Angiotensin-receptor blockers as therapy for mild-to-moderate hypertension-associated non-alcoholic steatohepatitis

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Abstract

AIM: To evaluate insulin resistance, cytolysis and non-alcoholic steatohepatitis (NASH) score (NAS) using the Kleiner and Brunt criteria in 54 patients with NASH and mild-to-moderate hypertension, treated with telmisartan vs valsartan for 20 mo.

METHODS: All patients met the NCEP-ATP III criteria for metabolic syndrome. Histology confirmed steatohepatitis, defined as a NAS greater than five up to 3 wk prior inclusion, using the current criteria. Patients with viral hepatitis, chronic alcohol intake, drug abuse or other significant immune or metabolic hepatic pathology were excluded. Subjects were randomly assigned either to the valsartan (V) group (standard dose 80 mg o.d., n = 26), or to the telmisartan (T) group (standard dose 20 mg o.d., n = 28). Treatment had to be taken daily at the same hour with no concomitant medication or alcohol consumption allowed. Neither the patient nor the medical staff was aware of treatment group allocation. Paired liver biopsies obtained at inclusion (visit 1) and end of treatment (EOT) were assessed by a single blinded pathologist, not aware of patient or treatment group. Blood pressure, BMI, ALT, AST, HOMA-IR, plasma triglycerides (TG) and total cholesterol (TC) were evaluated at inclusion and every 4 mo until EOT (visit 6).

RESULTS: At EOT we noticed a significant decrease in ALT levels vs inclusion in all patients and this decrease did not differ significantly in group T vs group V. HOMA-IR significantly decreased at EOT vs inclusion in all patients but in group T, the mean HOMA-IR decrease per month was higher than in group V. NAS significantly diminished at EOT in all patients with a higher decrease in group T vs group V.

CONCLUSION: Angiotensin receptor blockers seem to be efficient in hypertension-associated NASH. Telmisartan showed a higher efficacy regarding insulin resistance and histology, perhaps because of its specific PPAR-gamma ligand effect.

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Key words: Telmisartan; Valsartan; Non-alcoholic steatohepatitis; Hypertension; Insulin-resistance; Hepatic steatosis; Necroinflammation

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a condition pathogenically linked to the metabolic syndrome by the intervention of insulin resistance (IR), characterized by hepatic steatosis in the absence of significant alcohol use, hepatotoxic medications or other known liver disease[6]. Currently, NAFLD and non-alcoholic steatohepatitis (NASH) are well-recognized causes of progressive chronic liver disease leading to cirrhosis and hepatocellular carcinoma[7-9]. All theories present NAFLD/NASH as the hepatic component of the metabolic syndrome (MS), whose central features include obesity, peripheral insulin resistance, diabetes, dislipidemia, and hypertension[6-8]. Potential therapies tested in NASH treat only the consequences of this condition or try to eliminate excessive fat and target the IR. Reducing food intake can limit accumulation of liver fat and can reverse IR, but there are no well-controlled trials for weight control as a therapy for NAFLD[9]. Other therapeutic interventions, pointing on other features of MS like dislipidemia and impaired glucose tolerance, trying to promote hepatic cytoprotection, or reduction of fibrosis were also evaluated.

This article focuses on angiotensin receptor blockers (ARBs) as multivalent therapeutic agents for NASH, targeting not only hypertension, but also the mechanisms of IR and of hepatic injury via renin-angiotensin system (RAS) as prominent pathways of liver damage. The primary endpoints of the study were to prove that ARBs can improve IR in mild-to-moderate hypertensive patients with histologically confirmed NASH, and that monotherapy with ARBs, on regularly basis, can ameliorate cytolysis, while biochemical improvement in these patients correlates with amelioration of NASH activity score. The secondary endpoint was to prove certain superiority of telmisartan vs valsartan in NASH-hypertensive patients regarding IR, cytolysis, and necroinflammation, given its specific PPAR-γ modulatory effects.

MATERIALS AND METHODS

Study population and screening

The study conducted between May, 2006 and November, 2007 at Filantropia University Hospital from Craiova-Romania was in accordance with the Helsinki Declaration of 1975, and approved by the Review Ethics Board of the University Medicine and Pharmacy of Craiova and of the Filantropia University Hospital. We screened for MS by NCE-ATP III criteria, in subjects with mild-to-moderate hypertension documented by Holter evaluation up to 4 wk prior inclusion, with systolic BP between 180 mmHg and 135 mmHg and the diastolic between 120 mmHg and 85 mmHg and having ALT values more than 1.5-fold normal range (30 IU/dL maximum normality) for at least at 2 determinations, up to 4 weeks prior inclusion. All patients had to have a fasting plasma glucose (FPG) level less than 130 mg/dL, without any therapy or low-carbohydrate diet for at least three determinations up to two weeks prior inclusion, and histologically[11] confirmed NASH with a necroinflammatory score of 5 or more up to 3 wk before inclusion (according to the scoring system proposed and validated for use in clinical trials by the Pathology Committee of the NASH Clinical Research Network in 2005; available at http://tpis.upmc.com/TPIShome). Other inclusion criteria included acceptance of alcohol abstinence, acceptance of taking the study medication daily at the same hour, and answering at each visit an alcohol consumption questionnaire for monitoring alcohol intake, adapted from Behavioral Risk Factor Surveillance System[12] 2007 Questionnaire (available at http://www.cdc.gov/brfss/questionnaires/english.html). Little amounts of alcohol were allowed occasionally, but not more than two drinks/week (one standard US alcoholic drinks = 14 g pure alcohol). No dietary restrictions or lifestyle modifications were imposed in any case, except current recommendations made by the general practitioner at the regular visits, and no concomitant medication was allowed 1 mo before and after treatment as well as for the entire period of study.

