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Two angiotensin AT_1 receptor antagonists, irbesartan and losartan, effects in cholesterol-fed rabbits

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Abstract

This study was performed to examine the long-term effects of irbesartan and losartan, two angiotensin (AT_1) receptor antagonists, on lipoproteins and vascular responsiveness in vessels isolated from hypercholesterolemic rabbits. Four groups of rabbits ($n=40$) were used: Group 0 (control group), Group 1 [hypercholesterolemic group, 0.5% (wt./wt.) cholesterol-enriched diet], Group 2 (hypercholesterolemic + irbesartan 10 mg/kg/day) and Group 3 (hypercholesterolemic + losartan 10 mg/kg/day). After 17 weeks of treatment, total cholesterol and low-density lipoproteins levels in irbesartan- and losartan-treated groups were significantly lower than those of Group 1 ($\alpha=0.05$). Furthermore, levels of high-density lipoproteins were higher in the treated groups than in the hypercholesterolemic ($\alpha=0.05$) when we consider the same level of total cholesterol in the hypercholesterolemic and the treated groups. Despite the effect of the drugs on the abovementioned parameters, treatment with irbesartan or losartan did not improve endothelium-dependent and independent relaxation in aortic and mesenteric rings. Treatment with irbesartan and losartan decreased noradrenaline-induced contraction in aortic rings with respect to that in the hypercholesterolemic group ($\alpha=0.05$). In addition, irbesartan treatment improved the increase in serotonin-induced contraction in proximal coronary arteries with respect to that in the hypercholesterolemic group ($\alpha=0.05$). These results indicate that irbesartan and losartan restore noradrenaline-induced contraction in hypercholesterolemic rabbit-isolated arteries and improve lipoprotein profile in cholesterol-fed rabbits.

Keywords: Irbesartan; Losartan; Angiotensin II receptor antagonist; Cholesterol-fed rabbit; Aorta, coronary; Mesenteric (5th branch)

1. Introduction

Atherosclerosis is the result of a cascade of events in blood vessels, leading to remodelling of the arterial wall and a subsequent reduction in lumen size. Vascular endothelium regulates vascular function and structure through the release of numerous factors such as nitric oxide, endothelin-1, arachidonic acid derivatives, reactive oxygen species, monocytes adhesion molecules, growth factors and coagulation and fibrinolytic agents (Rubanyi, 1993; Jaffe, 1996). In the presence of hypercholesterolemia, dysfunctional endothelial cells lack their homeostatic role and mediate the functional and structural alterations associated with cardiovascular risk factors (Berliner et al., 1995). Endothelial dysfunction produced by hypercholesterolemia has been characterised by reduced endothelium-dependent relaxation, suggesting a reduced availability of nitric oxide (Jayakody et al., 1985; Chappel et al., 1987).

The rennin–angiotensin system has been shown clinically and experimentally to affect onset, progression and outcome of atherosclerosis. All components of the rennin–angiotensin system are expressed in the vessel wall. Angiotensin II is a potent vasoactive peptide and the major effector of the rennin–angiotensin system. The effects of angiotensin II are mediated by the G-protein-coupled receptors angiotensin AT_1 and AT_2 but most of the cellular effects of angiotensin II are mediated by the angiotensin AT_1 receptor and can be blocked by specific receptor antagonists. Angiotensin II in the arterial wall increases cellular adhesion molecules, which may act in concert with chemotactic molecules to induce adherence, invasion and proliferation of monocytes and T lymphocytes (Sasayama et al., 2000). In addition, proinflammatory cytokines induced by angiotensin II such as interleukin-6 may participate in smooth muscle cell proliferation and migration and destabilisation of the fibrous plaque (Han et al., 1999). Therefore, angiotensin II can be considered as a proatherogenic agent because it is able to stimulate most of the processes involved in the development of atherosclerosis.

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It has been reported that hypercholesterolemia, and specially oxidised low-density lipoproteins, augment angiotensin II production through the enhancement of angiotensin-converting enzyme activity (Gibbons, 1997). These facts justify the beneficial use of angiotensin-converting enzyme inhibitors in atherosclerotic patients and animals (Chobanian et al., 1990; Finta et al., 1993; Mancini et al., 1996; Hoshida et al., 1997; Hernandez et al., 1998; Sanz et al., 2000). Besides, in hypercholesterolemic rabbits, the density of angiotensin AT_1 receptors in the media of diseased blood vessels is increased compared to that in healthy animals (Yang et al., 1998). In this way, some studies have indicated that angiotensin II receptor block may have effects similar to those observed with angiotensin-converting enzyme inhibition (Hope et al., 1999; De las Heras et al., 1999).

The present study was undertaken to assess whether the drugs irbesartan and losartan, angiotensin II receptor antagonists, have antiatherosclerotic effects on cholesterol levels and vascular reactivity in arteries isolated from cholesterol-fed rabbits.

2. Materials and methods

2.1. Experimental design

Four groups ($n = 40$) of New Zealand White male rabbits (Biocentre, Barcelona, Spain), weighing 2.5 ± 0.5 kg (3 months old) at the beginning of the study, were used. The rabbits were housed identically in individual cages in an air-conditioned room under a 12-h light/dark cycle. Each group was fed according to the following scheme: Group 0: The control group, was maintained on a standard diet; Group 1: The high-cholesterol group, was maintained on a diet containing 0.5% cholesterol (UAR, Paris, France); Group 2: An experimental group, received a diet containing 0.5% cholesterol plus irbesartan (10 mg/kg/day); Group 3: Another experimental group, received a diet containing 0.5% cholesterol plus losartan (10 mg/kg/day). The experiment lasted 17 weeks. All the animals were initially fed a standard laboratory diet (Panlab, Barcelona, Spain) for at least 7 days after delivery to our laboratory. Tap water was available "ad libitum". Food intake was monitored daily for the first 7 days and each week thereafter.

