Role of renin angiotensin system and its inhibition by perindopril and irbesartan in non alcoholic steatohepatitis induced experimentally in rats

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ABSTRACT: Non alcoholic steatohepatitis (NASH) is considered to be a hepatic component of metabolic syndrome that includes obesity, type 2 diabetes mellitus, dyslipidaemia and hypertension where insulin resistance forms the corner stone in its pathogenesis. Recently, there is accumulating evidence that renin angiotensin system (RAS) plays an important role in development of insulin resistance, hepatic inflammation and fibrogenesis. Thus, it is of much interest to investigate whether therapy against RAS either by perindopril (as an angiotensin converting enzyme inhibitor; ACEI) or irbesartan (as an angiotensin II type 1 receptor blocker; ARB) could provide beneficial effects in the liver of an animal model of steatohepatitis. The study was conducted on 50 albino Wistar rats weighing 110-130g. The rats were equally classified into 5 groups where group 1 served as normal control group fed ad libitum on control diet (5% of energy derived from fat) and received vehicle of gum acacia for 12 weeks. Steatohepatitis was induced by fed ad libitum on high fat diet (58% of energy derived from fat) for either 4 weeks (group 2) or 12 weeks (group 3). The causal role of RAS in development of NASH was evidenced by the significant progressive increase in ACE activity and its positive correlation with final body weight, hepatic triglycerides, levels of serum ALT and TNF-α as will as HOMA-IR as an indicator for insulin resistance. Treatment of steatohepatitis by oral gavage of either perindopril 3 mg/kg/day (group 4) or irbesartan 50 mg/kg/day (group 5) started from the 5th till the 12th week. Perindopril administration exhibited significant reduction in ACE activity, while irbesartan caused non significant increase in ACE activity. Treatment with either perindopril or irbesartan led to significant decrease in final body weight and an overall amelioration in the biochemical parameters and insulin resistance with significant improvement in histopathological grading. The current study proved that RAS plays a major pathogenic role in development of NASH including insulin resistance, lipid accumulation, inflammatory cytokines and hepatic degeneration. In conclusion, intervention of the biological cascade of RAS by either an ACE inhibitor like perindopril or an ARB like irbesartan could provide a new therapeutic approach for non alcoholic steatohepatitis.

KEYWORDS: Non Alcoholic Steatohepatitis (NASH), Renin Angiotensin System, Perindopril, Irbesartan.

INTRODUCION

Non-alcoholic steatohepatitis (NASH) was initially thought to be a disease of the western world, but it is now clear that the prevalence is very high in many regions, including the area of Middle East. The major risk factors are over nutrition and its resultant disorders: obesity, insulin resistance, glucose intolerance and dyslipidemia (Larter and Yeh, 2008). It is a metabolic disorder related to non-alcoholic fatty liver disease (NAFLD) and characterized by severe fatty infiltration of the liver accompanied by necroinflammatory activity (Filippatos and Elisaf, 2010). In the past, NASH was considered as a benign condition however, it is now believed to cause progressive fibrosis and cirrhosis and unfortunately, the long-term prognosis is not better than that of hepatitis C cirrhosis (van der Poorten and George, 2007). NAFLD is considered the hepatic manifestation of metabolic syndrome; a condition refers to a cluster of features including obesity, glucose intolerance or diabetes, dyslipidemia and hypertension (Varela-Rey et al. 2009). The most accepted theory describing the pathogenesis of NASH is the two-hit theory (Day and James, 1998). According to this theory; high caloric intake, obesity and insulin resistance can be considered the initiating events of hepatic steatosis. Insulin resistance leads to loss of anti lipolytic effect of insulin with excessive release of free fatty acids (FFAs) from visceral adipose tissue to plasma then to the liver. The liver tries to compensate this increased load of FFAs by an increase in hepatic mitochondrial β oxidation but, this is not sufficient to handle the increased load of hepatic FFAs and the remaining FFAs are converted into triglycerides, which are stored in the cytoplasm, causing steatosis (Shifflet and