Uncontrolled hypertension or requiring more than a single drug to obtain BP control, history of or confirmed viral hepatitis at screening, drug or alcohol abuse and any other concomitant/pre-existing metabolic or immune hepatic disease were exclusion criteria, as well as normal ALT, HIV positivity and use of dietary supplements, or any other concomitant medication taken on a regularly basis. Dramatic lifestyle changes (e.g. low-calorie diets, intensive physical training, surgery for obesity) were not permitted during the study and patients were encouraged to keep their regular dietary habits and to avoid weight variations. Patients unable to give informed consent, refusing paired liver biopsies, or having any other severe associated organic or psychiatric pathology, neoplasia, or history of intolerance to ARBs were also excluded.

By checking the inclusion/exclusion criteria, only 72/89 patients continued the screening and underwent liver biopsy. The study design scheduled two liver biopsies: the first biopsy performed up to 3 wk prior inclusion (considered as the index biopsy) and the second biopsy obtained at maximum 2 wk after the end of treatment. A single pathologist, unaware of patient information, evaluated the histological features of both index and second biopsies. NAFLD activity scores (NAS) were assessed in each case, and patients with simple steatosis, or those not fully meeting all criteria for steatohepatitis (18/72), were excluded. We finally included 54 subjects (28M/26F) in the trial.

Subjects were randomly assigned using dedicated computer software either to the valsartan (V) group (receiving a standard dose of 80 mg o.d., n = 26), or to the telmisartan (T) group (standard dose 20 mg o.d., n = 28). Medication was blinded and treatment had to be taken daily at the same hour in the morning, with no concomi-
tant medication or alcohol consumption allowed. Neither the patient nor the medical staff was aware of the treatment group allocation.

**Biochemical analyses and histology**
A central laboratory used standard procedures to insure reproducibility. FPG, alanine-aminotransferase (ALT), aspartate-aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), bilirubin (B), total cholesterol (TC), and triglycerides (TG), were determined on fresh serum using an autoanalyzer Hitachi 917 Automate with Roche Diagnostics reagents. Serum samples obtained after an overnight fast of at least 12 h and immediately frozen at -20°C were used to determine the levels of immunoreactive insulin (IRI) by a chemiluminescence immunoassay (Elecsys Modular Analytics E170; Roche Diagnostics) using monoclonal antibodies with stated negligible cross-reactivity. We determined IR by the homeostasis model assessment (HOMA-IR) method[13] using the following equation: HOMA-IR = [FPG (mg/dL) × IRI (µU/mL)]/405.

The percutaneous liver biopsy technique was performed in all cases[14]. All biopsies were fixed, paraffin-embedded, and stained with hematoxylin-eosin and Masson’s trichrome/picrosirius red for collagen. Biopsies were evaluated by a single, experienced, blinded pathologist, not aware about allocation in one or another treatment group and about the clinical and biochemical parameters of any patient using the scoring system validated by Kleiner et al[11]. As known, this histology scoring system quantifies necroinflammatory and steatotic changes (steatosis, lobular inflammation, and ballooning) resulting NAFLD activity scores (NAS) that range between 0 and 8. Scores greater or equal to 3 are largely diagnostic for NASH, while scores less than 4 characterizing a fatty liver having simple steatosis, but not NASH. Fibrotic changes are evaluated separately from NAS, ranging from 0 (no fibrosis) to 4 (cirrhosis). Our study also assessed the fibrosis stage in all patients in order to evaluate the antifibrotic effects of the two ARBs.

**Study schedule and surveillance parameters**
After screening, the included patients were followed for 20 mo. The study flowchart previewed 6 visits (V1-V6) scheduled every 4 mo (112 d) with a ± 5 d deviation admitted. Each visit took place between 8.00 and 11.00 a.m. and consisted with a clinical examination, blood pressure (BP) and body mass index (BMI) determinations, serum sampling, and a questionnaire. An average of three successive determinations of systolic (sBP) and diastolic (dBP) BP was calculated and used in records each visit. BMI was computed using the formula: [weight (kg)/square of (height (meters)], while serum was collected for FPG, ALT, AST, GGT, B, TC, TG and IRI determinations. An alcohol consumption questionnaire was also administered each visit and study compliance was strictly monitored, including checking the returned medication. Additionally, V1 (inclusion) comprised recording of the result of the index liver biopsy which was performed -21 to -7 d previously, while V6 ended with the second liver biopsy, performed at maximum 2 wk after the end-of treatment (EOT).

