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Effects of Azilsartan, Aliskiren or their Combination on High Fat Diet-induced Non-alcoholic Liver Disease Model in Rats

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ABSTRACT

Introduction: In addition to its role in regulation of blood pressure, fluid and electrolyte homeostasis, the renin-angiotensin system (RAS) components were expressed in many other tissues suggesting potential roles in their functions. **Aim:** The present study aims to evaluate the protective effect aliskiren, when used alone or in combination with azilsartan against high fat diet-induced liver disease in rats. **Material and methods:** Thirty-two Wistar male rats, weighing 150-200 gm were allocated evenly into four groups and treated as follow: group I, rats were fed a specially formulated high-fat diet for 8 weeks to induce non-alcoholic liver disease and considered as control group; groups II, III and IV, the rats were administered azilsartan (0.5 mg/kg), aliskiren (25 mg/kg) or their combination orally via gavage tube once daily, and maintained on high fat diet for 8 weeks. The possible treatment outcome was evaluated through measuring serum levels of glucose, insulin, lipid profile, TNF-α, IL-1α and liver enzymes. Additionally, the liver tissue contents of glycogen and lipids and histological changes were also evaluated. **Result:** The results showed that azilsartan significantly improves the studied markers greater than aliskiren, and their combination o has no additive or synergistic effects on the activity of each one of them. **Conclusion:** Both azilsartan and aliskiren protects the rats against high-fat diet induced NAFLD with predominant effects for the former, and their combination showed no beneficial synergistic or additive effects.

Keywords: azilsartan, aliskiren, liver disease model, NAFLD, RAS.

1. INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) describes a wide range of hepatic disorders, ranging from simple steatosis to nonalcoholic steatohepatitis (NASH). It is mostly associated with many metabolic derangements of carbohydrates and lipids that include metabolic syndrome, obesity, insulin resistance, and dyslipidemia (1), with the possibility of subsequent progression to hepatic fibrosis and hepatic cancers (2). Although the renin-angiotensin system (RAS) regulates many physiological processes, it plays a vital role in the pathogenesis of various disorders including metabolic syndrome, diabetes mellitus, and fatty liver diseases (3, 4). Many in vitro and in vivo studies indicated exaggerated RAS activation impairs mitochondrial functions and can be positively correlated with hepatic cirrhosis (5). Many reports supported the concept that modulating RAS activity with angiotensin converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) might be of value in the control of hepatic steatosis (6-8); however, they are inconclusive and conflicting (9, 10), and the effects in NAFLD are speculative rather than definite (11). Moreover, elevated plasma renin and angiotensin II (Ang II) concentrations was reported in experimental animals maintained on high-fat diet (12, 13), while modulation of RAS through genetic knock-out technique attenuates body weight gain in mouse model of high fat diet-induced obesity (14). Additionally, similar results were reported through the use of ARBs and ACEIs in this regard (15, 16). The present study aims to evaluate the effects of aliskiren, azilsartan or their combination on the metabolic and inflammatory changes in rat's model of NAFLD.