Wu, 2009). The excess FFAs oxidation in the mitochondria acts as a source of reactive oxygen species (ROS), stimulating the immune response leading to infiltration by inflammatory cells and activation of Kupffer cells to release cytokines particularly tumor necrosis factor α (TNF-α). Inflammatory cells attract fibrogenic cell types such as hepatic stellate cells (HSCs) to initiate collagen deposition (Shifflet and Wu, 2009). Among NASH patients 50–70% are hypertensive, so there must be a link between hypertension and NASH. The overactive renin angiotensin system (RAS) seems to be that link (van der Poorten and George, 2007). Recently, there is accumulating evidence that renin angiotensin system (RAS) plays an important role in the development of insulin resistance, hepatic inflammation and fibrogenesis (Toblli et al. 2008). Angiotensin II interferes with insulin signaling through increasing serine/threrionine phosphorylation of insulin receptor substrates (IRS). Regarding oxidative stress Ang II stimulates NADPH oxidase inducing significant hepatic ROS generation, lipid peroxidation, inflammatory cell infiltration, apoptosis, and fibrosis (Wei et al. 2008; Olivares-Reyes et al. 2009). Considering these facts, it is of interest to investigate whether therapy against renin angiotensin system either by perindopril (as an angiotensin converting enzyme inhibitor) or by irbesartan (as an angiotensin II type 1 receptor blocker could provide beneficial effects in the liver of an animal model of steatohepatitis.

MATERIAL AND METHODS

Drugs and chemicals:

Perindopril (Coversyl®, 5 mg tablet) was purchased as a product of Servier Pharmaceutical Co., Egypt; dissolved in distilled water to a final concentration of 1.2 mg/ml. Irbesartan (Aprovel®, 150 mg tablet) was purchased as a product of Sanofi-Aventis Egypt; suspended in gum acacia (6 gm/100 ml) to a final concentration of 20 mg/ml. The other chemicals and reagents (Sigma-Aldrich) are of analytical gradient.

Animal groups and treatment protocols:

The study was carried out on 50 Albino Wistar rats weighing initially 110-130 g were kept in the department animal house with free access to water during the whole period of the work. The handling and experimental dealing with the used rats was conducted in accordance to the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) to minimize animal suffering. The rats were equally classified into 5 groups where group 1 served as normal control group fed ad libitum on control diet (5% of energy derived from fat, 18% from proteins, and 77% from carbohydrates; 3.3 kcal/g) and received vehicle of gum acacia for 12 weeks. The groups of untreated induced

steatohepatitis fed ad libitum on high fat diet (58% of energy derived from fat, 18% from protein, and 24% from carbohydrates; 5.6 kcal/g) for either 4 weeks (group 2) or 12 weeks (group 3). The groups of treated induced steatohepatitis for 12 weeks either received oral gavage of perindopril (group 4) in a dose of 3 mg/kg/day or irbesartan (group 5) in a dose of 50 mg/kg/day. The doses were selected based on a previous study by Toblli et al. (2008). The treatment protocol started from the 5th till the 12th week. The control and high fat diets were prepared every two weeks, where the ingredients of each diet were mixed, formed into dough with water, rolled into small pellets, kept in plastic bags and stored at -20º C until use to minimize oxidation (Surwit et al. 1996; Svegliati-Baroni et al. 2006).

At the end of the work, rats were weighted to determine final body weight then sacrificed by decapitation and blood samples were collected, centrifuged at 1,000 ×g for 10 minutes to obtain serum for biochemical analysis. Liver of each rat was dissected immediately, washed with ice cold saline. The right lobe was excised and stored at -20˚C for further assay of hepatic triglycerides level and the remained part was preserved in 10% formalin for histopathological examination and grading.

- Assay of serum angiotensin converting enzyme (ACE) activity (U/L) was measured spectrophotometrically according to method described by Ronca-Testoni (1983). Briefly, 50-100 μl of serum was added to 0.5 ml of a substrate-buffer solution [1.6-2.0 mmol FAPGG (2-furanacryloyl-L-phenylalanylglycylglycine), 0.6 mol of NaCl, 100 mmol of Tris, pH 8.2]. Distilled water was added to a final volume of 1 ml. Incubate at 37 °C and measure the absorbance after 3, 15 and 20 minutes at 328 nm. Determine ΔA/min and convert it into U/L according to the following formula: ACE activity (U/L) = (ΔA /min × Vt × 1000) \ (0.5 × Vs) where; Vt is the final assay volume (= 1 ml), Vs is the sample volume, 0.5 is the millimolar ΔA of FAPGG hydrolysis. One unit of ACE is the amount of enzyme that converts 1 μmol of FAPGG into FA-Phe and Gly-Gly per minute at 37°C.