The primary parameters at followed-up were s/dBP, BMI, ALT, AST, GGT, HOMA-IR, TC, TG, NAS and fibrosis scores. Additionally, we used in the analysis the following derive parameters: the mean monthly decreases of ALT (md*ALT), HOMA-IR (md*HOMA-IR), TC (md*TC) and TG (md*TG), the mean decrease for NAS (md*NAS) and fibrosis score (md*Fibrosis) and the mean decrease of s/dBP (md*s/dBP) and BMI (md*BMI). The md*ALT, md*HOMA-IR, md*TC and md*TG represent the difference between the average values of respectively, ALT, HOMA-IR, TC and TG, between V2 and V6 and their mean values at V1 divided by the number of months of follow-up (20 mo for the patients that fully completed treatment), mathematically expressed by the following formula (where “y” is the study parameter, “i” is the number of the visit, “Ny” is the number of months of follow-up and “Li” is the index of the visit):

$$m_{Md} \times y = \left[ \frac{\sum_{i=2}^{N_y} y(V_i) - y(V_1)}{N_y} \right]$$

The md*NAS and md*Fibrosis were calculated by subtracting the average NAS and, respectively, fibrosis scores at index biopsy from those recorded at V6, while the md*s/dBP and md*BMI represent the difference between the respective values of these parameters averaged from V2 to the last visit and their mean value at V1 without considering the number of months of follow-up, as in the subsequent formula (where “x” is the study parameter, “i” is the number of the visit, and “Lx” is the index of the visit):

$$m_{Md} \times x = \left[ \frac{\sum_{i=2}^{Lx} x(V_i) - x(V_1)}{L_x - 1} \right]$$

**Statistical analysis**
Data is presented as mean ± SE. Differences in the baseline parameters between groups T and V were tested by the Kruskal-Wallis test to check for any baseline bias. Normal distribution was tested using the Kolmogorov-Smirnov test while the Wilcoxon test was used to assess the differences between the paired observations. Other data recorded during the study from groups T and V were analyzed by one-way analysis of variance ANOVA. A statistically significant result was considered when P value was less than 0.05. All statistical analyses were performed using the MedCalc Software Version 10.0.2.0-2008 (MedCalc Software, Broekstraat 52, 9030 Mariakerke, Belgium).

**RESULTS**
Mean age for the included patients was 48.89 ± 1.41 (48 ± 1.98 in group T and 49.85 ± 2.05 in group V) while the average dose per BMI unit was 0.74 ± 0.01 mg telmisartan in group T and 2.92 ± 0.06 mg valsartan in group V. No statistically significant difference between the two groups regarding the demographic data, as well as among the survey parameters, existed at inclusion.
Table 1 shows a synopsis of all the survey parameters at baseline in all included patients, as well as in the two therapeutic groups.

All included patients finished the study. At the end of the study, we observed significant differences regarding biochemical, metabolic, histological and hemodynamic parameters in both study groups compared with inclusion data. Tables 2 and 3 review the main results of the study concerning both the primary parameters of survey as well as the derive ones.

**Cytolysis study**

ALT values at V6 were significantly lower versus inclusion in all patients (49.48 ± 1.16 IU/L vs 67.65 ± 2.01 IU/L, P < 0.001), although the values did not returned to normality in either group. Both therapeutic groups had significantly lower ALT levels at EOT compared to V1; however, in group T these values were significantly smaller than in group V (46.68 ± 1.42 IU/L vs 52.50 ± 1.70 IU/L, P = 0.011). Despite a constant decrease of ALT in both groups from V2 to
V6 with differences in favor of group T (Figure 1A), significantly lower values in this group, as compared to group V, were observed only at the last visit. As Table 2 shows, similar data with significantly lower values in group T vs V (47.57 ± 2.08 vs 52.50 ± 1.70, P = 0.044) were noticed for AST, but not for GGT and B which remained stable in both groups throughout the study.

The overall mMd*ALT value was -0.57 ± 0.05 IU/L per month, with -0.63 ± 0.09 IU/L/mo in group T and -0.52 ± 0.05 in group V (Figure 2A). No significant difference between groups regarding this aspect was observed either.

**Metabolic study**

BMI was stable during the study in all patients (27.42 ± 0.36 vs 26.96 ± 0.36, P = NS) with no difference between group T and V at V6 (26.93 ± 0.49 in group T vs 27.42 ± 0.36, P = NS) and no differences were found between the two groups regarding the mMd*BMI (0.11 ± 0.47 in group T vs -0.05 ± 0.57 in group V, P = NS). At EOT, HOMA-IR was 5.19 ± 0.18, significantly lower than 7.7 ± 0.24 as shown in Figure 1B, the HOMA-IR constantly decreased in groups T vs V from V2 to V6, proving a better insulin-sensitizing activity for telmisartan. Moreover, the mMd*HOMA-IR,
which was \(-9.63 \pm 0.94 \times 10^{-2}\) units/mo in overall patients, was more than two-fold higher in group T with \(-13.7 \pm 1.32 \times 10^{-2}\) vs \(-5.3 \pm 0.64 \times 10^{-2}\) units/mo in group V (\(P = 0.001\)), demonstrating a reliable effect of telmisartan to improve insulin resistance (Figure 2B).

