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Orignal Article

Ramipril significantly attenuates the development of non-alcoholic steatohepatitis in hyperlipidaemic rabbits

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Abstract: Background: Non-alcoholic fatty liver disease (NAFLD) is considered as the most frequent cause of chronic hepatic disease in adults. It is strictly correlated with insulin resistance, frequently associated with components of metabolic syndrome, and, similarly to the latter, it has been correlated with high risk of developing type 2 diabetes and cardiovascular diseases. Systemic arterial hypertension has been suggested to be associated with NAFLD in approximately 40% of the cases, and NAFLD has been independently associated with an increased risk of arterial hypertension in observational studies. Therefore, we can infer that treating arterial hypertension in NAFLD carriers will be often necessary and that the potential beneficial effects of the antihypertensive might, in this context, influence the choice of the respective drug. The renin-angiotensin system has been correlated to the whole basic physiopathogenic mechanism of NAFLD in experimental models. Based on these findings, we conducted this study to evaluate the effects of the ACE-inhibitor ramipril, used preventively, in NAFLD induced in rabbits fed hyperlipidaemic diet. Methods: Twenty-nine rabbits were divided into three groups (normal, placebo, and ramipril). The placebo and ramipril groups were fed a ration containing 0.925% cholesterol. The groups were orally administered 0.35 mg/kg/day of ramipril, and an equivalent volume of vehicle was administered to the placebo group. At the end of the 8th week, all rabbits underwent segmental hepatic resection and were euthanized. Blood samples were collected to determine glucose, insulin, creatinine, total cholesterol, triglycerides, HDL-C, and aminotransferase levels at baseline and euthanasia. Haematoxylin and eosin and Gomori trichrome-stained slides were analysed based on the histological scoring system for NAFLD. Sudan III-stained slides were analysed by morphometry and immunostained based on the Allred scoring system. Results: When compared with placebo, ramipril significantly diminished the development of steatosis (P=0.032), lobular inflammation (P=0.006), hepatocellular ballooning (P=0.023), and fibrosis (P=0.02). Based on the NAFLD activity score (NAS), ramipril significantly reduced the development of non-alcoholic steatohepatitis (NASH) (P=0.003). Conclusions: The preventive use of ramipril in rabbits fed hyperlipidaemic diet, attenuates the development of the whole NAFLD histopathological spectrum and based on NAS, ramipril significantly reduced the development of NASH.

Keywords: Non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, renin-angiotensin system, hypertension, atherosclerosis, insulin resistance

Introduction

Non-alcoholic fatty liver disease (NAFLD) is considered as the most frequent cause of chronic hepatic disease in adults [1, 2]. It is strictly correlated with insulin resistance (IR) [3-5], frequently associated with components of metabolic syndrome (MS) [5-7], and, similarly to the latter, it has been correlated with high risk of developing type 2 diabetes (T2DM) [5] and cardiovascular diseases (CVD) [8-13]. IR has been considered the basic cause of lipid accumulation in hepatocytes (first hit) which generates oxidative stress, lipid peroxidation, and inflammatory response (second hit) thus resulting in hepatocellular lesion and fibrotic substitution.

The renin-angiotensin system (RAS) has been correlated to the whole basic physiopathogenic mechanism of NAFLD in experimental models. Systemic infusion of angiotensin 2 (AT2) in rats induced IR [14] and hepatic oxidative stress, increased the serum levels and hepatic concentration of tumor necrosis factor-alpha (TNF-α), promoted the infiltration of inflammatory cells in the liver, increased aspartate aminotrans-

ferase (AST) and alanine aminotransferase (ALT) and the activation of hepatic stellate cells (HSC) [15]. In human HSC culture, AT2 increased the production of reactive oxygen species and the expression of genes involved in hepatic fibrogenesis [16]. In rat HSC culture, AT2 stimulated the release of type I collagen and transforming growth factor beta 1 (TGF-β1) by activating its type 1 receptor [16]. In transgenic rats with high AT2 tissue levels, a significant increase in hepatic oxidative stress associated with an enhanced NADPH oxidase activity was observed [17].