- Hepatic triglycerides level was assayed by processing a fragment weighted 200 mg from the right lobe of the liver by homogenization in 20 volumes of propan-2-ol, then shaken in an orbital shaker for 45 min and centrifuged at 3,000 ×g for 10 min (Raubenheimer et al. 2006). The obtained supernatant was assayed spectrophotometerically at 505 nm for triglyceride content using a commercial triglyceride kit (EliTech diagnostics, France) according to principle described by Fossati and Perencipe (1982). Results were expressed as mg/g liver tissue.

- Fasting blood glucose (mg/dl) was measured spectrophotometerically at 505 nm according to method of

Trinder (1969) using the available commercial kit (Spinreact, Spain).

- Fasting serum insulin (µU/ml) was measured based on the direct sandwich technique using ELISA kit for rats (Chrestal Chem Inc., USA) following procedure described in the protocol.

- Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) was used as an indicator for insulin resistance and calculated according to formula described by Matthews et al. (1985); [HOMA-IR = Fasting plasma insulin concentration (mU/l) × Fasting blood glucose (mmol/l) / 22.5].

- Serum alanine aminotransferase (ALT) (U/L) was measured spectrophotometerically at 546 nm according to method of Reitman and Frankel (1957) using available commercial kit (Spectrum Diagnostics, Germany).

- Tumor necrosis factor alpha (TNF-α) (pg/ml) was assayed based on the direct sandwich technique using ELISA kit for rats (Ray Biotech, Inc., USA) following procedure described in the protocol.

- Histopathological examination and grading: The extracted livers were fixed in paraffin impeded sections and stained with H&E for light microscopic examination. The severity (Mild, Moderate, Severe) of NASH was determined according to grading system described by Brunt et al. (1999) considering inclusion of steatosis, ballooning degeneration of hepatocytes, lobular and portal inflammation.

2.3. Statistical Analysis:

Values were presented as means±S.E.M. The values were tested for normality of distribution. Independent samples t-test was used to evaluate difference between two parametric arithmetic means and Mann-Whitney U test was used for non parametric values. The labeled independent frequencies of histopathological grading between different groups were estimated using Chi-square test. Pearson's correlation coefficient was used for parametric results, while non parametric results were correlated using spearman's rank correlation. The difference was considered to be statistically significant at P values <0.05. The statistical analysis was processed using Statistical Package for the Social Sciences (SPSS) for Microsoft windows, version 10.0.

RESULTS

When compared to control group, induction of non-alcoholic steatohepatitis by high fat diet feeding for 4 weeks (group 2) resulted in a significant increase in final body weight (210.5±6.3 Vs 174.2±5.6) (Fig. 1), serum ACE activity (48.4±1.6 Vs 37.9±1.4) (Fig. 2), hepatic triglycerides, fasting blood glucose, serum insulin, HOMA-IR, serum ALT and TNF-α (table 1). In comparison to group 2 that fed on high fat diet for 4 weeks, group 3 that fed on high fat diet for 12 weeks,

revealed non significant increase in final body weight (220.5±7.3 Vs 210.5±6.3), with further significant increase in serum ACE activity (53.2±1.5 Vs 48.4±1.6), hepatic triglycerides, fasting blood glucose, serum insulin, HOMA-IR, serum ALT and TNF-α. Serum ACE activity of untreated NASH either in group 2 or group 3 showed significant positive correlation with all of the studied parameters (Table 2).
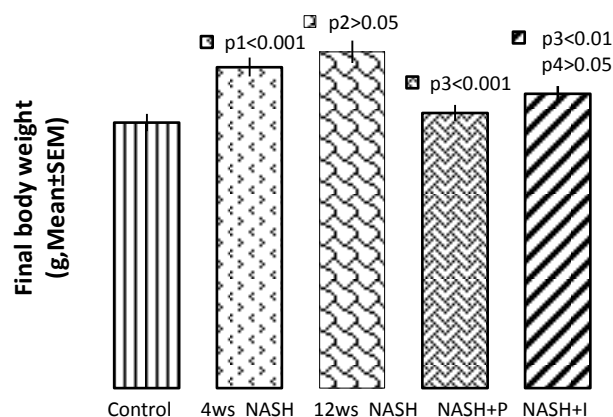