2. MATERIALS AND METHODS

Wistar rats initially weighing 160-200 g, obtained from the local bred

of the animal house, College of Pharmacy, University of Baghdad, were used in the present study. The research protocol and animal care procedures were approved by the local research ethics committee of this institution, in compliance with the standard requirements for the care and use of experimental animals reported elsewhere. The animals were acclimatized for one week before starting experiments under 12 h light-dark cycle and controlled room temperature (24°C±2) with standard rat chaw and drinking water *ad libitum*. After 1 week 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 orally 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 0.5 mg/kg azilsartan (Takeda, Japan), 25 mg/kg aliskiren (Novartis, Switzerland) or their combination (formulated as suspension in 5% CMC), one week before start feeding with high fat diet formula as mentioned previously for the control group for 60 days. All rats were euthanized on day 60, after overnight fasting, by intraperitoneal injection of 2% thiopental (80 mg/kg). Blood samples were collected and utilized to prepare the serum, which was either processed immediately or stored at -40°C until assay. Serum levels of glucose, insulin, the lipid profile, ALT, AST and ALP were measured as described previously (17). Moreover, serum concentrations of TNF-α and IL-1β were assayed utilizing ready-made kits (MyBiosource, Inc, USA) (18, 19). The liver glycogen content was estimated according to the method of Chan and Exton (20), while glucose was enzymatically estimated using hexokinase method. A portion of the liver was fixed in 10% formalin for histology. According to the method of Junqueira *et al* (1995) (21), various liver tissue sections were prepared and stained with hematoxylin and eosin or Mason Trichrome stain. The stained sections were evaluated in a blind fashion by senior pathologist. The data were evaluated using Graph Pad Prism 5.1 software (Graph Pad Software Inc, California, US); the changes were analyzed by repeated measures ANOVA. One-way ANOVA, followed by Bonferroni's *post hoc* test, was performed when appropriate. A probability value of $p<0.05$ was considered statistically significant.

3. RESULTS

Table 1 demonstrated that serum glucose was elevated in control group compared with reference normal range values. Azilsartan or its combination with aliskiren significantly decreased serum glucose levels compared with control (39% and 42%, respectively). However, aliskiren alone did not show significantly different effect compared with control group. Serum insulin was elevated in control group compared with reference normal range values, and all treatments significantly decreased serum insulin levels compared with control (40%, 17% and 44%, respectively). Table 1 also showed that lipid profile markers were dysregulated (except for HDL-c) in control group. Azilsartan or its combination with aliskiren significantly decrease serum TG compared with control (58% and 53%, respectively). All treatment approaches decreased serum cholesterol compared with control (48%, 24% and 48%, respectively). However, the effect of aliskiren was significantly lower than those reported in the other two treatment groups. Meanwhile, serum LDL-c levels were significantly decreased by all treatments compared with control (51%, 28% and 45%, respectively). In Table 2, Azilsartan or its combination with Aliskiren significantly and comparably decreased liver content of cholesterol (33% and 30%, respectively). Meanwhile, aliskiren significantly elevates liver cholesterol content compared with other groups. High-fat diet remarkably elevates liver TG content compared with reference range. Azilsartan

Treatment type	Glucose mg/dl	Insulin mU/ml	Cholesterol mg/dl	Triglyceride mg/dl	LDL-c mg/dl	HDL-c mg/dl
Control	257.5±41.7^a	27.7±4.0^a	129.1±16.6^a	133.8±44.2^a	99.1±8.4^a	28.4±11.3^a
Azilsartan	157.5±17.5^b	16.7±1.7^b	67.3±10.4^b	56.3±15.8^b	48.5±3.2^b	36.0±9.0^a
Aliskiren	189.4±12.7^b	22.9±2.8^c	93.6±13.2^c	98.5±16.1^c	71.5±11.0^c	24.1±8.7^a
Combination	148.1±18.5^b	15.4±2.4^b	67.5±6.5^b	63.4±15.9^b	54.8±8.3^b	32.8±6.2^a

Table 1. Effects of azilsartan, aliskiren or their combination on serum glucose, insulin and lipid profile of rats with high-fat induced NAFLD Values were expressed as mean±SD; n= 8 rats in each group; values with non-identical super scripts (a,b,c) were significantly different among different groups (P<0.05).

Treatment type	Cholesterol mg/g tissue	Triglyceride mg/g tissue	Glycogen mg/g tissue
Control	201.1± 25.8a	173.0± 37.7a	3.2± 0.7a
Azilsartan	134.8± 22.7b	120.8± 21.5b	6.0± 1.1b
Aliskiren	253.8± 21.1c	187.6± 32.2a	3.8± 0.4a
Combination	140.4± 25.8b	139.4± 24.6c	6.3± 1.0b

Table 2. Effects of azilsartan, aliskiren or their combination on liver contents of cholesterol, triglycerides and glycogen in high-fat induced NAFLD rats. Values were expressed as mean±SD; n= 8 rats in each group; values with non-identical super scripts (a,b,c) were significantly different among different groups (P<0.05).