In NAFLD animal models, namely rats, significant results were obtained with the use of angiotensin-converting enzyme (ACE) inhibitor perindopril [18] and angiotensin 2 type 1 receptor (AT1) antagonists irbesartan [18], telmisartan [19, 20], and olmesartan [21, 22]. A significant improvement was observed in steatosis, inflammatory response, and hepatic fibrosis with the aforementioned drugs [18-22]. IR, as assessed by the homeostasis model assessment index, improved with perindopril, irbesartan [18], and olmesartan [21]. Oxidative stress improved with telmisartan [19] and olmesartan [21, 22]. In hypercholesterolaemic rabbits, olmesartan prevented lobular inflammation and fibrosis, while reducing significantly steatosis and steatohepatitis (NASH), as assessed by the NAFLD activity score (NAS) [23].

A significant prevalence of MS in NAFLD carriers (> 40%) [1], an increased risk to develop NAFLD in MS carriers [6], and the correlation of this syndrome with NAFLD progression and severity have been reported [3]. Systemic arterial hypertension has been suggested to be associated with NAFLD in approximately 40% of the cases [1], and NAFLD has been independently associated with an increased risk of arterial hypertension in observational studies [24, 25]. Therefore, we can infer that treating arterial hypertension in NAFLD carriers will be often necessary and that the potential beneficial effects of the antihypertensive might, in this context, influence the choice of the respective drug.

Based on these findings, we conducted this study to evaluate the effects of the ACE-inhibitor ramipril, used preventively, in NAFLD induced in rabbits fed hyperlipidaemic diet.

Methods

Animals, and biochemical assays

Twenty-nine white adult male rabbits (New Zealand), with a mean age of 4 months, were selected for this study. The animals were handled in compliance with the Guiding Principles in the Care and Use of Animals, and protocol approval was obtained from the Pontifical Catholic University Animal Research Committee. The animals were divided into three groups: placebo group, 10 rabbits; ramipril group, 10 rabbits; and control group, 9 rabbits. During the 56 days of the study, the animals from control group were fed a specific diet (Nuvilab; Nuvital, Colombo, Brazil) that does not cause metabolic abnormalities. The animals from placebo group and ramipril group were fed a specific diet plus 0.925% cholesterol (cholesterol ≥ 92.5%; Sigma-Aldrich, St. Louis, USA). All groups were fed the respective diet ad libitum. With regard to the Ramipril group, 0.35 mg/kg/day of ramipril (commercially available from Sanofi Aventis) was administered orally from day 1 to day 56. The placebo (vehicle) was administered orally to placebo group throughout the study period at the same dosage. On day 56, the animals from the three groups underwent liver resection. Anaesthesia was induced with ketamine (30 mg/kg) and intramuscular xylazine (6 mg/kg). After the procedure, the rabbits were euthanized with a lethal dose of barbiturate. Blood samples were obtained on the first day of the experiment and immediately before euthanasia by cardiac puncture. Clinical laboratory assessment included fasting serum glucose, insulin, total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides, creatinine, aspartate aminotransferase (AST), and alanine aminotransferase (ALT). Measurements were obtained using two automated systems (Dimension RxL Max; Siemens, Munich, Germany. Abbott Architect i1000SR; Abbott, Chicago, USA).

Histological, morphometric, and immunohistochemistry analyses

The liver segments of the right lateral and left medial lobes were fixed with 10% formaldehyde buffered with phosphate and then paraffin embedded for haematoxylin and eosin staining and Gomori trichrome staining. Slides with the two histological sections (4 μm thick) were pre-