Lipid profiles were also modified at the EOT. We noticed a decrease of TG values in patients at V6 compared to V1 (153.96 ± 4.8 mg/dL vs 161.51 ± 4.98 mg/dL, \(P = 0.003\)) in both in male and female patients, as shown in Table 2. However, only in group T was the decrease of plasma TG found to be statistically significant by the Wilcoxon test for paired samples (154.14 mg/dL ± 6.79 vs 165.64 ± 6.95 mg/dL, \(P = 0.0013\)). Moreover, although the mean values for TG were similar in groups T and V at V6, the mMd\(^{*}\)TG was significantly higher in the telmisartan group, with \(-1.99 \pm 0.16\) mg/dL per month vs \(-1.58 \pm 0.11\) in group V (\(P = 0.001\)), irrespective of gender of patients (Figure 2C). TC decreased at V6 compared to V1 both in men (198.04 ± 1.39 mg/dL vs 200.82 ± 1.2 mg/dL, \(P = 0.006\)) as in women (189.73 ± 1.58 mg/dL vs 191.77 ± 1.5 mg/dL, \(P = 0.008\)) and in overall patients (194.04 ± 1.19 mg/dL vs 196.46 ± 1.13 mg/dL, \(P = 0.003\)). We did not notice any difference regarding these values at V6 between groups T and V, when analyzing the results either by gender or in overall patients (191.89 ± 1.64 mg/dL vs 196.35 ± 1.64 mg/dL, \(P = 0.06\)). However, at EOT group T had significant lower values compared with inclusion in both

| Units         | Overall | Group T | Group V |
|---------------|---------|---------|---------|
| mMd\(^{*}\)ALT | IU/dL per month | -0.57 ± 0.05 | -0.63 ± 0.09 | -0.52 ± 0.05 |
| mMd\(^{*}\)HOMA-IR | units/mo | -9.63 ± 0.94 × 10\(^{-2}\) | -13.7 ± 1.32 × 10\(^{-2}\) | -5.3 ± 0.64 × 10\(^{-2}\) |
| mMd\(^{*}\)TG | mg/dL per month | -1.79 ± 0.10 | -1.89 ± 0.16 | -1.58 ± 0.11 |
| mMd\(^{*}\)TC | mg/dL per month | -0.03 ± 0.03 | -0.12 ± 0.05 | 0.006 ± 0.02 |
| md\(^{*}\)BMI | units | 0.03 ± 0.36 | 0.11 ± 0.47 | -0.05 ± 0.57 |
| md\(^{*}\)sBP | mmHg | -21.13 ± 1.13 | -21.35 ± 1.68 | -20.90 ± 1.54 |
| md\(^{*}\)dBP | mmHg | -19.18 ± 1.43 | -19.65 ± 2.15 | -18.67 ± 1.91 |
| md\(^{*}\)NAS | point | -0.92 ± 0.14 | -1.43 ± 0.19 | -0.38 ± 0.17 |
| md\(^{*}\)Fibrosis | point | -0.46 ± 0.11 | -0.75 ± 0.13 | -0.15 ± 0.18 |

mMd\(^{*}\)ALT: Mean monthly decrease of ALT; mMd\(^{*}\)HOMA-IR: Mean monthly decreases for HOMA-IR; mMd\(^{*}\)TG: Mean monthly decrease of plasma triglycerides; mMd\(^{*}\)TC: Mean monthly decrease of total cholesterol; md\(^{*}\)BMI: Mean decrease of BMI; md\(^{*}\)sBP: Mean decrease of systolic blood pressure; md\(^{*}\)dBP: Mean decrease of diastolic blood pressure; md\(^{*}\)NAS: The mean decrease for NAS; md\(^{*}\)Fibrosis: Mean decrease for fibrosis score.

**Table 3** Derivate study parameters in treatment groups and in overall patients (mean ± SE)
male (195.79 ± 1.96 mg/dL vs 200.57 ± 1.3 mg/dL, P = 0.024) and female patients (188 ± 2.23 mg/dL vs 191.57 ± 2.13 mg/dL, P = 0.006) while in group V we did not observe the same aspect. Furthermore, as showed in Figure 2D, the mMd*TC was higher in group T than in group V (-0.12 ± 0.05 mg/dL vs -0.06 ± 0.02 mg/dL per month, P = 0.003) demonstrating a significant effect on the lipid profile by telmisartan, whereas valsartan seemed to lack this property.

**Histology study**

NAS score decreased at EOT in overall patients (5.89 ± 0.14 vs 4.96 ± 0.14, P < 0.01), but only steatosis and ballooning showed a significant reduction, while lobular inflammation rested unchanged. The NAS score at V6 was lower in group T vs V (4.57 ± 0.18 vs 5.38 ± 0.2, P = 0.004) demonstrating a significant efficacy of telmisartan to improve hepatic histology. Additionally, when comparing the evolution of the NAS elements in the two groups, we found that all these components significantly decreased in group T from V1 to V6 (P < 0.045 for any comparison V1 vs V6 concerning steatosis, lobular inflammation and ballooning), while in group V, only steatosis improved (P = 0.027) without significant changes for inflammation and ballooning (Figure 3A). Furthermore, the mMd*NAS was significantly higher in telmisartan group (-1.43 ± 0.19 vs -0.35 ± 0.17, P < 0.001) confirming that this ARB can effectively act as a factor promoting amelioration of the NAS activity score (Figure 3B).