Weight was determined before starting the treatment and then on days 15, 30, 60, 90 and 120. A blood sample was collected from each rabbit before starting the treatment and then also on days 15, 30, 60, 90 and 120 of treatment. Blood samples were collected from the ear vein, and serum concentrations of total cholesterol, high-density lipoprotein and low-density lipoprotein were determined with commercially available enzyme kits (BioMerieux, Marcy, France).

The animals were anaesthetised with ethyl ether and killed by exsanguination from the common carotid on the 17th week of the experiment. The thoracic aorta and proximal coronary arteries were rapidly removed and placed in Krebs–Henseleit solution of the following composition (mM): NaCl 119, KCl 4.7, $NaHCO_3$ 25, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5 and glucose 11.1. Adherent fat and surrounding tissue were cleaned off and then the aorta and proximal coronary arteries were cut into rings approximately 2–3 mm wide. The rings were then suspended between two stainless steel hooks in organ baths containing 10 ml of Krebs–Henseleit solution. The solution was kept at 36 ± 0.5 °C and gassed continuously with a 95% O_2–5% CO_2 gas mixture (pH to 7.35 ± 0.05). The aorta rings were mounted under 2 g tension and the coronary rings under 0.5 g. Each preparation was allowed to equilibrate for 90–120 min (aorta) or 30 min (proximal coronary). Contractile responses were measured isometrically by means of force-displacement transducers (Grass FT 03) and were recorded on a Grass polygraph as previously described (Tejerina et al., 1988). The isometric force was also digitalized by a MacLab A/D converter (Chart v3.2, AD Instruments, Castle Hill, Australia) and stored and displayed on a Macintosh computer (Ruiz and Tejerina, 1998).

Using a dissecting microscope, a segment of small mesenteric artery, approximately 2 mm in length, corresponding to a fifth-order branch of the superior mesenteric artery, was carefully dissected free from its vein and placed in physiological salt solution (PSS) of the following composition (mM): NaCl 139, KCl 5, $MgCl_2$ 0.98, $CaCl_2$ 0.15, glucose 9 and hepes 5. The artery was mounted in a small vessel myograph (Mulvany and Halpern, 1977). Two 40-μm tungsten wires were passed through the lumen of an isolated cylindrical segment (approximately 175 μm inside diameter), one wire was fastened with screws to a fixed tissue mount and the other was pulled out by parallel hooks, which were attached to a strain-gauge force transducer (U-gauge, Shinko), the position of which was adjusted with a micromanipulator.

The vessel was set to a tension equivalent to that generated at 0.9 times the diameter of the vessel at 100 mm Hg transmural pressure (Mulvany and Warshaw, 1977). Vessels were allowed to equilibrate at 36 ± 0.5 °C and gassed continuously with 95% O_2–5% CO_2 gas mixture to maintain pH at 7.4. Isometric force was also digitalized by a MacLab A/D converter (Chart v3.2, AD Instruments) and stored and displayed on a Macintosh computer (Ruiz and Tejerina, 1998).

After the equilibration period, aortic rings were contracted with noradrenaline 10^{-6} M and exposed to acetylcholine (10^{-8}–10^{-5} M) or to sodium nitroprusside (10^{-8}–10^{-4} M) when contraction had reached a consistent maximum, to test endothelium-dependent and -independent relaxation. Other aortic rings were contracted with KCl 80 mM.

Proximal coronary arteries were initially contracted with KCl 120 mM; then, 30 min after being washed out, they were used for a concentration–response curve with 5-hydroxytryptamine (10^{-9}–10^{-4} M).

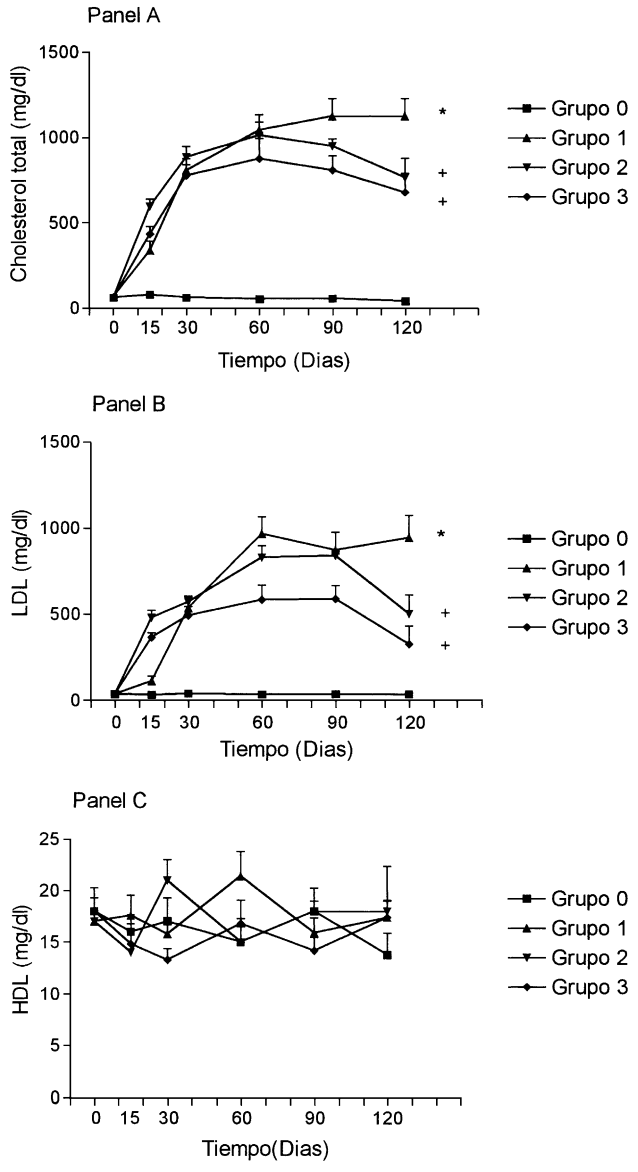

Fig. 1. Time course of total cholesterol (panel A), low-density lipoproteins (panel B) or high-density lipoproteins (panel C). Each data point shows the mean of 10 experiments and vertical lines indicate S.E.M. Group 0 (control group), Group 1 (hypercholesterolemic group, 0.5% cholesterol-enriched diet), Group 2 (hypercholesterolemic + irbesartan 10 mg/kg/day) and Group 3 (hypercholesterolemic + losartan 10 mg/kg/day). Significant differences at the 95.0% confidence level were found between group 0 and group 1 (*) and between either groups 2 or 3 and group 1 (+).