Table 1. Weight and serum biochemical parameters at baseline and eutanásia

PHASE	GROUP	n	WT	GLC	INS	CREAT
Baseline	Placebo	10	2996±250	141.0±32.6	5.00±1.58	1.16±0.20
	Ramipril	10	3080±314	123.7±48.0	4.59±1.09	1.23±0.22
	Control	9	3255±329	163.7±37.6	5.1±2.27	1.28±0.15
p-value (η^2)*			0.179 (η^2=0.12)	0.055 (η^2=0.20)	0.910 (η^2=0.01)	0.394 (η^2=0.07)
Euthanasia	Placebo	10	2989±255[a]	150.8±22.3	3.09±2.24[a]	1.44±0.29
	Ramipril	10	3182±197[a]	125.3±43.1	2.08±2.01[a]	1.47±0.26
	Control	9	3541±374	142.0±25.9	6.7±3.43	1.23±0.20
p-value (η^2)*			0.001 (η^2=0.42)	0.160 (η^2=0.13)	0.002 (η^2=0.39)	0.099 (η^2=0.16)
Baseline x Euthanasia	Placebo	10	0.904 (d=0.04)	0.234 (d=0.40)	0.045 (d=0.74)	0.007 (d=1.11)
p-value (Cohen's d)**	Ramipril	10	0.346 (d=0.31)	0.938 (d=0.03)	0.004 (d=1.19)	0.049 (d=0.72)
	Control	9	0.001 (d=1.79)	0.240 (d=0.42)	0.280 (d=0.39)	0.544 (d=0.21)

Data represent means ± standard deviations. WT, weight (grams); GLC, glucose (mg/dl); INS, insulin (μUI/ml); CREAT, creatinine (mg/dl); *oneway ANOVA and LSD post hoc test; η^2, eta-squared effect size measure; **paired Student's t test; d, Cohen's d effect size measure; [a]significant difference compared to control group (WT): placebo (P < 0.001, r=0.68), ramipril (P=0.010, r=0.54); INS: placebo (P=0.016, r=0.57), ramipril (P < 0.001, r=0.70).

Table 2. Serum aminotransferase and lipids at baseline and euthanasia

PHASE	GROUP	AST	ALT	CHOL	HDL	TGC
Baseline	Placebo	52.8±14.8	85.9±29.7	31.9±16.2	22.4±11.0	49.5±16.3
	Ramipril	51.2±18.1	68.7±29.2	38.4±28.0	22.5±9.2	45.7±19.4
	Control	70.0±43.7	92.2±47.8	18.4±10.7	14.7±6.6	70.7±34.6
p-value*		0.463	0.332	0.107	0.128	0.099
Euthanasia	Placebo	86.1±32.8[a]	98.5±47.9[a]	970.7±156.6[a]	306.2±72.1[a]	384.9±307.7[a]
	Ramipril	86.5±39.6[a]	104.3±54.1[a]	989.3±102.7[a]	234.2±80.6[a,b]	282.7±175.6[a]
	Control	50.6±23.0	92.7±37.7	18.1±6.19	13.0±6.2	76.2±48.6
p-value*		0.019	0.876	< 0.001	< 0.001	0.001
Baseline x Euthanasia	Placebo	0.023 d=0.87)	0.383 d=0.29)	< 0.001 d=6.29)	< 0.001(d=4.06)	0.007 (d=1.09)
p-value (Cohen's d)**	Ramipril	0.027 d=0.84)	0.120 d=0.54)	< 0.001 (d=10.3)	< 0.001 (d=2.67)	0.002 (d=1.34)
	Control	0.218 d=0.45)	0.976 (d=0.01)	0.919 (d=0.03)	0.479 (d=0.25)	0.769 (d=0.10)

Data represent means ± standard deviations. AST, aspartate aminotransferase (U/L); ALT, alanine aminotransferase (U/L); CHOL, total cholesterol (mg/dl); HDL, high-density lipoprotein cholesterol (mg/dl); TGC, triglyceride (mg/dl). *Oneway ANOVA and LSD post hoc test; **Paired Student's t test. [a]Significant difference compared to control group (AST): placebo (P=0.013, r=0.55), ramipril (P=0.013, r=0.50); CHOL: placebo (P < 0.001, r=0.98), ramipril (P < 0.001, r=0.99); HDL: placebo (P < 0.001, r=0.95), ramipril (P < 0.001, r=0.89); TGC: placebo (P=0.004, r=0.58), ramipril (P=0.042, r=0.64). [b]Significant difference compared to placebo group (HDL): P=0.018, r=0.44.

pared and analysed by blinded observers for steatosis, lobular inflammation, hepatocellular ballooning, and fibrosis based on the histological scoring system for NAFLD [26] using an Olympus BX 50 microscope (Tokyo, Japan).

