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EFFECT OF CHRONIC ASPIRIN ADMINISTRATION ON AN EXPERIMENTAL MODEL OF METABOLIC SYNDROME

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SUMMARY

1. The aim of the present study was to examine the effect of chronic administration of aspirin on metabolic and cardiovascular parameters in fructose-fed rats (FFR), an experimental model of metabolic syndrome.

2. Chronic treatment of FFR with aspirin (10 mg/kg per day for 6 weeks) partially reversed the increment in systolic blood pressure. In addition, chronic aspirin treatment normalized relative heart weight and vascular remodelling of renal and carotid arteries, measured as lumen diameter : medial thickness ratio.

3. Furthermore, chronic aspirin administration completely reversed glucose intolerance and decreased the oxidative status that characterizes the FFR model, as indicated by decreased plasma levels of thiobarbituric acid-reactive substances and aortic NAD(P)H oxidase activity.

4. Prevention of oxidative stress and vascular remodelling in FFR may contribute to the protective actions attributed to aspirin in the treatment of metabolic syndrome.

Key words: aspirin, fructose-fed rats, hypertension, metabolic syndrome, oxidative stress, vascular remodelling.

INTRODUCTION

The cluster of cardiovascular risk factors including hypertension, dyslipidaemia, hyperinsulinaemia, insulin resistance and other disease states has been called metabolic syndrome.[1,2] Changes in vascular wall components could be involved in the cardiovascular alterations associated with this state. Considerable experimental and clinical evidence links an enhanced production of reactive oxygen species (ROS) with certain diseases of the cardiovascular system, including hypertension and diabetes.[3]

Feeding carbohydrate-enriched diets to normal rats has been shown to induce metabolic syndrome.[4,5] Fructose-fed rats (FFR) have been used to assess the pathophysiological mechanisms involved in the development of this syndrome.[6,7]

Antiplatelet drugs have an established role in the primary and secondary prevention of cardiovascular events. Among these drugs, there is much evidence supporting a role for aspirin as an important pharmacological tool in treatment and prevention of metabolic syndrome, as well as an adjunct therapy for these patients.[8] Aspirin interferes with the metabolism of cyclic prostanoids by irreversible inhibition of cyclo-oxygenase (COX), a key enzyme in the pathway of synthesis of thromboxane (TX) A_2, a potent prothrombotic and vasoconstrictor substance. Postulated mechanisms of aspirin activity include inhibition of platelet TXA_2 production, increased production of nitric oxide, protection of low-density lipoproteins and fibrinogen against oxidation, scavenging of free radicals, facilitated inhibition of platelet activation by neutrophils, and protection of endothelial function and anti-inflammatory properties.[9]

Excessive production of superoxide anion (O_2^-) is associated with oxidative stress and subsequent cardiovascular tissue injury. Numerous clinical and experimental studies have shown that oxidative stress plays an important pathogenic role in cardiovascular diseases, including atherosclerosis[10] and hypertension.[3]

Aspirin is also effectively hydrolysed by the liver during its passage through the liver.[11] Most aspirin is present in the form of free salicylate in the plasma. Whether the effects of aspirin are mediated by intact aspirin or its hydrolytic metabolite salicylate remains to be established. Salicylate, like other simple phenols (and unlike aspirin, a blocked phenol) is an anti-oxidant and is expected to have an anti-inflammatory action.[12]

In the present study, we examined the effects of chronic administration of aspirin on metabolic and cardiovascular variables, including oxidative stress and vascular remodelling, in an experimental model of metabolic syndrome.

METHODS

Animals and experimental design

All procedures were performed according to institutional guidelines for animal experimentation. Thirty-day-old male Wistar rats were fed a standard commercial chow diet *ad libitum* and housed for the duration of the 12 week experimental period in a temperature-controlled (20°C) room, at 45% relative humidity and under a 12 h light–dark cycle.

Animals were randomly assigned to one of four groups ($n = 8$ in each group): (i) a control group, in which rats received food and water *ad libitum*; (ii) a control aspirin (C + A) group, in which rats received 10 mg/kg per day aspirin in their drinking water for the last 6 weeks of the experiment; (iii) a FFR group, in which rats were fed 10% (w/v) fructose (Parafarm, Buenos Aires, Argentina) solution in their drinking water for a period of 12 weeks; and (iv) a FFR aspirin-treated group (FFR + A), in which rats were fed fructose for 12 weeks in their drinking water and were given aspirin in their drinking water for the last 6 weeks.