Treatment type	ALT U/L	AST U/L	ALP U/L	ALT/ULN	ALP/ULN
Control	103.5±14.9a	208.8±25.2a	296.5±51.2a	3.4±0.5a	2.3±0.4a
Azilsartan	51.4±8.2b	69.1±10.9b	207.8±16.5b	1.7±0.3b	1.6±0.1b
Aliskiren	73.3±12.2c	97.0±11.5c	242.8±48.1b	2.4±0.4c	1.9± 0.4b
Combination	53.3±6.9b	69.1±11.9b	208.1±27.9b	1.8±0.2d	1.4±0.3b

Table 3. Effects of azilsartan, aliskiren or their combination on serum levels of liver enzymes, ALT/ULN and ALP/ULN ratios in high-fat induced NAFLD rats.. Values were expressed as mean±SD; n= 8 rats in each group; values with non-identical super scripts (a,b,c) were significantly different among different groups (P<0.05).

Treatment type	TNF-α pg/ml	IL-1β pg/ml
Control	45.4±7.9[a]	33.4±4.5[a]
Azilsartan	16.6±8.3[b]	16.4±3.5[b]
Aliskiren	34.9±12.4[a]	25.9±4.2[a]
Combination	18.6±4.2[b]	19.2±1.7[b]

Table 4. Effects of azilsartan, aliskiren or their combination on serum levels of TNF-α and IL-1β in high-fat induced NAFLD rats. Values were expressed as mean±SD; n= 8 rats in each group; values with non-identical super scripts (a,b) were significantly different among different groups (P<0.05).

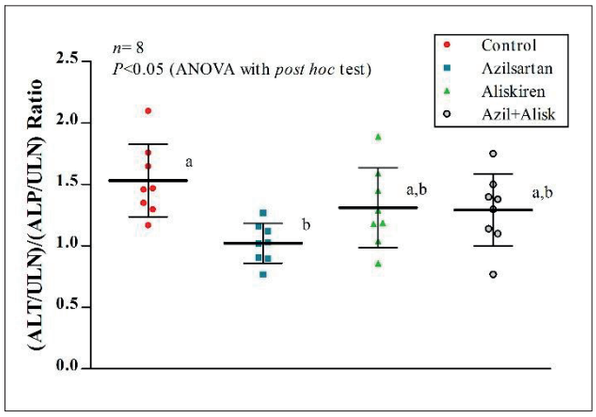

Figure 1. Effects of azilsartan, aliskiren, or their combination on (ALT/ULN)/(ALP/ULN) ratio in rat's model of high-fat diet induced NAFLD; n= 8 rats in each group; values with non-identical letters (a,b) are significantly different (p<0.05); ULN: Upper Limit Normal value.

significantly decreased liver TG content compared with control (30%) while aliskiren non-significantly elevates liver TG compared with control. Azilsartan or its combination with aliskiren significantly improves the already depressed glycogen liver content compared with control (89% and 103%, respectively), while aliskiren did not change this marker (Table 2). All treatment approaches significant decreased serum ALT activity compared with control (50%, 29% and 49%, respectively) (Table 3). Similar effects were reported on the elevated serum AST and ALP activities. In Figure 1, analysis of (ALT/ULN)/(ALP/ULN) ratio revealed that azilsartan significantly decreases this ratio compared with the control (33%). Although the other treatment approaches produced comparable effects compared to azilsartan, their effects did not significantly differ from that reported in control. In Table 4, azilsartan or its combination with aliskiren significantly decreased serum TNF-α levels compared with control group (63.4% and 59%, respectively). Meanwhile, all treatments significantly decreased serum IL-1β levels (51%, 22% and 43%, respectively). The histopathologic findings revealed profound fatty changes and intracytoplasmic water vacuoles with hazy boundaries, especially in the cetrilobular and midzonal areas (Figure 2A). Moreover, lobular coagulative necrosis was noticed in the control group with minimum spectrum and mostly associated with few multifocal areas of infiltration with inflammatory cells. Furthermore, early signs of fibrosis were reported in the control group (Figure