Fig. 1. Comparison between values of the final body weight (g) in the different studied groups. P1: compared to control group, P2: compared to 4 weeks NASH group, P3: compared to 12 weeks NASH group, P4: compared to perindopril treated group.
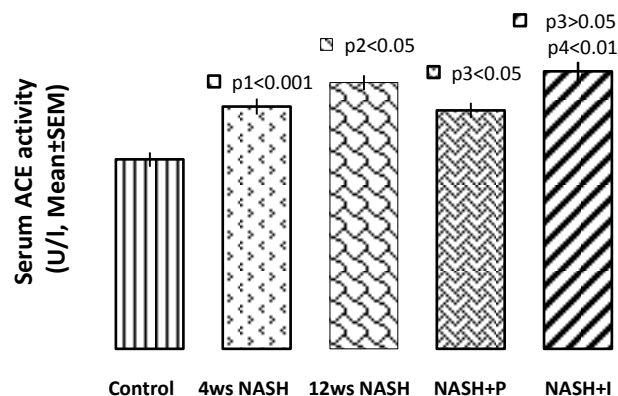

Fig. 2. Comparison between values of serum angiotensin converting enzyme (ACE) activity (U/L) in the different studied groups. P1: compared to control group, P2: compared to 4 weeks NASH group, P3: compared to 12 weeks NASH group, P4: compared to perindopril treated group.

Treatment by either perindopril (group 4) or irbesartan (group 5) caused a significant reduction in final body weight (180.4±5.2 Vs 220.5±7.3 and 193.0±5.0 Vs 220.5±7.3; respectively), hepatic triglycerides, fasting blood glucose, serum insulin, HOMA-IR, serum ALT and TNF-α when compared to 12 weeks untreated NASH

(group 3). Only perindopril treatment caused significant decrease in serum ACE activity (47.7±1.5 Vs 53.2±1.5), while irbesartan treatment resulted in non-significant change in it (55.4±1.9 Vs 53.2±1.5).

When comparing irbesartan treated group to perindopril treated group, there was a significant increase in serum ACE activity (55.4±1.9 Vs 47.7±1.5), with non significant difference in final body weight (193.0±5.0 Vs 180.4±5.2), hepatic triglycerides, fasting blood glucose, serum insulin, HOMA-IR, serum ALT and TNF-α levels.

Histopathological findings (Fig. 3-7) revealed that feeding on high fat diet led to diffuse macro vesicular steatosis, hepatocyte ballooning and degeneration with multiple foci of inflammatory cell infiltration. Treatment by either perindopril or irbesartan decreased hepatic fatty infiltration and caused significant improvement in the histopathological grading when compared to the untreated 12 weeks NASH group (Table 3).
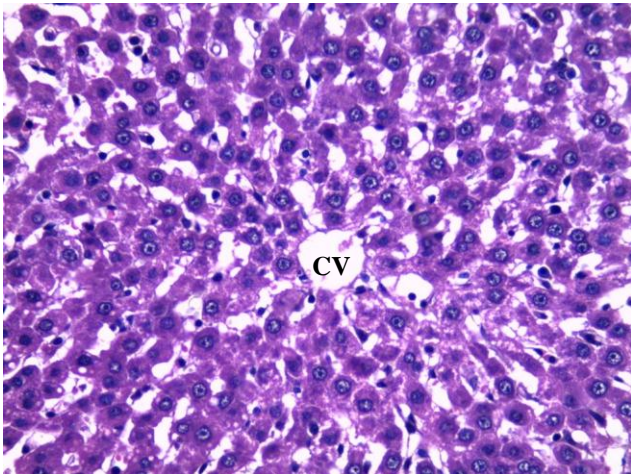

Fig. 5. Section in liver of group 3 (12 weeks NASH) showing severe steatosis with marked ballooning degeneration of hepatocytes (arrows) (HX&E ×100).

Fig. 3. Section in liver of group1 (control group) showing normal liver architecture of central vein (CV) surrounded by normal hepatocytes) (HX&E ×100).