Mesenteric arteries were contracted with KCl 80 mM and 30 min after washing out, the rings were exposed to a single concentration of noradrenaline (10^{-5} M) and when the plateau was reached, a concentration–response curve to acetylcholine (10^{-8}–10^{-4} M) was made.

2.2. Drugs

The following drugs were used: acetylcholine chloride (Sigma), noradrenaline bitartrate (Sigma), and sodium nitroprusside (Sigma). Irbesartan and losartan were a gift from Bristol-Myers Squibb and Merck Sharp and Dohme Laboratories, respectively. Stock solutions were prepared by dissolving the compound in distilled water daily and keeping it on ice until used. The concentrations are reported as the final molar concentration in the organ chamber solution. Ascorbic acid was added to each daily prepared solution of noradrenaline to avoid noradrenaline oxidation. Working solutions were made in Krebs–Henseleit solution.

All values used in analyses represent means ± S.E.M. for 10 rabbits in each group of experiments. Comparisons among the different groups, at the level corresponding to the maximum concentration, were performed by means of a Bonferroni multiple range test and differences were considered significant at the 95% confidence level ($1-\alpha=0.95$).

The Universidad Complutense of Madrid (EEC official registration 28079-15ABC) approved all protocols concerning animals.

3. Results

3.1. General results

Acceptance of the diet supplemented with irbesartan and losartan was rapid and there were no significant differences among the groups regarding daily consumption of the diet. Moreover, no significant differences in body weights were observed during the experimental period; initial weights were 2500 ± 500 g and at the end of the treatments (17 weeks), the weights were 3556 ± 115 g (control group; Group 0), 3685 ± 93 g (hypercholesterolemic group, Group 1), 3710 ± 78 g (hypercholesterolemic + irbesartan, Group 2) and 3696 ± 66 g (hypercholesterolemic + losartan, Group 3).

Serum concentration of total cholesterol was 61.4 ± 5.7 mg/100 ml at the beginning of the study and increased gradually during the first 8 weeks in all the groups, except for the group fed the standard diet. There were significant differences in the increase of total serum cholesterol among the treated groups, Groups 2 and 3 and the hypercholesterolemic group (Group 1) ($\alpha=0.05$) (Fig. 1, panels A–C), and also marked changes in the distribution of cholesterol between high-density lipoprotein and low-density lipoprotein (Table 1).

Table 1
TC, LDL and HDL levels among different groups

Group	TC (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
0 (Control)	39.8 ± 6.2	33.3 ± 5.2	13.8 ± 2.1
1 (Hypercholesterolemic)	1121.0 ± 104.0	946.1 ± 129.0	17.4 ± 5.0
2 (Irbesartan treated)	762.0 ± 114.0[a]	503.0 ± 109.0[a]	18.0 ± 1.0
3 (Losartan treated)	674.3 ± 112.0[a]	329.0 ± 105.8[a]	17.4 ± 1.7

Values are means ± S.E.M. TC: total cholesterol; LDL: low-density lipoproteins; HDL: high-density lipoproteins.

[a] Has been placed indicating statistically significant differences among groups 2 and 3 vs. group 1 at the 95.0% confidence level.

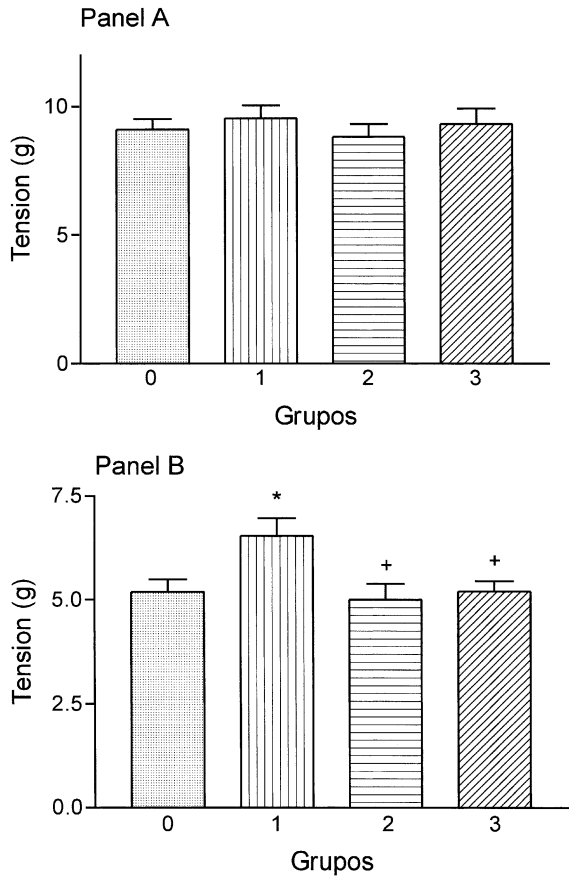

Fig. 2. Contractions induced by KCl 80 mM (panel A) or noradrenaline 10^{-6} M (panel B) in aortic rings. Each data point shows the mean of 10 experiments and vertical lines indicate S.E.M. Group 0 (control group), Group 1 (hypercholesterolemic group, 0.5% cholesterol-enriched diet), Group 2 (hypercholesterolemic + irbesartan 10 mg/kg/day) and Group 3 (hypercholesterolemic + losartan 10 mg/kg/day). Significant differences at the 95.0% confidence level were found between group 0 and group 1 (*) and between either groups 2 or 3 and group 1 (+).