For Sudan III staining, cryofixation and frozen sections of the liver segments was performed. The Sudan III stained slides were photographed with a digitizer (Axio Scan.Z1; ZEISS, Oberkochen, Germany), and images were analysed to estimate hepatic steatosis with an image analyser (Image-Pro Plus 4.5.0.29; Media Cybernetics, Rockville, USA). For each slide, approximately 600 images were obtained using a 20× objective. Approximately 500 images were excluded, resulting in approximately 100 satisfactory images per animal. The positive control was used as a "mask" displaying sufficient levels of tissue stained. The mask was then superimposed on images of the slides and an image analysis was performed to identify the positive areas in the slides based on the ideal pattern staining indicated by the mask, and these results were transformed to measures of positive areas in square micrometres (μm^2).

Immunohistochemistry was performed to estimate the expression of inducible nitric oxide synthase (iNOS) in liver tissue as an indirect parameter of nitrative/oxidative stress. Histological sections were stained with mouse mo-

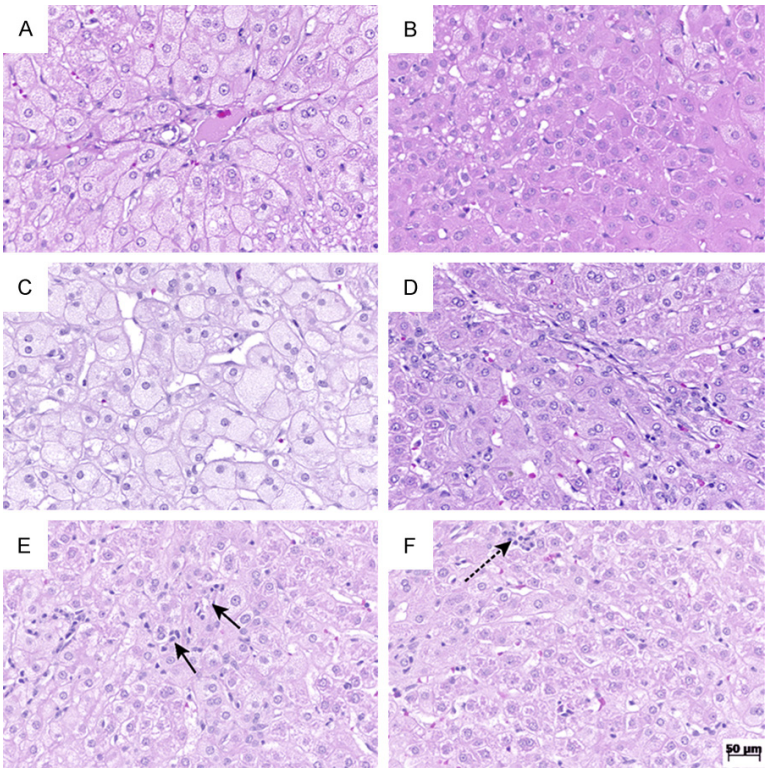

Figure 1. Hematoxylin and eosin stain at original magnification, 200×. A. Rabbit from placebo group with high score of ballooning; B. Liver from the ramipril group with preserved lobular architecture, showing no ballooning; C. Rabbit from placebo group with high score of steatosis; D. Rabbit from the ramipril group with minor degree of steatosis; E. Rabbit from the placebo group with high score of lobular inflammation; F. Rabbit from the ramipril group with score 1 of lobular inflammation.

noclonal anti-iNOS antibody (1/200 dilution; ab129372, Abcam), and immunostained slides were analysed blindly based on the Allred score [27], using an Olympus BX 50 microscope (Tokyo, Japan). The Allred score is defined as the sum of the proportion and intensity scores. The proportion score is determined by the overall proportion of stained cells (0= none; 1= < 1/100; 2=1/100 to < 1/10; 3=1/10 to < 1/3; 4=1/3 to 2/3; 5= > 2/3) and the intensity score by the intensity of staining in the positive cells (0= none; 1= weak; 2= intermediate; 3= strong).