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At the end of the experimental period, rats were anaesthetized with ether, blood samples were taken and arteries and organs were excised aseptically for the measurement of various parameters.

Biochemical determinations

Homeostatic model assessment index and intraperitoneal glucose tolerance test

Blood was collected from rats and plasma was separated. Fasting plasma insulin was assayed by an ACS:180 SE automated chemiluminescence system (Bayer, Leverkusen, Germany). Plasma glucose levels were assayed using a commercially available colourimetric kit (Wiener Laboratory, Rosario, Argentina). Homeostatic model assessment (HOMA) was used as an index of insulin resistance and was calculated as follows:[13,14]

$$\text{HOMA} = (\text{insulin } (\mu\text{U/mL}) \times \text{glucose (in mmol/L)})/22.5$$

Three days before the end of the experimental period, a glucose tolerance test (GTT) was performed. Rats were fasted overnight, anaesthetized with pentobarbital (35 mg/kg) and glucose was administered (2 g/kg, i.p.). Blood samples were taken by tail bleeding at 0, 30, 60 and 90 min after glucose injection to determine plasma glucose concentrations by an enzymatic glucose oxidase–peroxidase photocolourimetric method (Wiener Laboratory). The total area under the curve was calculated as mmol/L per 90 min.

Systolic blood pressure measurement

Systolic blood pressure (SBP) was monitored indirectly in conscious rats (prewarmed and restrained slightly in animal nose-cone acrylic holders) by the tail-cuff method and recorded on a Grass Model 7 polygraph (Grass Instruments, Quincy, MA, USA). Rats were accustomed to the apparatus several times before actual measurements were taken.

Relative heart weight

In order to evaluate cardiac hypertrophy, the heart was removed from the great vessels, placed in phosphated-buffered saline (PBS), blotted with tissue paper to remove blood and weighed. Total heart weight was normalized against bodyweight and is expressed as mg/100 g bodyweight.

Measurement of plasma thiobarbituric acid-reactive substances

In order to demonstrate the effect of increased oxidative stress at the vascular level, plasma lipid peroxidation was evaluated as the concentration of thiobarbituric acid-reactive substances (TBARS). This method is based on the reaction between plasma malondialdehyde, a product of lipid peroxidation, and thiobarbituric acid (TBA).[13] Heparinized plasma was added to the reaction mixture, followed by 30 min heating at 95°C. Butylated hydroxytoluene was used to prevent lipid peroxidation during heating. After cooling, the chromogen was extracted and measured photometrically.

Results are expressed as equivalents of malondialdehyde (μmol/L). No correction to sample protein content was necessary because of the type of sample.[13]

Measurement of vascular NAD(P)H oxidase activity

The lucigenin-derived chemiluminiscence assay was used to determine NAD(P)H oxidase activity in the aorta, as described previously.[15] A 2 cm segment of thoracic aorta was cut, cleaned, washed and transferred to a tube with 2 mL Jude's Krebs' buffer (JKB) containing (in mmol/L): HEPES 2; NaCl 11.9; KCl 0.46; $MgSO_4.7H_2O$ 0.1; Na_2HPO_4 0.015; KH_2PO_4 0.04; $NaHCO_3$ 0.5; $CaCl_2$ 1.2; glucose 5.5, pH 7.40. Tissues were equilibrated in JKB at 37°C for 30 min. Aortic segments were then transferred to a tube containing 1 mL JKB and lucigenin (5 μmol/L) and left in the dark at room temperature for 10 min. This concentration of lucigenin does not appear to be involved in redox cycling and specifically detects superoxide anion.[15] To assess NAD(P)H oxidase activity, βNAD(P)H (500 μmol/L) was added and chemiluminiscence was immediately measured in a liquid scintillation counter (Model 1219 Rack-Beta Scintillation Counter; LKB Wallac, Turku, Finland) set in the out-of coincidence mode. Time-adjusted and normalized against tissue weight scintillation counts were used for calculations. Measurements were repeated in the absence and presence of diphenylene iodinium (DPI; 10^{-6} mol/L), which inhibits flavin-containing enzymes, including NAD(P)H oxidase.[15]