In all groups, the fibrosis scores at V6 were lower than those observed at V1 (1.57 ± 0.09 vs 2.11 ± 0.11, P < 0.001); however, fibrosis scores at EOT were higher in group V than in group T (1.84 ± 0.11 vs 1.32 ± 0.13, P = 0.013). The decrease of the fibrosis score from V1 to V6 was statistically significant in group T (2.07 ± 0.16 to 1.32 ± 0.13, P < 0.001), but not in group V, demonstrating an antifibrotic effect of telmisartan that is not possessed by the other ARB (Figure 3C). Moreover, the mMd*Fibrosis was significantly higher in group T than in group V (-0.75 ± 0.13 vs -0.15 ± 0.18, P = 0.01), confirming the capacity of telmisartan to inhibit liver fibrosis (Figure 3D).

**Hemodynamic study**

A detailed analysis of the antihypertensive effect of the two ARBs was not the scope of this study. We only noticed that both drugs are equally potent in reducing both sBP and dBP. Telmisartan reduced BP from 157.42 ± 2.04/101.89 ± 1.8 mmHg at V1 to 133.21 ± 1.23/77.14 ± 1.25 mmHg at V6, while valsartan reduced BP from 157.42 ± 2.04/101.89 ± 1.8 at V1 to 135.35 ± 2.31/78.5 ± 1.7 at V6. No differences were noticed between groups regarding either sBP or dBP at any of the visits from V2 to V6, while the mMd*sBP and mMd*dBP values were, respectively, -21.35 ± 1.68 mmHg in group T vs -20.90 ± 1.54 in group V and -19.65 ± 2.15 mmHg in group T vs -18.67 ± 1.91 in group V (P = NS for both comparisons).

**DISCUSSION**

In brief, our study demonstrates that although it does not normalize ALT values, telmisartan can reduce cytolysis by 30.28% and can improve IR by decreasing HOMA-IR with 42.63% in patients with NASH and mild-to-moderate hypertension. This improvement is associated with a significant decrease of NAS and fibrosis scores and with an amelioration of the lipid profile demonstrated by lower values of plasma TG and TC in both men and women. On the other hand, despite a significant reduction of ALT levels by 23.22%
and of HOMA-IR by 21.4%, valsartan did not improve liver histology (except steatosis) and had no effect on plasma lipids. There is no statistically significant difference in ALT reduction between the two ARBs, but the higher rates of HOMA-IR reduction, as well as the improvement of NAS score and antifibrotic effect observed in group T, suggests that the effects of this ARB are driven not only through the angiotensin-1 receptor blockade, but also via its PPAR-γ modulator specific effects.

Telmisartan is an ARB possessing unique qualities of PPAR-γ modulation that makes it ideal for the treatment of NASH. Unfortunately, no major studies have been performed to confirm its efficacy in steatohepatitis, although a theoretical and experimental fundament exists[29]. Interestingly, a study by Fujita et al[30] tested the same compounds as we did in this study in a rat model of NASH, providing evidence that telmisartan, but not valsartan, improved both qualitatively and quantitatively hepatic steatosis, inflammation, and fibrosis. Furthermore, in both rats with choline-deficient diet-induced NASH (in vivo) and in primary hepatic stellate cells (in vitro), Jin et al[31] concluded that telmisartan is able to prevent liver fibrosis by increasing matrix-metalloproteinase (MMP) expression, down-regulation of transforming growth factor beta-1 (TGF-β1) and tissue inhibitor of matrix-metalloproteinases (TIMP), and by inhibition of hepatic stellate cell (HSC) activation and proliferation. A study by Sugimoto et al[32] provided evidence that in hepatic steatosis telmisartan (and not valsartan) reduces accumulation of visceral fat and hepatic triglyceride levels, decreases adipocyte size, and increases the muscle expression of certain important genes involved with energy metabolism. These properties of telmisartan are probably linked to its ability to modulate PPARγ activity. Indeed, in a recent study, Yoshida et al[33] demonstrated that telmisartan improves IR in advanced glycated end-product (AGE)-exposed human hepatoma (Hep3B) cells by decreasing serine phosphorylation and enhancing tyrosine phosphorylation of insulin-receptor substrate-1 but, when antagonized with an inhibitor of PPARγ, it loses these properties. Other animal studies[32,34] provided additional evidence of properties of telmisartan linking it to PPAR modulation that can account for its effects in steatohepatitis, for example a partial PPAR-γ agonist activity which seems to be restricted to the liver, regulating serum adipokines with increased adiponectin and decreased resistin levels, and even anti-inflammatory properties.

Human studies employing ARBs in NASH are quite rare[35-38], testing habitually losartan and lacking either a sufficient number of patients, either an adequate assessment of morphologic changes given the difficulty to obtain paired liver biopsies. The major strength of our study is that, from our knowledge, it is the first human blinded trial evaluating the effects of telmisartan and valsartan in steatohepatitis that uses paired liver biopsies simultaneously with cytolysis and IR assessment. Interestingly, although not pointing on steatohepatitis, a recent study by Ichikawa[39] demonstrated that in hypertensive patients with MS, receiving 20 mg telmisartan daily for 4 wk, resulted in a reduction of HOMA-R by 16%, while 40 mg valsartan/day did not show significant results on this parameter. There are differences between this study and our trial, including different dosage for valsartan (higher doses in our study), longer period of survey (20 mo vs 4 mo), younger study population (49 years vs 65 years), higher values of HOMA-IR (7.7 units vs 3 units), use of Japanese criteria for MS and permission for concomitant medication, but in all, our results confirm the insulin-sensitizing effect of telmisartan. Additionally, we demonstrated that this ARB has a favorable effect on plasma TG and TC in opposition to Ichikawa and other groups[39-41], but in accordance with others[42,43]. As for valsartan, again in contrast with Ichikawa, but in accordance with larger studies[44,45], we demonstrated that it also reduces IR, although it has no other effects on lipid profiles.