3.2. Contractile responses to KCl or noradrenaline in aortic rings

In the first group of experiments, the contractions induced by KCl (80 mM) or noradrenaline (10^{-6} M) were measured. In arteries obtained from Group 0 (control group), KCl induced a contraction of 9.1 ± 0.4 g, and in the hypercholesterolemic group (Group 1), this contraction was 9.5 ± 0.5 g. In Groups 2 and 3 (irbesartan- and losartan-treated groups), the contraction induced by high K^+ was not modified significantly (8.8 ± 0.5 and 9.3 ± 0.6 g, respectively). On the contrary, in Groups 0 and 1, noradrenaline (10^{-6} M) induced a contraction of 5.2 ± 0.3 and 6.5 ± 0.4 g, respectively) ($\alpha = 0.05$) and in irbesartan- and losartan-treated rabbits, this contraction was significantly reduced to the control level (5.0 ± 0.4 and 5.21 ± 0.3 g, respectively) (Fig. 2, panels A and B).

3.3. Responses to acetylcholine in aortic rings

Acetylcholine (10^{-8}–10^{-5} M) caused an endothelium-dependent relaxation in a concentration–response manner in all the groups studied. Endothelium-dependent relaxation decreased strongly in the hypercholesterolemic group (Group 1) with respect to the control group (Group 0), the maximum relaxation being induced by acetylcholine (10^{-5} M) ($65.0 \pm 4.0\%$ and $24.0 \pm 9.4\%$) ($\alpha = 0.05$) Groups 0 and 1, respectively. Irbesartan and losartan were not able to restore endothelium-dependent relaxation to normal (maximal relaxation: $13.5 \pm 4.5\%$ and $23.0 \pm 5\%$, respectively) (Fig. 3).

3.4. Responses to sodium nitroprusside in aortic rings

Endothelium-independent relaxation was also tested. Sodium nitroprusside induced a relaxation in all groups studied. The concentration–response curve for sodium nitroprusside was significantly shifted to the right in the hypercholesterolemic group (Group 1) with respect to the control group ($\alpha = 0.05$) but in the treated groups, this effect was not prevented (Fig. 4).

3.5. Contractile responses to KCl and 5-hydroxytryptamine in proximal coronary arteries

The irbesartan-treated group (Group 2) showed a contraction induced by KCl 120 mM in proximal coronary arteries that was not significantly different from that in the hypercholesterolemic group (Group 1) (Fig. 5, panel A).

On the other hand, in the hypercholesterolemic group, the concentration–response curve for 5-hydroxytryptamine was shifted with respect to the control group (Group 0). Moreover, the treatment with irbesartan partially restored this

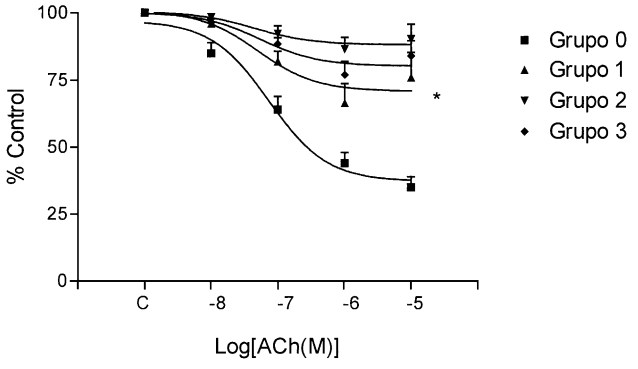

Fig. 3. Endothelium-dependent relaxation induced by acetylcholine (10^{-8}–10^{-5} M) in noradrenaline-precontracted aortas. Each data point shows the mean of 10 experiments and vertical lines indicate S.E.M. Group 0 (control group), Group 1 (hypercholesterolemic group, 0.5% cholesterol-enriched diet), Group 2 (hypercholesterolemic + irbesartan 10 mg/kg/day) and Group 3 (hypercholesterolemic + losartan 10 mg/kg/day). Significant differences at the 95.0% confidence level were found between group 0 and group 1 (*).

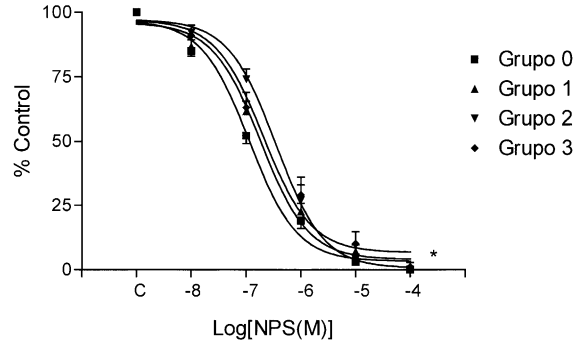

Fig. 4. Endothelium-independent relaxation induced by sodium nitroprusside (10^{-8}–10^{-4} M) in noradrenaline-precontracted aorta arteries. Each data point shows the mean of 10 experiments and vertical lines indicate S.E.M. Group 0 (control group), Group 1 (hypercholesterolemic group, 0.5% cholesterol-enriched diet), Group 2 (hypercholesterolemic + irbesartan 10 mg/kg/day) and Group 3 (hypercholesterolemic + losartan 10 mg/kg/day). Significant differences at the 95.0% confidence level were found between group 0 and group 1 (*).

effect to normal. Thus, the maximum contraction induced by 5-hydroxytryptamine 10^{-4} M was 14.0 ± 10.0% (percentage of KCl 120 mM-induced contraction); 71.0 ± 8.0% and 25.0 ± 9.0% in Groups 0, 1 and 2, respectively (Fig. 5, panel B). Significant differences were found between Groups 0 and 1 and between Groups 2 and 1 at $\alpha = 0.05$.