Tissue preservation

Tissue samples were processed for histopathology as reported previously.[16] Samples were used from all rats. Animals were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.) and then perfused for 10 min at 8 mL/min with PBS (298 mOsmol/kgH_2O, pH 7.40, 4°C) to clear blood. The kidneys were perfused *in vivo* with the same solution at a rate of 8 mL/min through the renal artery for a period of 5 min. For histological studies, left kidneys were perfused with 4% paraformaldehyde solution for 10 min, then fixed by immersion in the same solution for 48 h, before being placed in 30% sucrose solution and kept at –70°C until analysis. Sections (5 μm) were cut transversely through the entire kidney on a cryostat (HM 505E; Microm, Munich, Germany) at –26°C and then processed for histological analysis, as described below.

Common left carotid arteries were fixed and processed as described above for the kidneys.

Histopathology and morphometry

Lumen : media ratio in kidney arteries

Transverse slices from the common left carotid artery and left kidney were placed on microscope slides and stained with Masson's trichrome solution. Slides were scanned under a light microscope (Optiphot-2; Nikon, Kanagawa, Japan), images were digitalized (GP-KR222 colour CCD; Panasonic, Osaka, Japan) and then processed with the two-dimensional analysis system Scion Image 4.01 (Scion, Bethesda, MD, USA). To evaluate renal arterial wall thickening, images from three different artery types were studied in each kidney: interlobar, arcuate and interlobular. Interlobar arteries were localized towards the cortex along the columns of Bertin located between adjacent medullary pyramids. Arcuate arteries were identified along the corticomedullary junction and were surrounded by tubules. Interlobular arteries were identified as a single muscular artery within the inner cortex and, at times, lying close to the glomerulus.[17] Non-transverse sectioned arteries were excluded from investigation. The lumen : media ratio (i.e. internal diameter : medial thickness) was then calculated. Forty slices from each kidney were processed and five to 10 arteries of each type in each slice were analysed to obtain an average value for each rat. Average values were then used for final analysis.

Common left carotid arteries were sectioned transversely. The lumen : media ratio was calculated for 10 slices from each artery to obtain an average value for each rat, which was then used for final analysis.

Reagents

Unless noted otherwise, all reagents were purchased from Sigma Chemical (St Louis, MO, USA).

Statistical analysis

Data are expressed as the mean±SEM. The significance of differences was assessed by one-way ANOVA, followed by Student–Newman–Keuls' post-test

Table 1 Final bodyweight, variations in bodyweight throughout experimental period, basal glucemia, homeostatic model assessment index and total area under the glucose tolerance test curve measured at the end of the experimental period, final relative heart weight and systolic blood pressure from the start of experiment, at initiation of aspirin treatment (6 weeks) until the end of the study

	Control	C + A	FFR	FFR + A
Final bodyweight (g)	295 ± 3	286 ± 8	315 ± 3*	290 ± 7†
Increase in bodyweight (g)	180 ± 5	186 ± 8	210 ± 6**	184 ± 7†
Fasting glucose (mmol/L)	4.9 ± 0.1	5.1 ± 0.1	7.1 ± 0.1**	5.2 ± 0.1††
Fasting triglycerides (mmol/L)	0.99 ± 0.03	0.94 ± 0.06	1.56 ± 0.18**	0.98 ± 0.07††
HOMA	4.3 ± 0.2	4.0 ± 0.6	11.9 ± 0.2**	5.2 ± 2.4††
AUC_{GTT} (mmol/L per 90 min)	881 ± 64	886 ± 128	1292 ± 31**	839 ± 51††
Relative heart weight (mg/100 g bodyweight)	225 ± 4	234 ± 4	298 ± 18***	242 ± 4††
Systolic blood pressure (mmHg)				
Baseline	105 ± 3	102 ± 2	102 ± 1	105 ± 3
6 weeks	103 ± 2	114 ± 3	131 ± 3***	132 ± 3***
12 weeks	111 ± 4	110 ± 2	136 ± 3***	123 ± 3*†††

Data are the mean±SEM ($n = 8$ in each group). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with the control group; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$ compared with fructose-fed rats (FFR).