Statistical analysis

Data were analysed with software SPSS, version 20 (IBM, New York, USA). Results are described by means and standard deviations or by frequencies and percentages. The interaction between group (placebo, ramipril, and control) and time (baseline and euthanasia) was analysed using ANOVA (mixed model) with one between-subjects factor (group) and one within-subjects factor (time). Considering that most of the variables presented a significant interaction between group and time, the analysis was performed using the one-way ANOVA model (group comparisons at baseline and euthanasia) and paired Student *t*-test (time comparisons for each group). Effect size was reported by eta-squared measures, r-value, and Cohen's d. Categorical variables were analysed by Fisher's exact test. Normality distribution was evaluated using Shapiro-Wilk test, and data from variables that did not meet this condition were submitted to a logarithmic transformation. A *p*-value < 0.05 was indicative of statistical significance.

Results

Metabolic and lipid profiles

The weight of the animals at baseline was similar in the three groups. The average body weight of rabbits in the control group was higher than that in the other groups, and no difference was observed between the ramipril and placebo groups at euthanasia (**Table 1**).

Glucose, insulin, creatinine, aminotransferase, and lipid serum levels were also similar in the three groups at baseline, and these parameters were similar between the placebo and ramipril groups at euthanasia (**Tables 1** and **2**). Compared to the control group, there was a significant increase in lipid and AST levels, and a significant decrease in insulin levels in the placebo and ramipril groups at euthanasia (**Tables 1** and **2**). Insulin levels in the control group were similar at baseline and euthanasia (**Table 1**).

Histological, morphometric, and immunohistochemistry analysis

Steatosis was observed in all animals of the placebo group and in 90% of the ramipril group. The lowest steatosis score was observed in 40% of the ramipril group, which was not

Table 3. Results of histological analysis according to the histological scoring system for NAFLD

ITEM	DEFINITION	SCORE	PERCENTAGE FOR GROUP			P
			Placebo	Ramipril	Control	
Steatosis	< 5%	0	0%	10%	100%	0.032
Grade	5 a 33%	1	0%	40%		
	> 33 a 66%	2	50%	40%		
	> 66%	3	50%	10%		
Lobular	No foci	0	0%	0%	100%	0.006
Inflammation	< 2 foci per 200X field	1	20%	90%		
	2-4 foci per 200X field	2	40%	10%		
	> 4 foci per 200X field	3	40%	0%		
Hepatocellular	None	0	0%	10%	100%	0.023
Balooning	Few balloon cells	1	20%	70%		
	Many cells/prominent ballooning	2	80%	20%		
Fibrosis Stage	None	0	0%	0%	100%	0.020
	Perisinusoidal or periportal	1	0%	0%		
	Mild, zone 3, perisinusoidal	1A	20%	60%		
	Moderate, zone 3, perisinusoidal	1B	10%	30%		
	Portal/periportal	1C	50%	10%		
	Perisinusoidal and portal/periportal	2	20%	0%		
NAS	Not NASH	0-2	0%	20%	100%	0.003
	Borderline	3-4	0%	50%		
	NASH	≥ 5	100%	30%		

NAS, nonalcoholic fatty liver disease activity score; NASH, nonalcoholic steatohepatitis. p, *p* value: placebo vs ramipril (Fisher's exact test, after grouping the scores into two categories).

observed in the placebo group. The highest steatosis score was noted in 10% and 50% of the ramipril and placebo groups, respectively (**Table 3**). The comparison between the two groups showed a significant difference (P= 0.032), pointing to a decrease in steatosis with ramipril (**Figure 1**). All animals of both placebo and ramipril groups developed lobular inflammation. However, 90% of the ramipril group developed slight inflammation (score 1), and the highest inflammation score was not observed in any animal of this group (**Table 3**). A significant difference was found between the two groups (P=0.006), showing a decreased inflammatory response with ramipril (**Figure 1**).