There are interesting theories and experimental facts that can explain the intervention of the RAS in liver disease, leading to the theoretical conclusion that ARBs have the capacity to become the first-class option for a tailored therapy in NAFLD and NASH. The RAS is an enzymatic cascade in which renin, an aspartic protease released from juxtaglomerular cells, cleaves angiotensinogen to form a decapeptide, angiotensin I (Ang-1), which is in turn transformed to angiotensin II (Ang-II) by the angiotensin-converting enzyme (ACE). Ang-II can be further converted by aminopeptidases A and N in Ang-III (2-8) which is finally transformed in Ang-IV (3-8)[46]. Historically, Ang-II was first described as the primary effector of this system, but more recent research added new components as a result of the action of prolylendopeptidase and carboxypeptidases: angiotensin 1-5 (Ang-1-5), angiotensin 1-7 (Ang-1-7), and angiotensin 1-9 (Ang-1-9)[47]. Ang-1-7 is a heptapeptide generated from either Ang-1 or Ang-2 by a homologue of ACE, angiotensin converting enzyme 2 (ACE2) which has a catalytic domain different from ACE and acts antagonistically as a counter-regulatory factor. The biological actions of Ang-1-7 are both activation of peripheral vasodilatory mechanisms and antifibrotic effects mediated by the inhibition of protein synthesis[48,49].

Classically, Ang-II and Ang-III acts on two types of G-protein-coupled receptors, AT1 and AT2. The AT1 receptor is widely expressed in various tissues (heart, kidney, vessels, liver and adipocytes), while AT2 has low levels of expression after birth, but may play a role in activation of AT1, modulation of cell differentiation, tissue repair and apoptosis[50,51]. Ang-IV possesses its own receptors (AT4) distinct from AT1 and 2, and Ang-1-7 acts through a different G protein-coupled receptor (Mas) downregulating AT1[52].

Although consistent convergent data about the intervention of the RAS in NAFLD/NASH exists, the contribution of this factor in setting and promoting the hepatic consequences of MS is still not fully clarified. It is likely that the mechanisms by which RAS could inter-
fere with the pathogenic course that links IR to steatohepatitis might include interactions with insulin receptors and intracellular signalling, effects on adipogenesis, influences on cytokine and adipokine production, interferences with pancreatic β-cell insulin secretion and/or local hepatic effects interfering hepatocellular regulatory mechanisms.

Angiotensinogen is synthesized in the liver and adipocytes, but adipose tissue differs from liver given the differences in the AT1/AT2 receptor populations, the inhibitory effect of the AT2 receptors impairs excessive angiotensinogen production by the adipocytes. RAS is frequently activated in the patients with chronic liver diseases, promoting mainly fibrosis, with Ang-II stimulating contractility and proliferation of the activated HSCs, increasing TGF-β1 and promoting neovascularization and production of vascular endothelial growth factor. It is largely accepted that the local hepatic RAS system acts almost exclusively through the AT1 receptors localized to hepatocytes, bile duct epithelial cells, HSCs, myofibroblasts, Kupffer cells and vascular endothelium, as the AT2 receptors are not significantly expressed in liver. However, some data regarding the AT2 receptors exists suggesting that it may have protective effects against fibrosis.

Although the profibrotic effects of RAS begins to be unveil in various conditions including NASH, little is known about the inflammatory changes that precede fibrosis. Perfusion studies by Bataller and colleagues showed mild portal inflammation, thickening and thrombosis of small hepatic vessels, as well as accumulation of CD43-positive inflammatory cells and activated HSCs in pericentral areas following infusion of Ang-II, and concluded that liver injury is induced in this circumstance by oxidative stress, hepatic inflammation, and vascular damage. It is considered that Ang-II acts by amplifying the general inflammatory response that follows the chronic liver injury, inducing reactive oxygen species (ROS) generation as well as inflammatory cytokines like interleukins (IL) -6 and -1, monocyte chemoattractant protein-1 (MCP-1), TGF-β1 and tumor necrosis factor-α (TNF-α). More complex connections and interferences are, however, occurring in real conditions, like the crosstalk between TNF-α and RAS in the TNF-induced plasminogen activator inhibitor-1 (PAI-1) production in human hepatocytes. Accordingly, gene expression of RAS and that of PAI-1 are upregulated in the liver of patients with obesity and type 2 diabetes, and in non-malignant human hepatocyte cell lines, RAS-encoding genes are upregulated time-dependently by TNF-α while AT1-receptor blockade inhibits the TNF-induced PAI-1 production.