C + A, control group receiving aspirin for the last 6 weeks of the study; FFR + A, FFR receiving aspirin for the last 6 weeks of the study; HOMA, homeostatic model assessment (calculated as (μU/mL insulin × mmol/L glucose)/22.5)); AUC_{GTT}, area under the glucose tolerance test curve

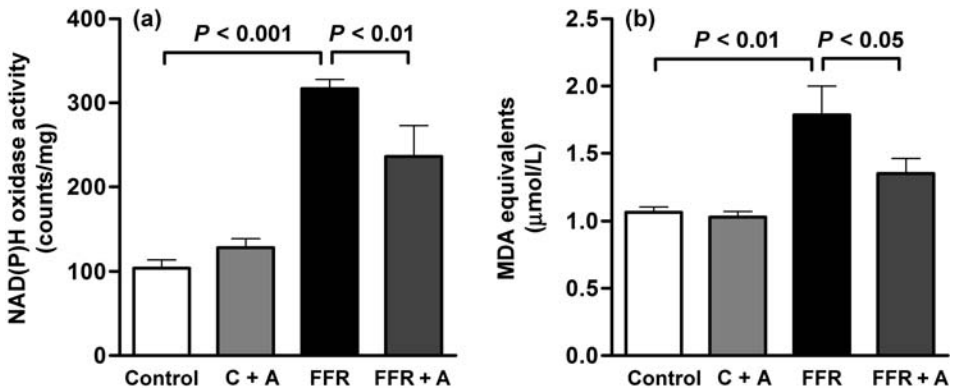

Fig. 1 (a) Aortic NAD(P)H oxidase activity, expressed as chemiluminiscence counts detected per mg aortic tissue, indicating superoxide production in the control, control + aspirin (C + A), fructose-fed (FFR) and fructose-fed + aspirin-treated (FFR + A) groups. (b) Plasma thiobarbituric acid-reactive substances (TBARS), expressed as malondialdehyde (MDA) equivalents. Significant differences were detected (one-way ANOVA and Newman–Keuls' post-test). Data are expressed as the mean±SEM ($n = 8$ in each group).

using GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego, CA, USA). $P < 0.05$ was considered significant.

RESULTS

Chronic administration of fructose induced several alterations included in the cluster of risk factors that characterizes metabolic syndrome. At the end of the experimental protocol, when animals were killed to obtain organs for histological examination, samples were taken of the gastric epithelium to check for gastric bleeding. Neither haemorrhagic lesions nor ulceration were observed. None of animals in the present study died. The bodyweight of rats in the fructose-fed group was increased compared with control ($P < 0.05$), but aspirin treatment of FFR reduced bodyweight to levels similar to those in the control group (Table 1). A significantly greater ($P < 0.01$) HOMA index and area under the GTT curve in FFR compared with control rats indicated that development of glucose intolerance in FFR. Chronic treatment with aspirin had no effect on either HOMA or GTT in the control group, but caused a significant reduction in both parameters in FFR ($P < 0.01$; Table 1).

Table 1 also gives the time-course of changes in SBP and relative heart weight at the end of the experimental period. By Week 6, the SBP of FFR was significantly increased compared with that in the control group ($P < 0.001$). Daily administration of aspirin to FFR during the last 6 weeks of the experiment partially reversed the increment in SBP ($P < 0.001$); however, SBP in the FFR + A group remained greater than that in the control group ($P < 0.01$). Aspirin treatment had no effect on SBP in control rats. Relative heart weight was significantly higher in FFR than in control rats ($P < 0.001$). Long-term treatment with aspirin reduced RHW in FFR ($P < 0.01$), but had no effect in the control group (Table 1).

Vascular oxidative status was assessed by measurement of the enzyme activity producing superoxide and its effects on plasma lipid peroxidation. Figure 1a shows that NAD(P)H oxidase activity was significantly higher in aortas from FFR compared with control ($P < 0.001$). Aspirin treatment partially, but significantly, reduced enzyme activity in FFR ($P < 0.01$), but had no effect in control rats.