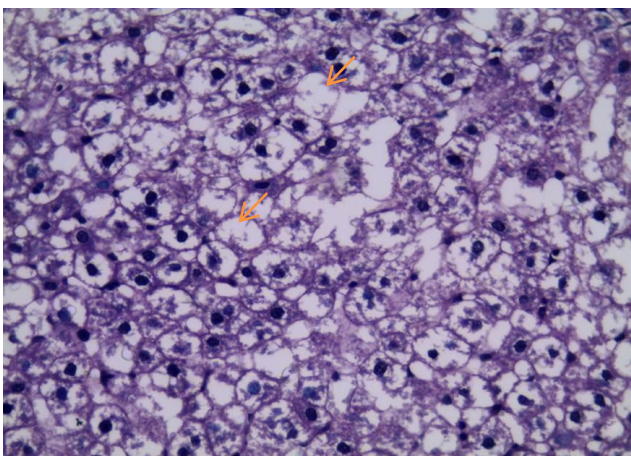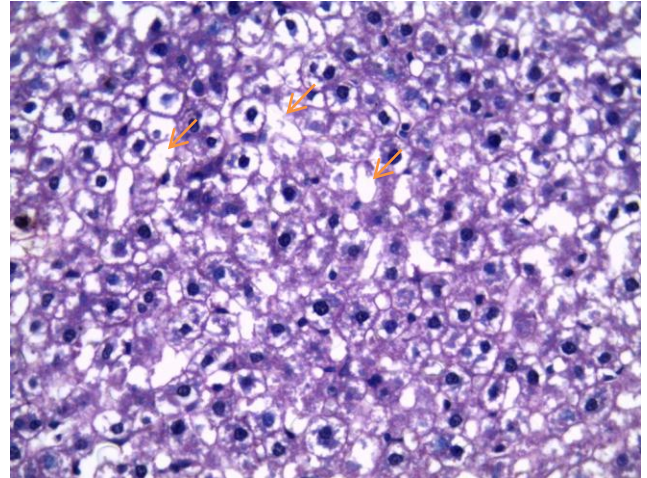**Fig. 6.** Section in liver of group 4 treated by perindopril showing mild steatosis (arrow) with no inflammatory infiltrate (HX&E ×100).

Fig. 4. Section in liver of group 2 (4 weeks NASH) showing moderate steatosis with ballooning degeneration of hepatocytes (arrows) (HX&E ×100).

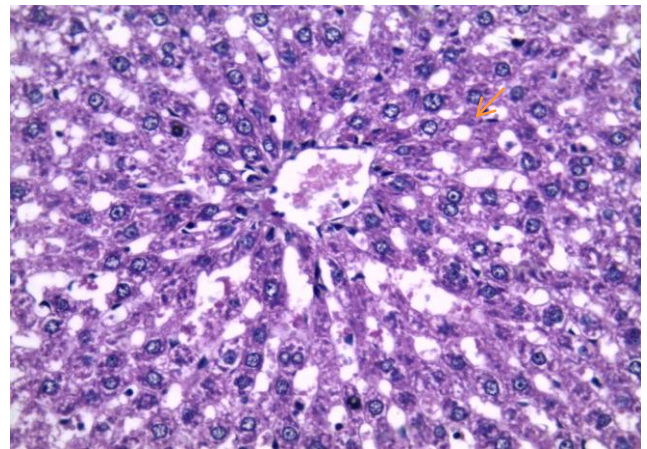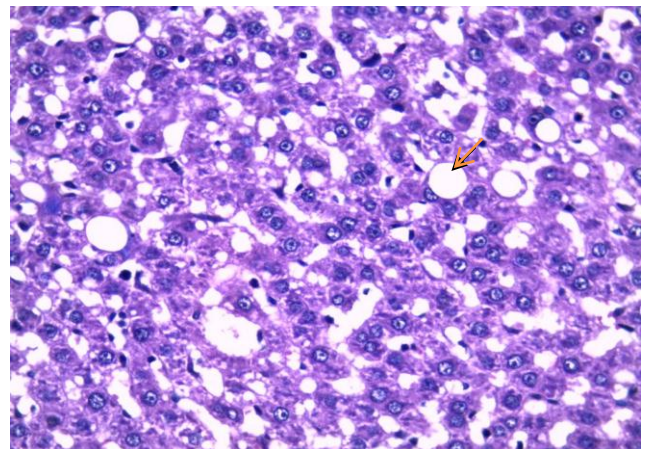**Fig. 7.** Section in liver of group 5 treated by irbesartan showing mild steatosis (arrow) with no inflammatory infiltrate (HX&E ×100).