The mechanisms of inflammatory activation induced by Ang-II are classic. AT1 receptor binding, with subsequent protein kinase C (PKC) activation followed by the intervention of intracellular signalling systems, like extracellular signal-regulated protein kinase (ERK) and c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK). c-Jun leads to the activation of nuclear factor-κB (NF-κB), preceded by the release of several transcription factors, like activator protein-1 (AP-1) and signal transducer and activator of transcription (STAT), the final result being transcription and delivery of proinflammatory cytokines. Additionally, other NF-κB-dependent inflammatory proteins such as cyclooxygenase-2 (COX2) and inducible nitric oxide synthase (iNOS) are upregulated by angiotensin. Jamaluddin et al. recently described in liver cells an alternate pathway for NF-κB activation similar to the signalling pathway that mediates antigen-induced lymphocyte proliferation by bridging T or B cell receptor. It can be, therefore, speculated that such a pathway can account for inflammatory changes that occur when “steatosis” turns to “steatohepatitis”. Moreover, as activation of NF-κB is also followed by further stimulation of angiotensinogen transcription in hepatocytes by Ang-II via the AT1 receptors, thus inducing expression of its own precursor and creating a biological “positive feedback loop”, it is possible that this pathway represents one of the key factors that contributes to the vicious cycle of liver damage.

The key factors in promoting hepatic fibrosis are the HSCs, together with the portal myofibroblasts and cells of bone marrow origin which exhibit fibrogenic potential. In liver fibrosis, the resident HSCs appears to be the primary source of myofibroblasts, although bone-marrow-derived cells can also contribute. Chemokines attracting mononuclear phagocytes like MIP-1α (CCL3) and MCP-1 (CCL2) are considered as main pro-fibrotic mediators, while TGF-β1 and Th2 cytokines (IL-4, IL-5, IL-13 and IL-21) have distinct roles in the regulation of tissue remodelling and fibrosis. TGF-β1 is the best known pro-fibrotic cytokine, being stored in macrophages as an inactive homodimer that needs to be dissociated by several enzymes, like cathepsin, plasmin, integrins and MMPs, to bind to the specific receptors and to trigger intracellular intermediates (SMAD proteins) which induce procollagen I and III synthesis.

HSCs are the main source of extracellular matrix (ECM) in liver, residing in the space of Disse. When activated, HSCs express contractile, proinflammatory, and fibrogenic factors, migrate, secrete ECM, and regulate ECM degradation by expressing MMPs. Activated HSCs are also a major source for additional proinflammatory mediators and cytokines and are able to de novo generate Ang-II, being the key factor to maintain the vicious cycle which links inflammation to fibrosis. There is a consensus that Ang-II and local RAS are major pro-fibrotic agents in liver, inducing all the pro-fibrogenic properties of HSCs. Consequently, there are multiple points in which Ang-II, acting on the AT1 receptors, increases the ability of HSCs to generate fibrosis, including stimulation of chemoattractant factors, activation of contractile and secretory properties of HSCs and imbalance of the production and removal of ECM.

Further, with the stimulatory effects of Ang-II on MCP-1 and TGF-β1, with the implication of the AT1 receptor-mediated NF-κB-dependent pathway in this phenomenon, and its effects on TGF-β1 secretion and
activation, Ang-Ⅱ also enhances HSCs' intracellular signalling by increasing SMAD levels and the nuclear translocation of phosphorylated SMAD with subsequent production of collagens, fibronectin and proteoglycans. The contractile functions of activated HSCs, derived from intracellular smooth muscle actin expression, are also stimulated by Ang-Ⅱ which increases intracellular Ca²⁺[47,60]; its proliferative capacity is also enhanced as shown recently by Liu et al[61] who observed that Ang-Ⅱ prompts HSC proliferation and DNA synthesis and also facilitates its contraction and collagen synthesis. These properties of HSCs are expressed through the mitogen-activated protein kinases (MAPK), a family of ubiquitous proline-directed, protein-serine/threonine kinases, which participate in signal transduction pathways that control intracellular events including apoptosis, cell growth, prostanoïd formation, and other cellular dysfunctions when induced by oxidants or pro-inflammatory cytokines. These events are reversed by AT1 receptor blockade. By acting on the AT1 receptors in activated HSCs, Ang-Ⅱ also stimulates, via PKC intracellular signalling cascade, TIMP-1. This effect inhibits the activity of MMP which are responsible for collagen degradation and thus facilitates the progression of hepatic fibrosis[62].

Almost all the functions of HSCs, including the induction of proinflammatory cytokines, expression of NF-κB and production of ECM, are largely mediated by ROS generated by a nonphagocytic form of NADPH oxidase, which also plays a role in the inflammatory actions of Kupffer cells. NADPH oxidase is expressed at higher levels in response to cytokines and under inflammatory conditions, generating more free radicals[58], while Ang-Ⅱ also can induce supplementary production of ROS, providing a potentiating mechanism and creating an autocrine loop in which liver injury increases Ang-Ⅱ production that in turn perpetuates liver damage and fibrosis.