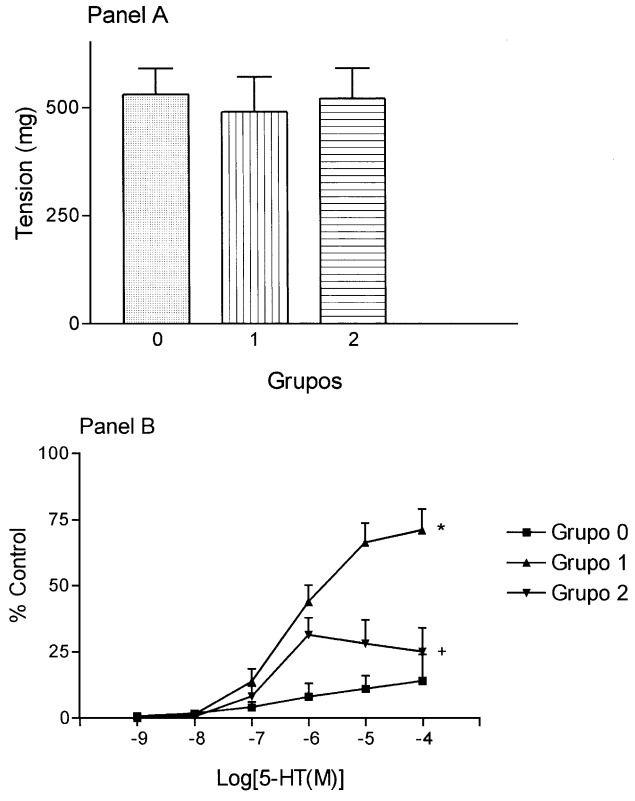

Fig. 5. Contractions induced by a single concentration of KCl (120 mM) (panel A) or by a cumulative concentration–response curve induced by 5-hydroxytryptamine (10^{-9}–10^{-4} M) (panel B) in proximal coronary arteries. Each data point shows the mean of 10 experiments and vertical lines indicate S.E.M. Group 0 (control group), Group 1 (hypercholesterolemic group, 0.5% cholesterol-enriched diet) and Group 2 (hypercholesterolemic + irbesartan 10 mg/kg/day). Significant differences at the 95.0% confidence level were found between group 0 and group 1 (*) and between either groups 2 or 3 and group 1 (+).

3.6. Contractile responses induced by KCl and noradrenaline in small mesenteric arteries

Fig. 6 (panels A and B) shows the values of contractions induced by high K^+ or by a submaximal concentration of noradrenaline (10^{-5} M) in the different groups studied. The contractions obtained were similar in all the groups when the contraction was induced by high K^+ (753 ± 250, 700 ± 285, 812 ± 176 and 669 ± 115 g, Groups 0, 1, 2 and 3, respectively). In addition, when the contractions were induced by noradrenaline (10^{-5} M), they were similar in all the groups studied (870 ± 180, 913 ± 172, 843 ± 193 and 894 ± 173 g, Groups 0, 1, 2 and 3, respectively).

3.7. Responses to acetylcholine in small mesenteric arteries

Cumulative addition of acetylcholine induced a concentration-dependent and sustained relaxation during contrac-

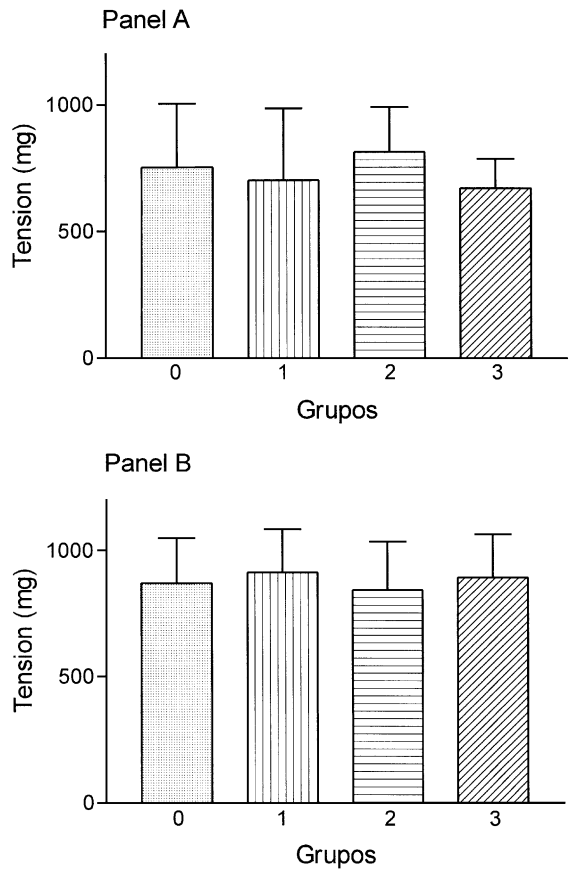

Fig. 6. Contractions induced by KCl 80 mM (panel A) or noradrenaline 10^{-5} M (panel B) in small mesenteric arteries. Each data point shows the mean of 10 experiments and vertical lines indicate S.E.M. Group 0 (control group), Group 1 (hypercholesterolemic group, 0.5% cholesterol-enriched diet), Group 2 (hypercholesterolemic + irbesartan 10 mg/kg/day) and Group 3 (hypercholesterolemic + losartan 10 mg/kg/day).