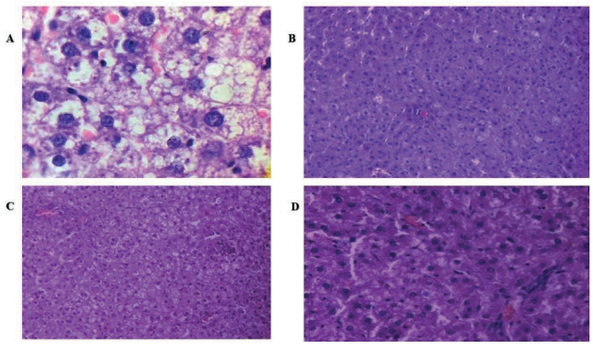

Figure 2. Histopathological features of liver tissue samples stained with Hematoxylin & Eosin. A: Control group showed fatty liver changes in rats on high fat diet, with ballooned hepatocytes containing Mallory's hyaline-like material and Kupffer cells were also observed (100X); B: Azilsartan treated group (20X); C: Aliskiren treated group (20X); D: Combination treated group (40X).

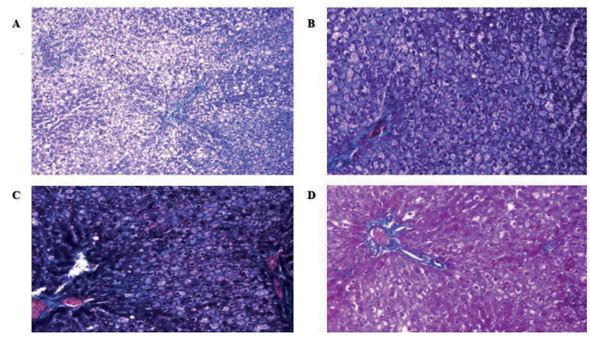

Figure 3. Histopathological features of liver tissue samples stained with Masson trichrome stain. A: Control group showed hepatic periportal fibrosis and macrovesicular steatosis (10X); B: Azilsartan treated group (20X); C: Aliskiren treated group (20X); D: Combination treated group (40X).

3A). Compared with the control, the azilsartan-treated group showed best protection against the previously mentioned histopathologic changes (Figure 2B). The groups treated with aliskiren and combination showed less steatosis and ballooning but with mild inflammation (Figures 3C and 3D).

4. DISCUSSION

In addition to its influence on fluid homeostasis and regulation of blood pressure, the RAS plays a role in the pathophysiology of insulin resistance and NAFLD (22), while blockade of RAS improves insulin signaling and regulates adipocyte proliferation (23). In the present study, glycemic control was in tune with the previously reported data (24, 25), where azilsartan and aliskiren improve insulin sensitivity and decreases serum glucose level. This may be due to the attenuation of Ag II-mediated vasoconstriction and/or elevating the levels of vasodilators like prostaglandins or nitric oxide, associated with increased blood flow and glucose delivery to the tissues (26). Moreover, sensitivity of liver to insulin was effectively improved through decreasing Ag-II levels, which can be attributed to various mechanisms including interference with insulin signaling cascade, ROS production or TNF-α overproduction (27). It has been