Table 1. Comparison between the different studied groups for the studied parameters.

Groups / Parameter	Group 1 Control	Group 2 4 weeks NASH	Group 3 12 weeks NASH	Group 4 NASH + perindopril	Group 5 NASH + Irbesartan
Hepatic triglycerides (mg/g liver tissue)	14.6± 0.5	23.5± 0.3 P1<0.001	26.6± 0.4 P2<0.001	18.6± 0.7 P3<0.001	19.1±0.7 P3<0.001 P4>0.05
Fasting blood glucose (mg/dl)	90.2 ±1.8	162.8± 8.8 P1<0.001	192.7± 7.6 P2<0.05	131.0±2.9 P3<0.001	118.6± 5.2 P3<0.001 P4>0.05
Serum insulin (µU/ml)	18.2 ± 1.1	31.2± 0.5 P1<0.001	33.6± 1.0 P2<0.05	29.1± 0.6 P3<0.01	29.7± 0.6 P3<0.01 p4>0.05
(HOMA-IR)	4.0± 0.3	12.5± 0.7 P1<0.001	15.9± 0.4 P2<0.001	9.4± 0.2 P3<0.001	8.7± 0.2 P3<0.001 P4>0.05
Serum ALT (U/L)	30.7±3.1	41.2±2.1 P1<0.05	46.9±1.6 P2<0.05	36.4±1.8 P3<0.001	38.7±2.2 P3<0.01 P4>0.05
Serum TNF-α (pg/ml)	30.7± 1.0	51.6± 2.2 P1<0.001	61.5± 1.2 P2<0.01	44.1± 1.5 P3<0.001	45.7± 1.3 P3<0.001 P4>0.05

The values expressed as mean±S.E.M. P1: compared to control group, P2: compared to 4 weeks NASH group, P3= compared to 12 weeks NASH group 3, P4=compared to perindopril treated group.

Table 2. Correlation between serum ACE activity and the different studied parameters in the untreated NASH groups

Parameter	r value of 4 weeks NASH group	r value of 12weeks NASH group
Final body weight	0.966[**]	0.748[*]
Hepatic triglycerides	0.756[*]	0.874[**]
HOMA-IR	0.746[*]	0.821[**]
Serum ALT	0.795[**]	0.733[*]
Serum TNF-α	0.831[**]	0.724[*]

* Significance at P< 0.05, ** Significance at P< 0.01

Table 3. Comparison between histopathological grading in high fat diet and drug treated groups

Groups / Parameter	Group 2 4 weeks NASH	Group 3 12weeks NASH	Group 4 NASH + Perindopril	Group 5 NASH + Irbesartan
Histopathological grading (Mild/Moderate/Severe)	(1 / 8 /1)	(1 / 4 /5) P1>0.05	(8 / 2 / 0) P2<0.01	(7 / 3 / 0) P2<0.01 P3>0.05

P1: compared to 4 weeks NASH group, P2: compared to 12 weeks NASH group, P3: compared to perindopril treated group.

DISCUSSION

The present study evidenced the implication of renin-angiotensin system in the underlying pathogenesis of NASH, where there was a significant increase in angiotensin converting enzyme (ACE) activity in rats received high-fat diet for 4 weeks with a progressive worsening in the group received this diet for 12 weeks, a result pointing to the extended role of RAS during the entire course of the disease. This causal role of RAS in NASH was further confirmed by its significant positive correlation with the biochemical parameters including levels of serum ALT and TNF-α, hepatic triglycerides as well as HOMA-IR as an indicator of insulin resistance. This role was investigated in a previous study by Velkoska et al. (2010). Also, in concordance with our results, other studies reported that NASH was established in a progressive way by high fat diet for 4 weeks and 12 weeks; respectively (Svegliati-Baroni et al., 2006; Koppe et al., 2009). This was indicated by the significant progressive increase in hepatic triglycerides accompanied