Hepatocellular ballooning occurred in all animals of the placebo group and in 90% of the ramipril group. However, the lowest ballooning score occurred in 70% and 20% of the ramipril and placebo groups, respectively (**Table 3**). The comparison between the two groups showed a significant reduction (P=0.023) of ballooning degeneration with ramipril (**Figure 1**). Fibrosis scores between 1A and 1C and between 1A and 2 were observed in the placebo and ramipril groups, respectively. Fibrosis scores 1C and 2 were observed in 90% of the placebo group, and lower scores (1A e 1B) in 70% of the animals belonging to the ramipril group (**Table 3**). A significant difference was found between the two groups (P=0.02), pointing to a reduction of hepatic fibrosis with ramipril (**Figure 2**). NAS correlated with the presence of NASH (score ≥ 5) in the entire placebo group and in 30% of the ramipril group. Scores ≤ 2 correlated with the absence of NASH, which occurred in 20% of the ramipril group (**Table 3**). Comparison between the two groups showed a significant difference, suggesting an attenuating effect of ramipril in the development of NASH (P=0.003). None of the histological alterations that compose the histological scoring system for NAFLD were observed in the control group. Therefore, the control group was characterized as normal (**Table 3**; **Figure 3**).

The mean area of positivity was estimated by morphometry of Sudan III-stained slides, in the placebo (19.9±10.6 μm^2) and ramipril (11±8.2

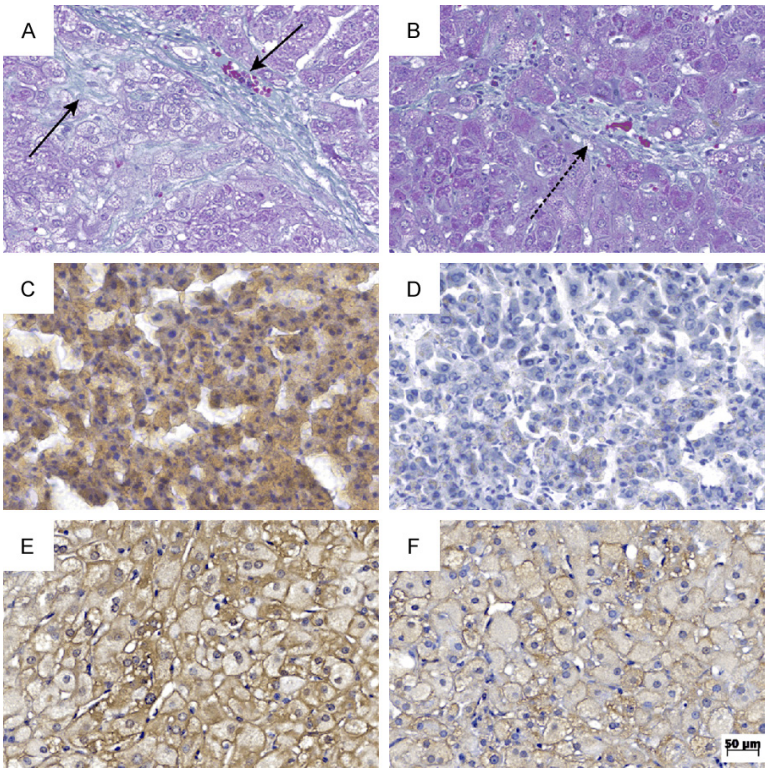

Figure 2. Photomicrographs at original magnification, 200×. A. Liver from the placebo group with score 2 of fibrosis (Gomori staining); B. Fibrosis score 1A observed in the ramipril group (Gomori staining); C. Rabbit from the placebo group with high score of steatosis (Sudan III stain); D. Rabbit from the ramipril group with minor degree of steatosis (Sudan III stain); E. Rabbit from the placebo group with high Allred score (iNOS immunostaining); F. Rabbit from the ramipril group with Allred score 5 (iNOS immunostaining).