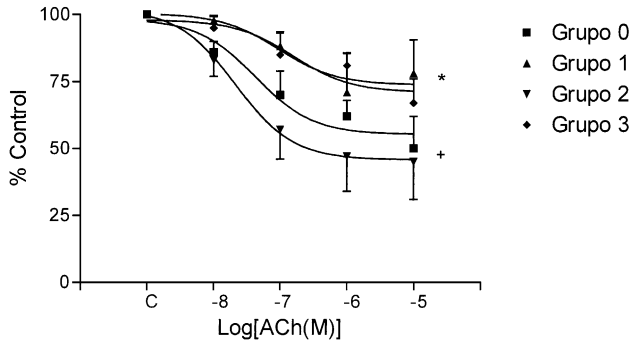

Fig. 7. Endothelium-dependent relaxation induced by acetylcholine (10^{-8}–10^{-5} M) in noradrenaline-precontracted small mesenteric arteries. Each data point shows the mean of 10 experiments and vertical lines indicate S.E.M. Group 0 (control group), Group 1 (hypercholesterolemic group, 0.5% cholesterol enriched diet), Group 2 (hypercholesterolemic + irbesartan 10 mg/kg/day) and Group 3 (hypercholesterolemic + losartan 10 mg/kg/day). Significant differences at the 95.0% confidence level were found between group 0 and group 1 (*) and between either groups 2 or 3 and group 1 (+).

tion by noradrenaline (10^{-5} M). The concentration–relaxation curve for acetylcholine was shifted significantly to the right in the small mesenteric arteries when the hypercholesterolemic group was compared to the control group (Fig. 7) ($\alpha = 0.05$).

The treatment with irbesartan completely restored endothelium-dependent relaxation in small mesenteric arteries with respect to the hypercholesterolemic group ($\alpha = 0.05$).

4. Discussion

In the present study, feeding rabbits 0.5% cholesterol for 17 weeks resulted in marked hyperlipidemia. In this experiment, we have shown that irbesartan and losartan could lower the levels of serum total cholesterol and low-density lipoproteins, and increase high-density lipoprotein levels. Results of preliminary studies by Li et al. (1999) also support our results.

During the atherosclerotic process, alterations in vascular reactivity have been observed, including impaired endothelial-dependent vasodilatation and increased responsiveness to contractile agonists (Howes et al., 1997).

One could speculate that either the elevation of high-density lipoprotein cholesterol or an associated decrease in low-density lipoprotein cholesterol could act at the tissue level to preserve the integrity of endothelial function. An increase in intracellular calcium mediated by low-density lipoproteins has been reported by Orlov et al. (1993). When we inhibit the effects of angiotensin II-mediated calcium entrance by means of losartan and irbesartan, the number of low-density lipoprotein receptors could be increased to take up calcium, thus decreasing plasma low-density lipoprotein levels.

In our study with Trandolapril (non-sulphydryl angiotensin-converting enzyme inhibitor, Sanz et al., 2000), we did not find any significant effects on plasma cholesterol, high-density lipoprotein or low-density lipoprotein between the hypercholesterolemic group and the group treated with the drug. These data suggest, together with the present data, that the effects on low-density lipoproteins must have a close relationship with the angiotensin AT_1 receptor than with the action of angiotensin itself.

Our data confirm previously reported studies that show endothelial dysfunction results from hypercholesterolemia (Jayakody et al., 1985; Verbeuren et al., 1986; Chappel et al., 1987). In the present study, relaxation in response to acetylcholine, an endothelium-dependent vasodilator, was clearly blunted in arteries from animals with experimental hypercholesterolemia. Comparable results have previously been reported for experimental animals and humans, indicating that during hypercholesterolemia and during the early stages of atherosclerosis, the most common vascular functional alteration is a reduction of endothelium-dependent relaxation (Jayakody et al., 1987; Zeiher et al., 1991; Chowienezyk et al., 1992). This effect could depend on a diminished availability of nitric oxide as the result of an enhanced production of oxidised-low density lipoproteins, which has been demonstrated to reduce the expression of nitric oxide synthase by endothelial cells (Hernandez-Perera et al., 1998). Another alteration that could contribute to the reduced relaxing response to acetylcholine in hypercholesterolemic rabbits is intimal thickening, which constitutes a physical barrier, preventing nitric oxide from reaching smooth muscle cells (Ross, 1999). However, irbesartan and losartan, angiotensin AT_1 receptor antagonists, were not able to normalise this endothelial dysfunction in aortic rings. In contrast, De las Heras et al. (1999) found that the diminished response to acetylcholine observed in hypercholesterolemic rabbits was enhanced by valsartan treatment, another angiotensin AT_1 receptor antagonist.

Some studies have shown that hypercholesterolemia impairs endothelium-dependent relaxation in small arteries, vessels which do not develop atherosclerotic lesions (Yamamoto et al., 1988; Sellke et al., 1990; Zeiher et al., 1991). However, this does not agree with a study done by Simonsen et al., (1991) which found that endothelium-dependent acetylcholine-induced relaxation of cerebral, femoral and mesenteric-isolated small arteries in vitro was not affected by feeding rabbits a cholesterol-rich diet. In our study, the hypercholesterolemic diet impaired endothelium-dependent relaxation in mesenteric rings. Since, in resistance vessels, endothelium-dependent relaxation has been attributed to a diffusible endothelium-derived hyperpolarizing factor (Harasawa et al., 1997), this pathway should be altered in hypercholesterolemia. We found that treatment with irbesartan normalised endothelium-dependent relaxation in small arteries.

In our model of hypercholesterolemia, we also found that endothelium-independent relaxation induced by sodium nitroprusside was significantly diminished in aortic rings

in contrast to that found by Finta et al. (1993). Sodium nitroprusside releases a nitric oxide-like compound that acts via cGMP to cause vascular smooth muscle relaxation, and one can conclude from our data that this part of the cascade is altered in rabbits fed an atherogenic diet. However, neither losartan nor irbesartan treatment serves to normalise this dysfunction.