with overweight, fasting blood glucose, serum insulin, TNF-α in addition to development of insulin resistance. Varela-Rey et al. (2009) emphasized that the sustained release of FFAs by adipocytes as well as high insulin and glucose levels due to insulin resistance augments load of hepatic FFAs with a subsequent increment in hepatic mitochondrial β-oxidation and the remaining FFAs are converted into triglycerides, which are stored in the cytoplasm, causing steatosis. In an attempt to explain the link between renin-angiotensin system and development of NASH, De Kloet et al. (2010) reported that white adipose tissue -rich in triglycerides- expresses all elements of renin-angiotensin system including, angiotensinogen, renin, ACE, as well as AT1 and AT2 receptors. On the other hand, RAS contributes to adipocyte hypertrophy and also increases triglyceride content through increasing activities of the enzymes that promote lipogenesis namely, glycerol-3-phosphate dehydrogenase and fatty acid synthase (De Kloet et al. 2010). In regard to the close association of triglycerides to the severity of NASH (Kashyap et al. 2009); therefore, when mass of white adipose tissue increases due to overweight, it can act as a local generator of Ang II. Ang II was demonstrated to decrease insulin sensitivity by several proposed mechanisms, one mechanism of them is that Ang II interferes with insulin signaling through increasing serine/threrionine phosphorylation of insulin receptor substrates (IRS), receptor internalization and receptor dephosphorylation by specific tyrosine phosphatases leading to inhibition of insulin action (Olivares-Reyes et al. 2009). Another mechanism for Ang II to impair insulin sensitivity is contributed to its hemodynamic effect, where Ang II is a potent vasoconstrictor and the decrease in blood flow can decrease insulin-mediated glucose uptake by adipose tissue and skeletal muscle. Other proposed mechanisms for Ang II to reduce insulin sensitivity include decreasing adiponectin, increasing inflammation and reactive oxygen species (De Kloet et al. 2010). In this context, Ang II has a pro inflammatory activity and it is capable of increasing leucocytic infilteration, chemokines, cytokines and growth factors, hence activation of RAS is thought to be one of the factors that upregulates production of TNF-α (Larter and Yeh 2008). In progression from steatosis, the inflammatory process and release of pro-inflammatory cytokines represent the pathognomonic feature of steatohepatitis. The association of TNF-α to steatohepatitis was reported by Cano et al. (2009). Importantly, as fat accumulates and obesity develops, the secretion of many of adipokines including TNF-α and angiotensin increases (De Kloet et al. 2010). Elevated TNF-α level in obesity induce insulin resistance by impairing insulin signaling. TNF-α is known to activate intracellular signaling molecules, including stress related kinases such as Jun N-terminal kinase and inhibitor kappa beta kinase beta, that make cells resistant to the actions of insulin (Diehl et al. 2005). TNF-α also stimulates hepatic

fatty acid synthesis and increases triglyceride levels, both lead to further release of TNF-α, thus creating a vicious cycle of inflammation and insulin resistance (Feingold et al. 1990).

Till now there is no established therapy exists for NASH, the implication of renin-angiotensin system in the development of metabolic syndrome and NASH makes angiotensin converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) offer a potential therapeutic approach for NASH (Calamita and Portincasa, 2007). Herewith, the results of the present study exhibited that treatment of NASH with perindopril caused significant decrease in final body weight accompanied with significant amelioration in hepatic triglyceride content, fasting blood glucose, levels of serum insulin and TNF-α, that reflected as an overall improvement of insulin resistance and decrease in the histopathological grading. These results coincide with that obtained by Weisinger et al. (2009), who attributed the decrease in body weight because of ACE inhibitor treatment resulted in lowering blood glucose level 40-45% lower than controls with two-fold increase in plasma adiponectin level leading to increased energy expenditure. They excluded the decreased energy intake as a cause of weight loss because food intake of treated animals was not different from that of control animals. On contrary, Velkoska et al. (2010) found that treatment of high-fat fed animals by perindopril in a low dose of (0.3mg/kg/day) for 12 weeks led to decrease in food intake but did not decrease body weight. Such contradictory results could be contributed to be dose-dependent effects. It must be noted that complete (100%) ACE inhibition is unlikely to occur by usual recommended doses of ACE inhibitors. At the beginning of treatment, ACE activity is maximally inhibited, but thereafter with prolonged administration ACE upregulation will develop due to interference with the negative feedback by Ang II (Danser et al. 2007). Decrease in body weight and body fat by perindopril reduced adipocyte release of TNF-α. Improvement of insulin resistance, decreased FFAs delivery to the liver, decreased FFAs and Ang II mediated ROS generation and inflammatory cell activation, all contributes to improvement in TNF-α level by perindopril (Toblli et al. 2008). The mechanisms whereby ACE inhibitors improve glucose metabolism and protect against development of insulin resistance may be through improvement of blood flow through the microcirculation to adipose tissue and skeletal muscle and/or the improvement of insulin action at the cellular level by interfering with the Ang II-induced alteration of insulin signaling and inhibition of other Ang II induced actions (De Kloet et al. 2010). In this context, inhibition of bradykinins degradation (Ernsberger and Koletsky, 2007), production of angiotensin (1-7) may contribute to insulin sensitizing effect of perindopril. Ang (1-7) is a heptapeptide, present in the circulation and in many tissues including heart, blood vessels, kidney and liver. It is the