μm^2) groups. Comparison between the two groups showed a significant difference (P= 0.049), in agreement with the histological analysis of haematoxylin and eosin-stained slides (**Figure 2**).

The expression of iNOS, estimated by Allred score, in the placebo group was significantly higher compared with ramipril (P < 0.001), suggesting a reduction of iNOS expression with ramipril (**Figure 2**).

Discussion

Approximately four decades after the description of NAFLD, despite growing interest and efforts of the scientific community, its pharmacological therapy remains undefined. No drugs have been approved by the regulatory organs for NAFLD or NASH treatment [28-30]. The present recommendation is based on the approach used for associated diseases such as obesity, insulin resistance, diabetes, and dyslipidaemia [28, 31]. Hypertension is significantly prevalent in adults and is frequently associated with NAFLD [1, 24, 25]. Therefore, we can infer that criteria for the choice of antihypertensive drugs in this condition are needed.

In the present study, ramipril was used in a dosage calculated by allometric extrapolation, equivalent to approximately 5 mg per day for humans [32]. Compared with placebo, ramipril significantly decreased steatosis, lobular inflammation, and hepatic fibrosis (**Table 3**). Similar results were observed in other rat models of NAFLD with the ACE-inhibitor perindopril and the AT1 blockers irbesartan, telmisartan, and olmesartan [18-22]. Nevertheless, in these studies, NASH was not adequately characterised according to established histological criteria [31]. In the present study, placebo group rabbits developed histological alterations resembling human NASH, and in the ramipril group all these histological alterations, including steatosis, lobular inflammation, hepatocellular ballooning, and fibrosis were significantly attenuated. Furthermore, histological analysis was performed based on a validated [26] and recognized [31] histological scoring system for NAFLD.

NAS is defined as an unpondered sum of steatosis, lobular inflammation, and ballooning scores. In the validation study, scores ≤ 2 and ≥ 5 were correlated with the absence and presence of NASH, respectively. Scores 3 and 4 were distributed nearly evenly among the three defined diagnostic categories (not NASH, borderline, and NASH) [26]. In our study, the presence of NASH, estimated by NAS, was observed in all rabbits of the placebo group and in 30% of the ramipril group. NAS correlated with the absence of NASH in 20% of the ramipril group.

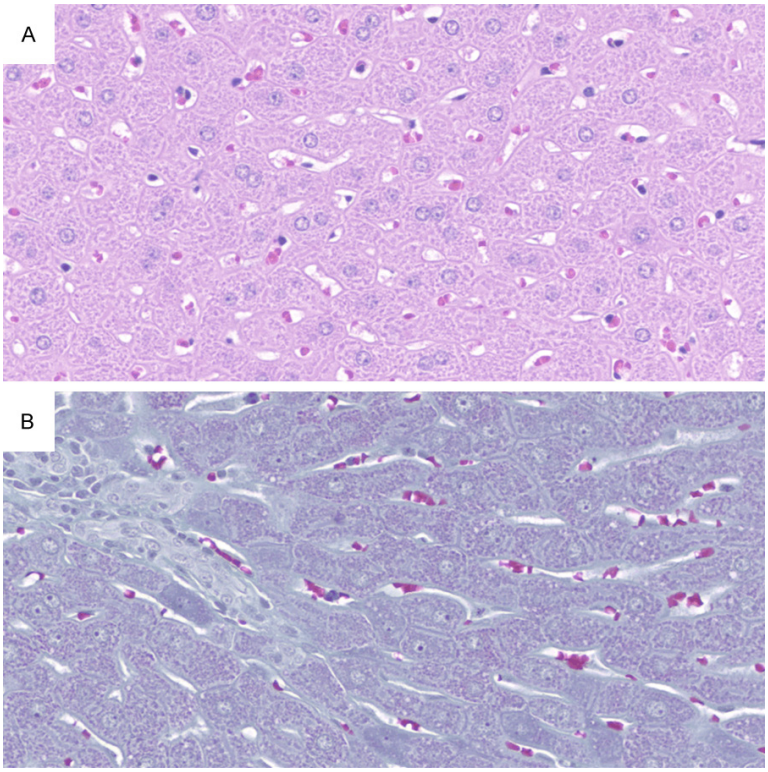

Figure 3. Photomicrographs from the control group at original magnification, 200×. Liver with preserved lobular architecture, showing no histopathological changes. A. Hematoxylin and eosin stain; B. Gomori staining.

