in body weight compared with control ($P>0.05$), and azilsartan alone or its combination with aliskiren did not significantly differ in this respect ($P>0.05$) (Figure 3). Table 1 indicates that liver, kidney and heart weights of groups treated with azilsartan, aliskiren and their combination were not significantly different compared with control after 60 days ($P>0.05$), and they did not differ with each other. Meanwhile, adipose tissue weights in treated groups were significantly lower ($P<0.05$) than that reported in control group (45%, 49% and 48%, respectively); however, although aliskiren produces greatest decrease in adipose tissue weight, they were not significantly different when compared with each other ($P>0.05$).

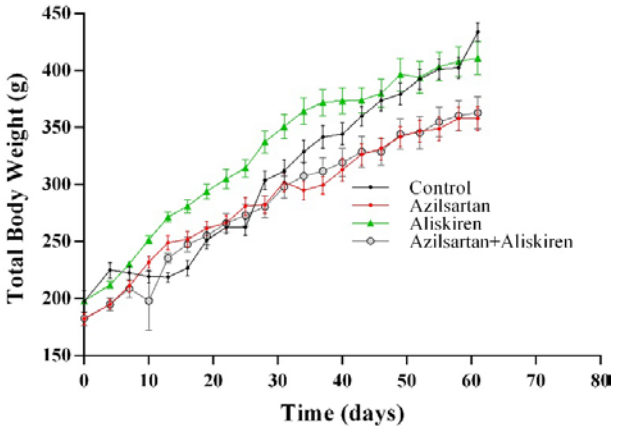

Figure 1. Effects of azilsartan, aliskiren or their combination on the daily increase of body weight in rat's model of high-fat diet induced NAFLD

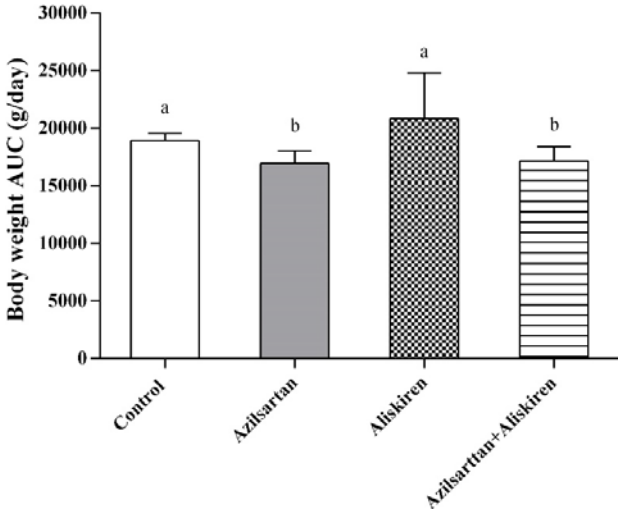

Figure 2. Influence of RAS blockade with azilsartan, aliskiren, or their combination on AUC of body weight changes vs time in rat's model of high-fat diet induced NAFLD; n= 8 rats in each group; values with non-identical letters (a,b) are significantly different ($P<0.05$)

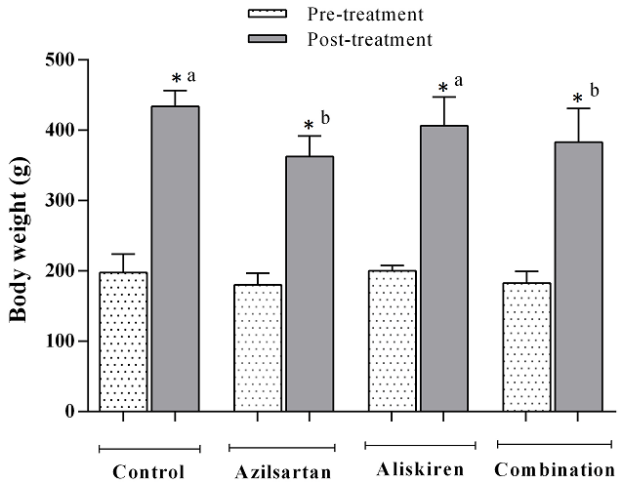

Figure 3. Comparison of body weights pre- and post-treatment with azilsartan, aliskiren, or their combination in rat's model of high-fat diet induced NAFLD; n= 8 rats in each group, * significantly different compared with pre-treatment within the same group ($P<0.05$); values with non-identical letters (a,b) are significantly different among different groups post-treatment ($P<0.05$)