reported that azilsartan have insulin sensitizing effect in obese Koletsky rats, which was attributed to activation of PPARγ in adipose tissues (25); meanwhile, aliskiren improves systemic insulin resistance in mice maintained on high fat diet and ameliorates fatty changes in the liver (28). These data supported the finding of the present study. In this regard, Lastra *et al* showed that aliskiren may improve insulin signaling in muscles through increased GLUT4 expression and attenuated oxidative stress (29, 30). Accordingly, blockade of RAS in hepatic tissues might be of value to improve lipid profile and limits progression of NAFLD. In this regard, telmisartan decreases triglycerides and increases HDL-c without significant effects on LDL-c in rat model of high-fat diet induced obesity (31), while treatment of Dahl salt-sensitive rats maintained on a high-fat with azilsartan decreases total cholesterol with minor influence on triglycerides (32). The results obtained in the present study were in tune with the previously reported evidence (33). Local RAS was expressed by many tissues and organs including the liver, which may generate Ang II level three to five times more than that found in plasma (34). Therefore, locally elevated Ang II may enhance lipid disorders that may definitely predispose to hepatic degenerations (35). The present study clearly showed the protective effects of azilsartan and aliskiren against high-fat diet induced hepatic damage, which were in tune with those reported for various RAS modulators, where they effectively ameliorate NAFLD symptoms in experimental and clinical settings (36-38). However, failure to achieve synergistic or even additive effect when azilsartan and aliskiren are concomitantly administered may be attributed to the complex nature of both activation and blockade of RAS, where many factors and signaling pathways may be directly or indirectly involved (39). It may also be attributed to insufficient time for effective inhibition of renin activity. In the present study, the ALT/ALP ratio indicates that liver damage is mostly of cholestatic nature rather than hepatocellular, and the use of azilsartan alone produced significant changes unlike the two other groups, which might be attributed to different mode of RAS blockade and other unrevealed mechanisms. The pleotropic effect of azilsartan impacts triglyceride and cholesterol content, and was in tune with the previously reported data that genetic alteration in RAS components dampens hepatic steatosis in rodent model (14). Interestingly, the use of aliskiren alone did not affect a hepatic lipid which was in tune with a previously reported data (33). The elevated liver content of cholesterol and triglyceride may be attributed to compensatory negative feedback increment in renin synthesis. Furthermore, it was found that aliskiren decreases steatosis only at higher doses with up-regulation PPAR-α (38). In the animal model of NAFLD used in this study and due to insulin resistance, glycogen content was significantly reduced. However, blocking RAS function with azilsartan or its combination with aliskiren elevated the liver tissue glycogen content, indicating improvement in insulin sensitivity. Meanwhile, aliskiren alone did not elevate glycogen content significantly, which may be attributed to the different ways in the mode of RAS blockade and other not yet explored mechanisms. Adipose tissue is one of the major sources of many cytokines including TNF-α, and its expression was highly exaggerated in NAFLD and potentially involved in inflammatory and metabolic changes (40, 41). The present study clearly demonstrates elevation of TNF-α and IL-1β in control group of high-fat diet induced NAFLD. However, RAS blockade significantly decreases the elevated serum levels of these inflammatory mediators, and seems consistent with those previously reported by others (42, 43). Furthermore, blocking of local AT1R may prevent fibrosis and attenuate the cycle that links steatosis with necrotizing inflammation (23). The liver lesions of NAFLD include steatosis, apoptosis, and fibrosis. In the present study, the control group developed NASH reflected by the presence of Mallory-Denk bodies, with early signs of fibrosis. These two features were absent in the treated groups, with less steatosis and hepatic ballooning, which indicate the protective effect of azilsartan, aliskiren and their combination against the disease progress. These results were in tune with many previous data (28, 44). Noteworthy, azilsartan alone demonstrates more protective effect compared with aliskiren and shows no lobular inflammation, which may be attributed to the difference in RAS blockade and other not yet revealed mechanisms.

5. CONCLUSION

Azilsartan and aliskiren can effectively protect the liver against high-fat diet induced NAFLD in rats, and their combination offers no additive or synergistic benefits in this respect.