A comparison between both groups showed a significant difference, suggesting an attenuating effect of ramipril in the development of steatohepatitis (**Table 3**). A similar result was observed in an experiment with olmesartan in the same animal model using the same histological analysis criteria [23]. Although these studies evaluated different forms of renin-angiotensin system blockade, these results may be considered consonant with our results.

The weight of the animals in the ramipril and placebo groups, as well as the total cholesterol and triglyceride levels, was similar at baseline and euthanasia (**Tables 1** and **2**). Therefore, these variables did not influence the results. Glucose, insulin, aminotransferases and creatinine levels were also similar in both groups (**Tables 1** and **2**), suggesting that these parameters had no influence in the histological results. The behaviour of the glucose and aminotransferases serum levels was similar in a study using olmesartan in the same animal model [23], suggesting an inherent pattern to the model used. The lack of difference between the placebo and ramipril groups in the analysis of glucose, insulin, and aminotransferases variables might characterise a limitation of the model used in the present study. However, the whole histopathological spectrum of NAFLD, as well as the difference between the groups was well characterised. The disease was induced by hyperlipidaemia. This was associated with NAFLD, found in 69.16% of the cases in a recently published meta-analysis [1]. Moreover, no standard animal model for NAFLD exists currently. In our study, peripheral IR was not assessed by euglycaemic hyperinsulinaemic clamp, considered the gold standard for this evaluation. However, the glucose levels were similar in the three groups, and the levels of insulin were lower in the placebo and ramipril groups compared with the control group at euthanasia. Furthermore, insulin levels in the control group were similar at baseline and at euthanasia (**Table 1**). Therefore, we can infer that peripheral IR was not characterised in our study, raising the possibility that histological alterations compatible with NAFLD developed in the model used were related with hepatic insulin resistance. In other animal models, the increase of iNOS hepatic expression has been associated with hepatic IR [33-35] and nitro-oxidative stress [33, 34]. In the present study, iNOS expression, estimated by the analysis of immunostained slides, was significantly higher in the placebo group compared with the ramipril group ($P < 0.001$), suggesting that a reduction of hepatic iNOS expression was observed with ramipril.

The widely described correlation between NAFLD and IR [3-5], frequent association of this hepatopathy with MS components [5-7] in 39.34% of hypertension cases [1], increased risk of NAFLD-carriers to develop T2DM [10] and CVD [8-13], increased mortality rate due to CVD in NAFLD carriers [36, 37], and the

wide association of RAS with the pathophysiology of this hepatic disease [14-17] might influence a possible definition of criteria for the choice of drugs to treat patients with NAFLD and hypertension.

ACE inhibitors (captopril, enalapril, lisinopril, ramipril, and trandolapril) and AT1 blockers (losartan, valsartan, and candesartan) significantly decreased T2DM incidence in randomised and controlled clinical trials, selected in two meta-analyses [38, 39]. Ramipril, in randomised clinical trial, controlled placebo, reduced CVD mortality, the incidence of acute myocardial infarction, and stroke in high-risk patients [40].

In the present study, ramipril significantly reduced the development of steatosis, lobular inflammation, and hepatic fibrosis. Based on the criteria of NAS, ramipril significantly diminished the development of NASH. Therefore, we can conclude that in rabbits fed hyperlipidaemic diet, the preventive use of ramipril attenuates the development of the whole NAFLD histopathological spectrum. Further studies are required to confirm these findings, increase the knowledge on the association of NAFLD and hypertension, and consolidate the criteria to choose the most appropriate drugs or therapeutic classes for the treatment of arterial hypertension in NAFLD carriers.

Disclosure of conflict of interest

None.

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