In opposition to the effects driven by the AT1 activation by Ang-Ⅱ, ACE2 and its product Ang-1-7, Mas receptors may counteract the adverse effects of Ang-Ⅱ in liver disease. Herath et al[63] examined the expression of these novel components of RAS and the production of Ang-1-7 in the bile duct ligated rats and observed that hepatic ACE2 gene and activity, plasma Ang-1-7 and Mas receptor expression increased after bile duct ligation. Moreover, perfusion experiments confirmed that bile-duct ligated livers produced increased Ang-1-7 from Ang-Ⅱ and this was augmented by ACE inhibition, leading to the conclusion that the RAS activation in chronic liver injury is associated with upregulation of ACE2, Mas and hepatic conversion of Ang-Ⅱ to Ang-1-7. These results support the theory that the presence of an ACE2-Ang-1-7-Mas axis in liver injury may moderate the effects of Ang-Ⅱ. Furthermore, Mas receptor antagonists have been tested in male Wistar rats subjected to sham-surgery or bile duct ligation[64]. Plasma renin activity and RAS components, as well as liver hydroxyproline and total TGF-β1 have been assessed, showing that renin activity, Ang-Ⅰ, Ang-Ⅱ and Ang-1-7 were progressively increased. Changes in RAS profile correlated with histological signs of fibrosis and deterioration in liver function while pharmacological blockade of the (Ang-1-7) receptor aggravated fibrosis with a significant elevation in hydroxyproline and total TGF-β1, suggesting that Ang-1-7 plays a protective role in hepatic fibrosis.

By observing in clinical conditions significant reduction of insulin resistance by both ARBs, as well as a moderate decrease of cytolysis in patients having NASH and mild-to-moderate hypertension, our study confirms, at least in part, these existing experimental data. There is, however, some unexplained issues, for example, why only telmisartan showed significant antifibrotic effects and why only this drug was able to improve the NAS score. Of course, a reasonable explanation could be the specific PPARγ modulatory activity of this ARB, but also other unique properties of this drug can contribute to this effect. As extensively discussed elsewhere[53], it seems that various ARBs have different “second-level” pharmacologic effects, unrelated to presence or absence of certain PPAR-modulating activity, as for example candesartan, which shows capacities to decrease liver fibrosis and diminish portal pressure in Child A cirrhotic patients[65], but do not have significant PPAR-modulating activity. It is subsequently possible that the better clinical results observed for telmisartan are driven through some undisclosed mechanism(s) that further studies will undoubtedly unveil.

Nevertheless, the limitations of our study are linked to the small number of patients, lack of a complementary analysis of plasma fibrosis markers and of serum leptin and adiponectin levels, and even a more complex evaluation of the lipid profile of the patients. Additionally, despite the fact that a rigorous analysis of antidiislipidaemic effects of the two ARBs was out of our scope, we can, however, question as others[66] did, if the lipid-lowering effects observed for telmisartan, although statistically significant, have any clinical relevance and if the cytolysis improvement noticed for both ARBs has any impact for the clinical outcome of NASH. However, as the renin-angiotensin system plays a central role in IR and subsequently in NAFLD/NASH as the hepatic expression of MS, an attempt to block the deleterious effects of its overexpression seems correct and further studies are certainly needed to confirm whether an ARB can be a first-option drug for controlling IR, cytolysis and liver fibrosis in hypertension-associated NASH.

**COMMENTS**

**Background**

Non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) are well-recognized causes of progressive chronic liver disease leading to cirrhosis and hepatocellular carcinoma. These conditions, considered hepatic components of the metabolic syndrome (MS) are triggered by insulin resistance. To date, no therapy provided evidence of significant efficacy, and as a consequence, no approved therapeutic options are available worldwide.

**Research frontiers**

Although IR plays a pivotal role in NASH/NAFLD, potential therapies tested for these conditions treat only its consequences or try to eliminate excessive fat. As the renin-angiotensin system (RAS) plays a central role in IR and subsequently
in NAFLD/NASH, an attempt to block the deleterious effects of its overexpression seems an attractive breakthrough. By inhibiting RAS they can achieve an improvement of intracellular insulin signalling pathway, a better control of adipose tissue proliferation and adipokine production and a more balanced production for various cytokines. At the same time, by controlling the local RAS in the liver, they might be able to prevent at least fibrosis and to slow down the vicious cycle that links steatosis to necroinflammation. By targeting pancreatic effects of angiotensin they would be able to preserve an adequate insulin secretion and acquire a better metabolic balance.

**Innovations and breakthroughs**

This is the first human blinded trial evaluating the effects of telmisartan and valsartan in steatohepatitis that uses paired liver biopsies with NASH score (NAS) evaluation, simultaneously with cytokysis, IR and lipid profile assessment. Although serum aminotransferases did not normalized, telmisartan can reduce cytokysis by 30.28% and can improve IR by 42.63% consequently with a significant decrease of NAS and fibrosis scores and an amelioration of the lipid profile. Conversely, despite a significant reduction of cytokysis levels by 23.22% and of IR by 21.4%, valsartan did not improved liver histology (except steatosis) and had no effect on plasma lipids.

**Terminology**

ARBs are angiotensin receptor blockers, non-peptide compounds that have a binding affinity to the receptor AT1 of angiotensin thus inducing an irreversible or competitive blockade of the physiologic agonists.

**Peer review**

By observing in clinical conditions significant reduction of IR by both ARBs, as well as a moderate decrease of cytokysis, the study confirms that ARBs can act as an elegant tool for adequate correction of various imbalances that act consensually in steatohepatitis. ARBs not only can correct hypertension, but also can act on IR and the hepatic RAS, preventing and treating steatohepatitis as an end-organ effect of MS. On the other hand, ARBs can prevent collagen synthesis and further progression to cirrhosis. As equally cheap, effective and well-supported antifibrotic therapies are hard to be found we can predict that this property will put ARBs in the pole position for treating at least the liver fibrosis.

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