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REFERENCES

1. Cohen JC, Horton JD, Hobbs HH. Human fatty liver disease: Old questions and new insights. Science. 2011; 332(6037): 1519-23. doi: 10.1126/science.1204265.
2. Malhotra N, Beaton MD. Management of non-alcoholic fatty liver disease in 2015. World J. Hepatol. 2015; 7(30): 2962-7. doi: 10.4254/wjh.v7.i30.2962.
3. Moreira de Macedo S, Guimaraes TA, Feltenberger JD, Sousa Santos SH. The role of renin-angiotensin system modulation on treatment and prevention of liver diseases. Peptides. 2014; 62: 189-96. doi:10.1016/j.peptides.2014.10.005.
4. Wang CH, Li F, Takahashi N. The renin angiotensin system and the metabolic syndrome. Open Hypertens. J 2010; 3: 1-13. doi:10.2174/1876526203010001.
5. Matthew Morris E, Fletcher JA, Thyfault JP, Rector RS. The role of angiotensin II in nonalcoholic steatohepatitis. Mol. Cell. Endocrinol. 2013; 378: 29-40. doi: 10.1016/j.mce.2012.04.013.
6. Jonsson JR, Clouston AD, Ando Y, Kelemen LI, Horn MJ, Adamson MD, et al. Angiotensin converting enzyme inhibition attenuates the progression