Plasma TBARS values are shown in Fig. 1b. Plasma TBARS concentrations were significantly greater in FFR compared with control rats ($P < 0.01$). Aspirin treatment significantly reduction

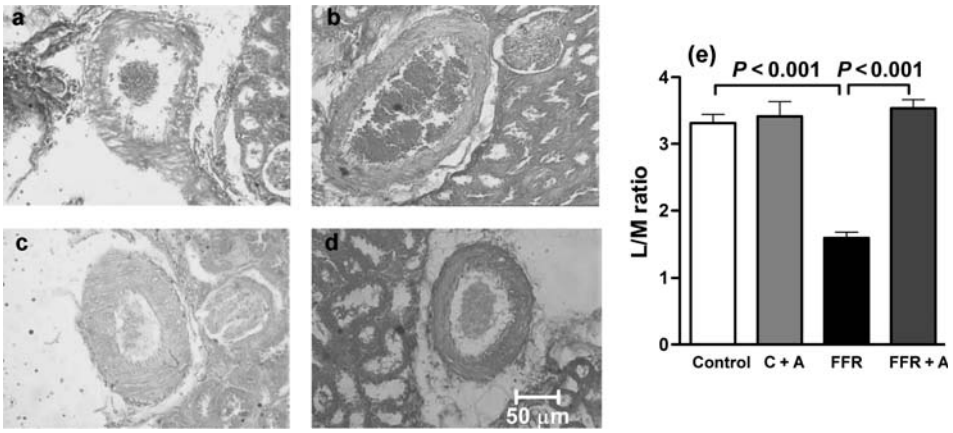

Fig. 2 (a–d) Microphotographs of renal lobar arteries (Masson's trichrome stain) in the (a) control, (b) control + aspirin (C + A), (c) fructose-fed (FFR) and (d) fructose-fed + aspirin-treated (FFR + A) groups. (e) Lumen : media (L/M) ratio of renal interlobar arteries. Data are the media±SEM ($n = 8$).

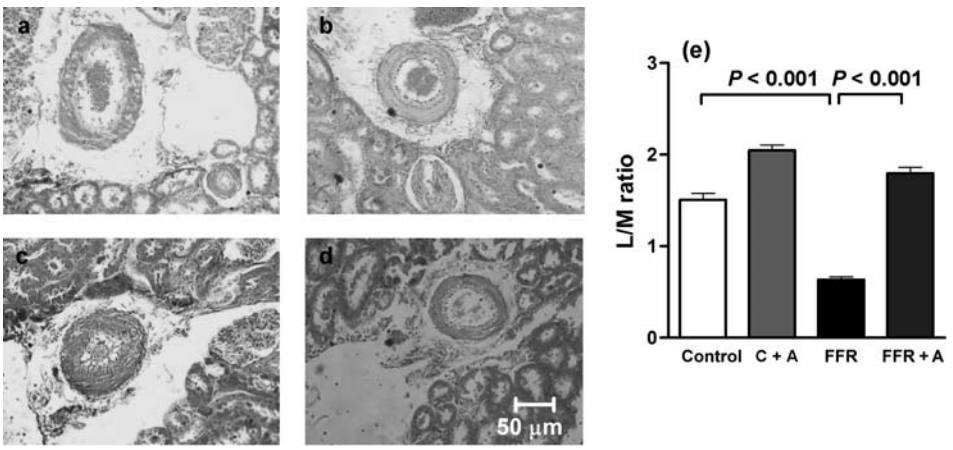

Fig. 3 (a–d) Microphotographs of renal arcuate arteries (Masson's trichrome stain) in the (a) control, (b) control + aspirin (C + A), (c) fructose-fed (FFR) and (d) fructose-fed + aspirin-treated (FFR + A) groups. (e) Lumen : media (L/M) ratio of renal arcuate arteries. Data are the media±SEM ($n = 8$).