In this study, we found an enhancement of vasoconstrictor responses to noradrenaline in aortic rings from hypercholesterolemic animals. Increased endothelin production may play a role, but a significant component of the increased vasoconstrictor responsiveness in animals appears to be independent of the endothelium. The mechanism underlying the enhancement of vasoconstrictor responses may be due to oxidised low-density lipoprotein stimulation of calcium uptake in vascular muscle cells (Orlov et al., 1993; Thorin et al., 1994). Short-term changes in low-density lipoprotein levels, and probably in the extent of oxidation of low-density lipoproteins, could modify altered vasoconstrictor responses. Thus, irbesartan and losartan treatment restored vasoconstrictor response to normal.

Proximal coronary arteries of hypercholesterolemic rabbits examined in the present study exhibited an increased response to 5-hydroxytryptamine. This agrees with earlier studies of hypercholesterolemic rabbits (Vrints et al., 1990; Simonsen et al., 1992) and non-human primates (Chilian et al., 1990). The abnormal response to by 5-hydroxytryptamine of the proximal coronary arteries is associated with an increase in the number of by 5-hydroxytryptamine receptors due to the presence of atherosclerotic lesions (Nanda and Henry, 1982). Irbesartan treatment restored 5-hydroxytryptamine-induced contraction to normal, probably as a result of diminished plaque development.

In summary, the present investigation showed that the major changes after irbesartan and losartan treatment in a hypercholesterolemic model take place in the cholesterol levels, reducing total cholesterol and low-density lipoproteins and increasing high-density lipoproteins, and in the noradrenaline and by 5-hydroxytryptamine-induced contractions in aorta and proximal coronary arteries respectively, which were restored to normal.

Acknowledgements

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References

Berliner, J.A., Navab, M., Fogelman, A.M., Frank, J.S., Demer, L.L., Edwards, P.A., Watson, A.D., Lusis, A.J., 1995. Atherosclerosis: basic mechanisms: oxidation, inflammation and genetics. Circulation 91, 2488–2496.

Chappel, S., Lewis, M., Henderson, A., 1987. Effect of lipid feeding on endothelium dependent relaxation in rabbit aortic preparations. Cardiovasc. Res. 21, 34–37.

Chilian, W.M., Dellsprenger, K.C., Layne, S.M., Eastman, C.L., Amstrong, M.A., Marcus, M.L., Heistad, D.D., 1990. Effects of atherosclerosis on the coronary microcirculation. Am. J. Physiol. 258, H529–H539.

Chobanian, A.V., Haudenschild, C.C., Nickerson, C., Drago, R., 1990. Antiatherogenic effect of captopril in the Watanabe heritable hyperlipidemic rabbit. Hypertension 15, 327–331.

Chowienezyk, P.J., Wats, G.F., Cockcroft, J.R., Ritter, J.M., 1992. Impaired endothelium-dependent vasodilation of forearm resistance vessels in hypercholesterolemia. Lancet 340, 1430–1432.

De las Heras, N., Aragoncillo, P., Maeso, R., Vazquez-Pérez, S., Navarro-Cid, J., DeGasparo, M., Mann, J., Ruilope, L.M., Cachofeiro, V., Lahera, V., 1999. AT_1 receptor antagonism reduces endothelial dysfunction in atherosclerotic rabbits. Hypertension 34, 969–975.

Finta, K.M., Fischer, M.J., Lee, L., Gordon, D., Pitt, B., Webb, R.C., 1993. Ramipril prevents impaired endothelium-dependent relaxation in arteries from rabbits fed an atherogenic diet. Atherosclerosis 100, 149–156.

Gibbons, G.H., 1997. Endothelial function as a determinant of vascular function and structure: a new therapeutic target. Am. J. Cardiol. 79, 3–8.

Han, Y., Runge, M.S., Brasier, A.R., 1999. Angiotensin II induces interleukin-6 transcription in vascular smooth muscle cells through pleiotropic activation of nuclear factor-kappaB transcription factors. Circ. Res. 84, 695–703.

Harasawa, U.L., Shimokawa, H., Nakashima, M., Egashira, K., Takeshita, A., 1997. Importance of endothelium-derived hyperpolarizing factor in human arteries. J. Clin. Invest. 100, 2793–2799.

Hernandez, A., Barberi, L., Ballerio, R., Testini, A., Ferioli, R., Bolla, M., Natali, M., Folco, G., Catapano, A.L., 1998. Delapril slows the progression of atherosclerosis and maintains endothelial function in cholesterol-fed rabbits. Atherosclerosis 137, 71–76.

Hernandez-Perera, O., Perez-Sala, D., Navarro-Antolín, J., Sanchez-Pascuala, R., Hernandez, G., Diaz, C., Lamas, S., 1998. Effects of the 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors, atorvastatin and simvastatin, on the expression of endothelin-1 and endothelial nitric oxide synthase in vascular endothelial cells. J. Clin. Invest. 101, 2711–2719.

Hope, H., Brecher, P., Chobanian, A.V., 1999. Comparison of the effects of AT_1 receptor blockade and angiotensin converting enzyme inhibition on atherosclerosis. Am. J. Hypertens. 12, 28–34.

Hoshida, S., Nishida, M., Yamashita, N., Igarashi, J., Aoki, K., Hori, M., Kuyuza, T., Tada, M., 1997. Vascular angiotensin-converting enzyme activity in cholesterol-fed rabbits: effects of enalapril. Atherosclerosis 130, 53–59.

Howes, L.G., Abbot, D., Straznicky, N.E., 1997. Lipoproteins and cardiovascular reactivity. Br. J. Clin. Pharmacol. 44, 319–324.

Jaffe, E.A., 1996. Physiologic functions of normal endothelial cells. In: Loscalzo, J., Crager, M.A., Dzau, V. (Eds.), Vascular Medicine. A Textbook of Vascular Biology and Diseases. Little, Brown & Co., Boston, MA, pp. 3–46.