of rat hepatic fibrosis. Gastroenterology. 2001; 121: 148-55.
7. Kishina M, Koda M, Kato J, Tokunaga S, Matono T, Sugihara T, et al. Therapeutic effects of the direct renin inhibitor, aliskiren, on non-alcoholic steatohepatitis in fatty liver Shionogi ob/ob male mice. Hepatol Res. 2014; 44(8): 888-96. doi: 10.1111/hepr.12186.
8. Frantz ED, Penna-de-Carvalho A, Batista Tde M, Aguila MB, Mandarim-de-Lacerda CA. Comparative effects of the renin-angiotensin system blockers on nonalcoholic fatty liver disease and insulin resistance in C57BL/6 mice. Metab Syndr Relat Disord. 2014; 12(4): 191-201. doi: 10.1089/met.2013.0129.
9. Cholongitas E, Vibhakorn S, Lodato F, Burroughs AK. Angiotensin II antagonists in patients with recurrent hepatitis C virus infection after liver transplantation. Liver Int 2010; 30: 334-5. doi: 10.1111/j.1478-3231.2009.02059.x.
10. Corey KE, Shah N, Misdraji J, Abu Dayyeh BK, Zheng H, and Bhan AK, et al. The effect of angiotensin-blocking agents on liver fibrosis in patients with hepatitis C. Liver Int. 2009; 29: 748-53. doi: 10.1111/j.1478-3231.2009.01973.x.
11. Paschos P, Tziomalos K. Nonalcoholic fatty liver disease and the renin-angiotensin system: Implications for treatment. World J. Hepatol. 2012; 4: 327-31. doi: 10.4254/wjh.v4.i12.327.
12. Benson SC, Pershadsingh HA, Ho CI, Chittiboyina A, Desai P, Pravenec M, et al. Identification of telmisartan as a unique angiotensin II receptor antagonist with selective PPARγ-modulating activity. Hypertension. 2004; 43: 993-1002. doi: 10.1161/01.HYP.0000123072.34629.57.
13. Boustany CM, Bharadwaj K, Daugherty A, Brown DR, Randall DC, Cassis LA. Activation of the systemic and adipose rennin-angiotensin system in rats with diet-induced obesity and hypertension. Am J Physiol. 2004; 287: 943-9. doi:10.1152/ajpregu.00265.2004.
14. Takahashi N, Li F, Hua K, Deng J, Wang CH, Bowers RR, et al. Increased energy expenditure, dietary fat wasting, and resistance to diet-induced obesity in mice lacking renin. Cell Metab. 2007; 6(6): 506-12. doi:10.1016/j.cmet.2007.10.011.
15. Velkoska E, Warner FJ, Cole TJ, Smith I, Morris MJ. Metabolic effects of low dose angiotensin converting enzyme inhibitor in dietary obesity in the rat. Nutr Metab Cardiovasc Dis. 2010; 20(1): 49-55. doi:10.1016/j.cmet.2007.10.011.
16. Utba, RM, Hussain SA, Fadhil AA, Ahmed A. Effect of azilsartan, aliskiren or their combination on body and organs weight and adipogenesis of high-fat diet induced non-alcoholic fatty liver disease in rats. Am J Pharmacol Sci. 2016; 4(3): 31-4. doi:10.12691/ajps-4-3-1
17. Qi NR, Wang J, Zidek V, Landa V, Mlejnek P, Kazdova L, et al. A new transgenic rat model of hepatic steatosis and the metabolic syndrome. Hypertension. 2005; 45: 1004-11.
18. Brenner DA, O'Hara M, Angel P, Chojkier M, Karin M. Prolonged activation of JUN and collagenase genes by tumor necrosis factor-alpha. Nature. 1989; 337: 661-3. doi:10.1038/337661a0.
19. Pathak S, Goldofsky E, Vivas EX, Bonagura VR, Vambutas A. IL-1-beta is overexpressed and aberrantly regulated in corticosteroid non-responders with autoimmune inner ear disease. J Immunol. 2011; 186(3): 1870-9. doi: 10.4049/jimmunol.1002275.
20. Chan TM, Exton JH. A rapid method for the determination of glycogen content and radioactivity in small quantities of tissue or isolated hepatocytes. Anal Biochem. 1976; 71: 96-105.
21. Videla LA, Smok G, Troncoso P, Simon KA, Junqueira VB, Fernandez V. Influence of hyperthyroidism on lindane-induced hepatotoxicity in the rat. Biochem Pharmacol. 1995; 50(10): 1557-65.
22. Giacchetti G, Sechi LA, Rilli S, Carey RM. The renin-angiotensin-aldosterone system, glucose metabolism and diabetes. Trends Endocrinol. Metab. 2005; 16(3): 120-6. doi:10.1016/j.tem.2005.02.003.
23. Georgescu EF. Angiotensin receptor blockers in the treatment of NASH/NAFLD: could they be a first-class option? Adv Ther. 2008; 25(11): 1141-74. doi: 10.1007/s12325-008-0110-2.
24. Rong X, Li Y, Ebihara K, Zhao M, Naowaboot J, Kusakabe T, et al. Angiotensin II type 1 receptor-independent beneficial effects of telmisartan on dietary-induced obesity, insulin resistance and fatty liver in mice. Diabetologia. 2010; 53: 1727-31. doi: 10.1007/s00125-010-1744-6.
25. Zhao M, Li Y, Wang J, Ebihara K, Rong X, Hosoda K, et al. Azilsartan treatment improves insulin sensitivity in obese spontaneously hypertensive Koletsky rats. Diabetes Obes Metab. 2011; 13: 1123-9.