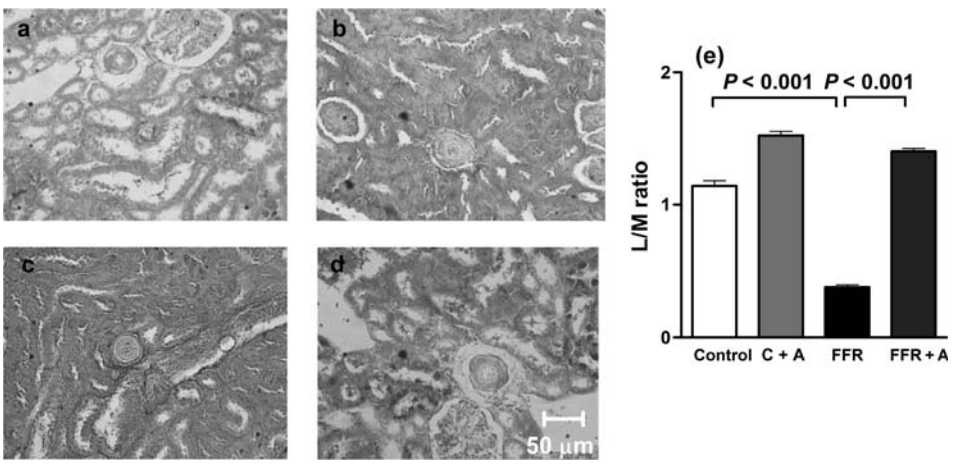

Fig. 4 (a–d) Microphotographs of renal interlobular arteries (Masson's trichrome stain) in the (a) control, (b) control + aspirin (C + A), (c) fructose-fed (FFR) and (d) fructose-fed + aspirin-treated (FFR + A) groups. (e) Lumen : media (L/M) ratio of renal interlobular arteries. Data are the media±SEM ($n = 8$).

plasma TBARS levels in FFR ($P < 0.05$), but had no effect in control rats.

Figures 2–4 show lumen : media ratios for the interlobar, arcuate and interlobular renal arteries. In FFR, the lumen : media ratio was significantly less than that of corresponding arteries from control rats. Aspirin treatment for the last 6 weeks of the experiment increased the lumen : media ratio for all three types of renal arteries from FFR to levels close to those seen in control rats, indicating an effect on vascular structure. In control rats, aspirin treatment for the last 6 weeks of the experiment increased the lumen : media ratio of arcuate and interlobular arteries ($P < 0.01$), but not that of interlobar arteries. Representative microphotographs from slices stained with Masson's trichrome are also shown for all four groups in Figs 2–4. Structural analysis using histological methods allows us to observe a pattern of structural modifications to the arterial wall, which appears in different locations and in arteries of different calibre.

Figure 5 shows the lumen : media ratio in digitalized images from transverse sections of common left carotid arteries, where significant

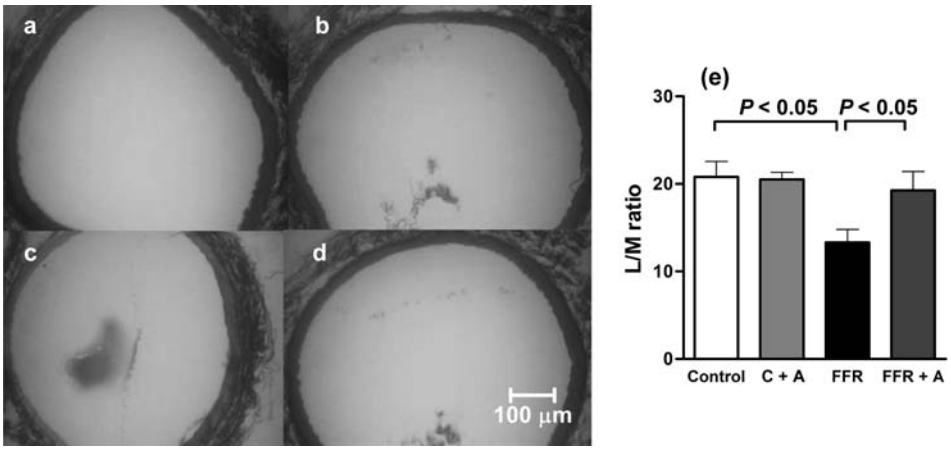

Fig. 5 (a–d) Microphotographs of renal radial arteries (Masson's trichrome stain) in the (a) control, (b) control + aspirin (C + A), (c) fructose-fed (FFR) and (d) fructose-fed + aspirin-treated (FFR + A) groups. (e) Lumen : media (L/M) ratio of common left carotid arteries. Data are the media±SEM ($n = 8$).

changes are observed in the lumen : media ratio of arteries from FFR compared with control. Arteries from the FFR + A group are similar to control, demonstrating the ability of aspirin to reverse the vascular remodelling associated with this experimental model.