main product formed from Ang I through angiotensin converting enzyme 2(ACE2) and degraded by ACE to angiotensin(1-5). ACE inhibitors elevate plasma Ang (1-7) concentrations by both increasing Ang I and by preventing Ang (1-7) degradation (Ribeiro-Oliveira et al. 2008). Mice lacking the Mas receptor, which is activated by angiotensin (1–7) and whose actions oppose traditional AT2 receptor activation, have increased abdominal fat mass, dyslipidemia, glucose intolerance and reduced insulin sensitivity (Ribeiro-Oliveira et al. 2008).

When the current study investigated treatment with irbesartan, it exhibited significant improvement in high-fat induced NASH that is similar to treatment with perindopril. Although is still significant, but irbesartan seems to be less effective than perindopril treatment in reducing body weight. A result that can be explained by compensatory renin rise leading to high Ang II level due to the disruption of the feedback inhibition of renin production secondary to blocking of AT1 receptors (Paul et al. 2006) which in turn stimulates the non blocked AT2 receptors that claimed in promoting adipocyte differentiation. This result is in agreement with Toblli et al. (2008). In their study perindopril treatment induced better improvement in final body weight than irbesartan treatment. Regarding restoring of insulin sensitivity, Iwai et al. (2011) found that the expression of insulin receptor was increased by irbesartan treatment. In addition, the insulin-sensitizing actions of irbesartan can be explained by vasodilation leading to increased glucose uptake, interference with Ang II actions on insulin receptor and insulin receptor substrates. In a study by de las Heras et al. (2009), irbesartan treatment of high fat fed rats led to improvement of insulin sensitivity as a consequence to normalization of leptin/adiponectin ratio and protein expression in lumbar adipose tissue. In proving role of AT1 receptors in mediating the inflammatory process in NASH; Nabeshima et al. (2009) found that AT1 deficient mice had no significant increase in liver enzymes when fed on MCD diet in contrast to wild type mice and that AT1 blockade by irbesartan led to significant decrease in TNF-α. AT1 blockade has been reported to attenuate several deleterious effects of the fatty diet systemically and locally in adipose tissue leading to decrease in TNF-α gene expression, macrophage infiltration of isolated adipocytes and circulating cytokines (Cole et al. 2010). Independent of its established AT1 receptor blocking actions, irbesartan was found to be a partial agonist at the peroxisome proliferator-activated receptor-γ (PPAR-γ), this novel action of irbesartan can help the improvement of insulin sensitivity. It was claimed that the beneficial effects of irbesartan on glucose and lipid metabolism are related only to its PPAR-γ partial agonist action but, this is a weak effect requiring high doses of irbesartan. However, the observed up-regulation of hepatic PPAR-α in AT1 deficient mice led to the speculation that PPAR-α might play a role in the anti-steatotic effect of AT1 blockade.

Interestingly, treatment with olmesartan, which lacks an effect on PPAR-γ receptors has been proved to attenuate the development of hepatic steatosis, suggesting possibility that the blockade of AT1 receptors itself improves hepatic lipid metabolism. These findings indicated that individual ARBs are different in their selective PPAR-γ modulating properties (Ernsberger and Koletsky, 2007; Nabeshima et al. 2009).

In conclusion, the present study pointed that intervention of the biological cascade of RAS by either an ACE inhibitor like perindopril or an ARB like irbesartan could provide a new therapeutic approach for non alcoholic steatohepatitis especially if accompanied with hypertension that considered a primary indication for these drugs.

ACKNOWLEDGMENT

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