26. Blackburn DF, Wilson TW. Antihypertensive medications and blood sugar: Theories and implications. Can J Cardiol. 2006; 22(3): 229-33.
27. Grasso A, Malfatti F, De Leo P, Martines H, Fabris P, Toscanini F, et al. Insulin resistance predicts rapid virological response in non-diabetic, non-cirrhotic genotype 1 HCV patients treated. J Hepatol. 2009; 51(6): 984-90. doi: 10.1016/j.jhep.2009.07.008.
28. Lee KC, Hsieh YC, Yang YY, Chan CC, Huang YH, Lin HC. Aliskiren reduces hepatic steatosis and epididymal fat mass and increases skeletal muscle insulin sensitivity in high-fat diet-fed mice. Sci Rep. 2016; 6: 18899. doi: 10.1038/srep18899.
29. Lastra G, Habibi J, Whaley-Connell AT, Manrique C, Hayden MR, Rehmer J, et al. Direct renin inhibition improves systemic insulin resistance and skeletal muscle glucose transport in a transgenic rodent model of tissue renin overexpression. Endocrinology. 2009; 150(6): 2561-8. doi: 10.1210/en.2008-1391.
30. Wu Y, Ma KL, Zhang Y, Wen Y, Wang GH, Hu ZB, et al. Lipid disorder and intrahepatic renin-angiotensin system activation synergistically contributes to non-alcoholic fatty liver disease. Liver Int. 2016; 36(10): 1525-34. doi: 10.1111/liv.13131.
31. Muller-Fielitz H, Hubel N, Mildner M, Vogt FM, Bakrhausen J, Raasch W. Chronic blockade of angiotensin AT1 receptors improves cardinal symptoms of metabolic syndrome in diet-induced obesity in rats. Br J Pharmacol. 2014; 171: 746-60. doi: 10.1111/bph.12510.
32. Jin C, O'Boyle S, Kleven DT, Pollock JS, Pollock DM, White JJ. Antihypertensive and anti-inflammatory actions of combined azilsartan and chlorthalidone in Dahl salt-sensitive rats on a high-fat, high-salt diet. Clin Exp Pharmacol Physiol. 2014; 41: 579-88. doi: 10.1111/1440-1681.12250.
33. Kang YS, Lee MH, Song HK, Hyun YY, Cha JJ, Ko GJ, et al. Aliskiren improves insulin resistance and ameliorates diabetic vascular complications in db/db mice. Nephrol Dial Transplant. 2011; 26(4): 1194-1204. doi: 10.1093/ndt/gfq579.
34. Paul M, Poyan Mehr A, Kreutz R. Physiology of local renin-angiotensin systems. Physiol Rev 2006; 86: 747-803. doi:10.1152/physrev.00036.2005.
35. Zhang Y, Ma KL, Ruan XZ, Liu BC. Dysregulation of the low-density lipoprotein receptor pathway is involved in lipid disorder-mediated organ injury. Int J Biol Sci. 2016; 12(5): 569-79. doi: 10.7150/ijbs.
36. Kudo H, Yata Y, Takahara T, Kawai K, Nakayama Y, Kanayama M, et al. Telmisartan attenuates progression of steatohepatitis in mice: role of hepatic macrophage infiltration and effects on adipose tissue. Liver Int. 2009; 29: 988-96. doi: 10.1111/j.1478-3231.2009.02006.x.
37. Mohamed AS, Emam HT, Elsayed Shaltout SA. The potential protective effect of captopril and irbesartan on experimentally induced non-alcoholic fatty liver in rats. IJAR. 2015; 12(3): 1168-81.
38. Lee KC, Chan CC, Yang YY, Hsieh YC, Huang YH, Lin HC. Aliskiren attenuates steatohepatitis and increases turnover of hepatic fat in mice fed with a methionine and choline deficient diet. PLoS One. 2013; 8(10): e77817. doi: 10.1371/journal.pone.0077817.
39. Lu H, Balakrishnan A, Howatt DA, Wu C, Charnigo R, Liau G, et al. Comparative effects of different modes of renin angiotensin system inhibition on hypercholesterolemia-induced atherosclerosis. Br J Pharmacol. 2012; 165(6): 2000-8. doi: 10.1111/j.1476-5381.2011.01712.x.
40. Greenberg AS, McDaniel ML. Identifying the links between obesity, insulin resistance and β-cell function: potential role of adipocyte-derived cytokines in the pathogenesis of type 2 diabetes. Eur J Clin Invest. 2002; 32(Suppl. 3): 24-34.
41. Milic S, Mikolasevic I, Krznaric-Zrnic I, Stanic M, Poropat G, Stimac D, et al. Nonalcoholic steatohepatitis: emerging targeted therapies to optimize treatment options. Drug Design Devel Ther. 2015; 9: 4835-45. doi: 10.2147/DDDT.S64877.
42. Dowman JK, Tomlinson JW, Newsome PN. Pathogenesis of non-alcoholic fatty liver disease. Q J Med. 2010; 103: 71-83.
43. de Araújo AA, Varela H, de Medeiros CA, de Castro Brito GA, de Lima KC, de Moura LM, et al. Azilsartan reduced TNF-α and IL-1β levels, increased IL-10 levels and upregulated VEGF, FGF, KGF, and TGF-α in an oral mucositis model. PLoS One. 2015; 10(2) :e01167. doi: 10.1371/journal.pone.0116799.
44. Fayez MFA, Abd El-Rahman MN, Abdin AA, Taha AA. Role of renin angiotensin system and its inhibition by perindopril and irbesartan in non-alcoholic steatohepatitis induced experimentally in rats. Asian J Med Pharm Res. 2011; 1(1): 39-47.