Jayakody, R.L., Senaratne, M.P.J., Thomson, A.B.R., Kappagoda, C.T., 1985. Cholesterol feeding impairs endothelium-dependent relaxation in rabbit aorta. Can. J. Physiol. Pharmacol. 63, 1206–1209.

Jayakody, L., Senaratne, M., Thompson, A., Kappagoda, T., 1987. Endothelium-dependent relaxation in experimental atherosclerosis in the rabbit. Circ. Res. 60, 251–264.

Li, J., Hirose, N., Kawamura, M., Arai, Y., 1999. Antiatherogenic effect of angiotensin converting enzyme inhibitor (benazepril) and angiotensin II receptor antagonist (valsartan) in the cholesterol-fed rabbits. Atherosclerosis 143, 315–326.

Mancini, G.B., Henry, G.C., Macaya, C., O'Neill, B.J., Pucillo, A.L., Carere, R.G., Wargovich, T.J., Mudra, H., Lüscher, T.F., Klibaner, M.I., Haber, H.E., Uprichard, A.C.G., Pepine, C.J., Pitt, B., 1996. Angiotensin-converting enzyme inhibition with quinapril improves endothelial vasomotor dysfunction in patients with coronary artery disease: the TREND (Trial on Reversing Endothelial Dysfunction) Study. Circulation 94, 258–265.

Mulvany, M.J., Halpern, W., 1977. Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. Circ. Res. 41, 19–26.

Mulvany, M., Warshaw, D., 1977. The active tension-length of vascular smooth muscle related to its cellular components. J. Gen. Physiol. 74, 85–104.

Nanda, V., Henry, P.D., 1982. Increased serotonergic and alpha-adrenergic receptors in aortas from rabbits fed a high cholesterol diet. Clin. Res. 30, 209A.

Orlov, S., Resink, T., Bernhardt, J., Ferracin, F., Buhler, F., 1993. Vascular smooth muscle cell calcium fluxes. Regulation by angiotensin II and lipoproteins. Hypertension 21, 195–203.

Ross, R., 1999. Atherosclerosis. An inflammatory disease. N. Engl. J. Med. 340, 115–126.

Rubanyi, G.M., 1993. The role of endothelium on cardiovascular homeostasis and diseases. J. Cardiovasc. Pharmacol. 2 (Suppl. 4), S1–S14.

Ruiz, E., Tejerina, T., 1998. Relaxant effects of L-citrulline in rabbit vascular smooth muscle. Br. J. Pharmacol. 125, 186–192.

Sanz, M., Ganado, P., Ruiz, E., Tejerina, T., 2000. Effect of trandolapril on vascular responsiveness in cholesterol-fed rabbit-isolated arteries. Eur. J. Pharmacol. 397, 359–365.

Sasayama, S., Okada, M., Matsumori, A., 2000. Chemokines and cardiovascular diseases. Cardiovasc. Res. 45, 267–269.

Sellke, F.W., Armstrong, M.L., Harrison, D.G., 1990. Endothelium-dependent vascular relaxation is abnormal in the coronary microcirculation of atherosclerotic primates. Circulation 81, 1586–1593.

Simonsen, U., Ehrnrooth, E., Gerdes, L.U., Faergemann, O., Buch, J., Andreasen, F., Mulvany, M.J., 1991. Functional properties in vitro of systemic small arteries from rabbits fed a cholesterol-rich diet for 12 weeks. Clin. Sci. (London) 80 (2), 119–129.

Simonsen, U., Prieto, D., Mulvany, M.J., Ehrnrooth, E., Korsgaard, N., Nyborg, N.C.B., 1992. Effect of induced hypercholesterolemia in rabbits on functional responses of isolated large proximal and small distal coronary arteries. Atheroscler. Thromb. 12, 380–392.

Tejerina, T., Sesin, J., Delgado, C., Tamargo, J., 1988. Effect of milrinone contractility and $^{45}Ca^{2+}$ movements in the isolated rabbit aorta. Eur. J. Pharmacol. 148, 239–245.

Thorin, E., Hamilton, C., Pruncizak, A., Reid, J., 1994. Change in endothelial cell membrane properties after chronic exposure to oxidised low density lipoproteins. J. Hypertens. 12 (Suppl. 3), S180–S183.

Verbeuren, T.J., Jordens, F.H., Zonnekeyn, L.L., VanHove, C.E., Coene, M.C., Herman, A.G., 1986. Effect of hypercholesterolemia on vascular reactivity in the rabbit. Circ. Res. 58, 552–564.

Vrints, C., Verbeuren, T., Snoeck, J., Herman, A.G., 1990. Effects of hypercholesterolemic on coronary vascular reactivity: impaired endothelium-dependent vasodilatation leads to unmasking of 5-HT_2-serotonergic vasoconstriction in hypercholesterolemic rabbits. In: Rubanyi, G.M., Vanhoutte, P. (Eds.), Endothelium-Derived Contracting Factors. S Karger, AG, Basel, Switzerland, pp. 162–169.

Yamamoto, H., Bossaller, C., Cartwright, J., Henry, P.D., 1988. Videomicroscopic demonstration of defective cholinergic arteriolar vasodilation in atherosclerotic rabbit. J. Clin. Invest. 81, 1752–1758.

Yang, B.C., Phillips, M.I., Mohuczy, D., Meng, H., Shen, L., Mehta, P., 1988. Increased angiotensin II type 1 receptor expression in hypercholesterolemic atherosclerosis in rabbits. Arterioscler., Thromb., Vasc. Biol. 18, 1433–1439.

Zeiher, A.M., Drexler, H., Wollschläger, H., Just, H., 1991. Modulation of coronary vasomotor tone in humans: progressive endothelial dysfunction with different early stages of coronary atherosclerosis. Circulation 83, 391–401.