DISCUSSION

Many studies in humans and experimental animal models have demonstrated an association between hypertension, insulin resistance and changes in the circulating lipid profile, a cluster of risk factors referred as 'metabolic syndrome', where insulin resistance has been implicated as a central pathogenic feature.[18] The growing prevalence and high-risk nature of this syndrome highlights the need to identify this condition and to treat it with an assertive, multitargeted approach.[19,20]

Over the past 10 years, it has become clear that cardiovascular disease and atherosclerosis have a 'micro-inflammatory' component and are often associated with low levels of inflammatory markers. In particular, diseases that predispose to cardiovascular events, such as metabolic syndrome and type 2 diabetes, appear to have a very strong inflammatory component.[21]

Growing evidence supports the role for increased oxidative stress and associated oxidative damage as mediators of vascular injury. Reactive oxygen species act as inter- and intracellular mechanisms of signal transduction in physiological and pathophysiological processes. In addition, ROS modulate vascular tone and structure,[15] are pro-inflammatory and stimulate the migration of monocytes, as well as the formation of oxidized low-density lipoproteins, which impair vascular endothelial function. Consequently, excessive ROS may underlie pathological processes associated with endothelial dysfunction and vascular remodelling, which are characteristic features of hypertension.[12,22–25]

The benefits of long-term aspirin therapy in cardiovascular diseases have been demonstrated by several trials.[26,27] It has recently been shown that aspirin exerts biological COX-independent effects.[28] The development of hypertension in several experimental models can be prevented or attenuated by chronic treatment with potent antioxidant therapies, such as aspirin.[3] Aspirin has been used for many years as an antithrombotic and anti-inflammatory agent. Evidence suggests that the efficacy of aspirin in the primary and secondary prevention of ischaemic cardiovascular events in patients with hypertension and atherosclerosis depends not only on its platelet inhibitory function, but also on its additional biological actions on the vasculature, including anti-inflammatory effects.[29,30]

Previous studies have indicated that angiotensin (Ang) II increases cardiovascular tissue NAD(P)H oxidase activity and stimulates cardiovascular O_2^- production.[15] This oxidative mechanism is thought to play an important role in Ang II-mediated trophic cardiovascular changes, such as cardiovascular tissue hypertrophy and hypertension.[7,15]

As described by Nascimento-Silva *et al.*,[31] lipoxins and their aspirin-triggered carbon-15 epimers have emerged as mediators of key events in endogenous anti-inflammation and resolution. However, the effects of these novel lipid mediators on cardiovascular diseases, such as hypertension, atherosclerosis and heart failure, have not been investigated. One of the major features shared by these pathological conditions is an increased production of ROS, generated by activation of vascular NAD(P)H oxidase. Using an aspirin-triggered lipoxin A analogue resulted in modulation of ROS generation by endothelial cells and suppressed NAD(P)H oxidase-mediated ROS generation in the endothelium,[31] strongly indicating that lipoxins may have a protective role against the development and progression of cardiovascular diseases. These findings may help explain our observations of decreased NAD(P)H oxidase activity following chronic administration of aspirin.

The FFR provides a useful model of dietary induced metabolic syndrome.[7] Endothelial dysfunction and changes in vascular smooth muscle cell (VSMC) proliferation at different levels of the vascular system are present in this experimental model.[5] Furthermore, there is evidence of the involvement of the renin–angiotensin system (RAS) in the FFR model of metabolic syndrome, because pharmacological block of the RAS lowered blood pressure, reduced heart hypertrophy and reversed changes in VSMC proliferation and endothelial nitric oxide synthase activity.[32,33] Similar observations were made following the chronic administration of resveratrol, a polyphenol with anti-oxidant properties, to FFR and resveratrol further reversed increases in TBARS levels.[34] Effects of aspirin *in vivo* following its long-term administration on variables related to oxidative stress, which are altered in FFR, have also been reported previously.[3,32,33] Besides, the increment of plasma TBARS, indicating a greater lipid peroxidation in this model, has previously been confirmed.[34]

In the present study, aspirin was administered orally to rats at a dose of 10 mg/kg per day. Using an interspecies dose conversion factor based on equal body surface (7 : 1 for conversion from rat to