Table 1. Effects of azilsartan, aliskiren or their combination on the organ weight/total body weight ratio of liver, kidney, heart and adipose tissue of high-fat induced NAFLD rats

Treatment type	Organ weight/Body weight (ratio x 10^{-2})			
	Liver	Kidney	Heart	Adipose tissue
Control	3.9±0.47[a]	0.57±0.04[a]	0.3±0.03[a]	2.58±0.4[a]
Azilsartan	3.5±0.42[a]	0.63±0.09[a]	0.27±0.04[a]	1.41±0.43[b]
Aliskiren	3.9±0.46[a]	0.62±0.08[a]	0.32±0.02[a]	1.32±0.41[b]
Combination	3.7±0.32[a]	0.59±0.05[a]	0.29±0.04[a]	1.35±0.46[b]

Values are expressed as mean±SD; *n*= 8 rats in each group; values with non-identical super scripts (a,b) are significantly different among different groups (P<0.05).

4. Discussion

In this project, we are interested in the role of multiple site blockade of the RAS in adipogenesis and body weight gain. For this aim we used the animal model of NAFLD, which represents most of the metabolic changes observed during obesity, dyslipidemia and insulin resistance. It has been previously reported that long-term maintenance of animals on high-fat diet increased body weight and adipose tissue [17]. The results of the present study seems consistent with the previously reported data. Regarding the effect of azilsartan, a major finding of the present study is that this imidazole-based ARB can interfere with the increase in both body weight and adipogenesis after exposure of rats to high-fat diet, compared with control and aliskiren-treated rats. This metabolic effect of azilsartan might be attribute to many well-known properties of this ARB, including enhanced phosphorylation of several critical distal insulin signaling elements in many insulin-sensitive tissues [18-20]. Moreover, exaggerated RAS activation in conditions like obesity has been linked to the development of insulin resistance [21]. The present data in high-fat diet treated rats are in tune with previous studies indicating that specific increases in Ang II exposure are associated with dysregulations in whole-body and muscle glucose homeostasis [22]. Additionally, the results of the present study using the novel ARB azilsartan are compatible with previous reports showing that interventions which suppress the RAS, whether involving direct renin inhibition [23], ACE inhibition [24] and ARB treatment [25], improve carbohydrate and lipid homeostasis, probably with positive influence on body weight gain and adipogenesis. In the present study, inhibition of renin by aliskiren effectively decreases adipose tissue without influencing the increase in total body weight; this could be attributed to its ability to reduce Ang I and Ang II in visceral tissue and associated with decreased plasma leptin levels [26]. The influence of using azilsartan and aliskiren on adipose tissue and body weight is consistent with other previously reported data, where the use of AT1R blockers [15] and ACEIs [16] decreases synthesis of adipose tissue, and this effect was linked with decreased leptin synthesis and release in adipose tissue. This finding supports the vital role of adipose RAS in regulating adipocyte metabolism and endocrine functions. Moreover, azilsartan is found to improve glucose transport in the muscles of Ang II-induced insulin resistant rats [18], which could be linked with improving carbohydrates and lipid metabolism, and the associated adipogenesis. This finding may explain the influence of azilsartan on both body weight gain and adipogenesis reported in the present study. Similarly, we previously reported that administration of ARBs and ACEIs in non-hypertensive diabetic patients ameliorates insulin resistance and decrease body mass index [27]. With obesity, the adipose tissue mass was increased through hypertrophy and differentiation processes. Many researchers have evaluated the effects of Ang II on adipocyte differentiation, with conflicting outcomes [28,29]. Many reports have demonstrated the roles of RAS blockade during experimentally-induced obesity in animal models. Administration of ARBs to type 2 diabetic mice resulted decreases plasma glucose levels and improves insulin sensitivity [30]. Such activity were associated with increased glucose uptake in adipose tissue, decreased adipocyte size, and elevated expression of peroxisome proliferator–activated receptor (PPARγ) in adipose tissue. Meanwhile, similar effects were reported in rats treated with ARBs and fed a normal diet [31]. However, the effects of blocking RAS in multiple sites, i.e. the use of renin antagonists and ARBs need further studies to explore influence on both local and systemic RAS, and the association with obesity and adipogenesis. Collectively, the presented data are preliminary evidence for the role of this concept in NAFLD model in rats.

5. Conclusion

Administration of azilsartan attenuates adipogenesis and obesity in rat's model of high-fat diet induced NAFLD, while aliskiren affects adipogenesis only.

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Conflict of Interest

None declared.

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