human),[35] the dose of aspirin used in the present study is equivalent to 1.43 mg/kg for humans or 100 mg for a 70 kg human, which is within the range of low-dose aspirin used in the prevention of cardiovascular diseases.[35] Previous reports[12,36,37] have demonstrated a similar effect of this dose of aspirin on oxidative status, which was not attributable to its anti-inflammatory effect. Inflammatory reactions can constitute a significant source of oxidative stress and damage by markedly increasing the number of activated leucocytes, which then increase levels of ROS. Aspirin is a potent anti-inflammatory and inhibitor of COX. However, a previous study reporting that ibuprofen, another anti-inflammatory and non-selective COX inhibitor, does not modify superoxide production suggests that the inhibition of COX enzymes *per se* does not account for the anti-oxidant effects of aspirin.[12] Similarly, an earlier paper[38] showed that, in normal rats, a similar dose of aspirin (10 mg/kg, p.o.) partially inhibits platelet TXA_2 formation (measured as serum TXB_2) and does not inhibit glomerular and medullary synthesis of prostaglandin (PG) I_2 and PGE_2. In the present study, chronic treatment with aspirin (10 mg/kg per day, p.o.) ameliorated insulin resistance and glucose intolerance in FFR. However, aspirin did not modify these variables in control rats. At the cardiovascular level, aspirin was able to partially prevent the increase in SBP and relative heart weight in FFR. Again, these effects were not observed in control animals.

The effects of aspirin on oxidative stress related to cardiovascular diseases have been studied extensively. Chronic oral treatment of spontaneously hypertensive rats (SHR) or AngII-infused hypertensive rats with aspirin reduced aortic and cardiac production of superoxide and aortic NAD(P)H oxidase activity.[12,36] Similar protective effects were observed in VSMC, with AngII-induced increases in superoxide production and protein synthesis totally prevented by the concurrent administration of aspirin.[12,36] In chronically glucose-fed rats treated with 100 mg/kg per day aspirin, the development of hypertension was inhibited, insulin resistance was reduced and increased basal aortic superoxide production was prevented.[37] In addition, the results of the present study show other beneficial effects of aspirin that are related to its anti-oxidant activity.

Aortic NAD(P)H oxidase activity, as another indicator of oxidative stress, was evaluated in the present study. Evidence supporting an important role for vascular NAD(P)H oxidase in generating ROS in different models of hypertension has been reviewed recently.[3,39] Previously, we reported that basal and AngII-induced NAD(P)H oxidase generation of ROS are enhanced in VSMC from SHR during the development of hypertension.[15] The results of the present study clearly show a significant increase in NAD(P)H oxidase activity in aortas from FFR, indicating participation of vascular ROS generation in the pathophysiological mechanisms associated with this model. Aspirin treatment partially reduced aortic NAD(P)H oxidase activity in FFR but had no effect in the control group. These results suggest an anti-oxidant effect of aspirin, which can be postulated as an important mechanism contributing to its efficacy in the primary and secondary prevention of cardiovascular events.

The results of the present study also demonstrate that structural changes in renal and common left carotid arteries occur in the insulin resistance model, as described previously.[16] The lumen : media ratio of interlobar, arcuate and interlobular arteries from the kidneys, as well as the lumen : media ratio of carotid arteries, was reduced in FFR compared with the control group, indicating arterial remodelling characterized by luminal narrowing and media thickening. It is well known that arteries are capable of structural and functional changes in response to changes in haemodynamic conditions. Arterial remodelling is mediated via the synthesis and release of locally produced growth and vasoactive factors and is an adaptative process occurring in response to chronic changes in arterial pressure or flow. In hypertension, the arterial system undergoes structural remodelling that is characterized by hypertrophy of the arterial wall, a decreased lumen : media ratio and associated decreased arterial distensibility.[22,40] In the present study, chronic administration of aspirin was able to markedly reduce vascular structural changes in these three types of renal arteries, as well as in the carotid artery, from FFR and improved the lumen : media ratio in arcuate and interlobular renal arteries from control rats.

Several studies have reported an antiproliferative effect of aspirin on VSMC from different origins,[41–43] as well as on endothelial cells.[44] This action of aspirin is likely to be involved in our finding of the prevention of vascular remodelling by aspirin.

In conclusion, the present study has demonstrated that chronic treatment with a low dose of aspirin in an experimental (fructose-fed) model of metabolic syndrome prevented insulin resistance, partially prevented increases in blood pressure and cardiac hypertrophy, reduced both vascular NAD(P)H oxidase activity and lipid peroxidation driven by ROS generation, that characterize this syndrome, and was effective in reversing vascular remodelling. These results suggest that aspirin could be of clinical use in the prevention and treatment of the metabolic syndrome. In addition, the results provide new insights into the cardiovascular benefits of aspirin in cardiovascular diseases and suggest that its administration may prevent or delay the occurrence of atherogenic cardiovascular diseases in the insulin-resistant state.

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