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Prevention of arterial structural alterations with verapamil and trandolapril and consequences for mechanical properties in spontaneously hypertensive rats

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Abstract

We compared the chronic effects in spontaneously hypertensive rats (SHR) of low doses of an angiotensin converting enzyme inhibitor, trandolapril, a Ca^{2+} channel antagonist, verapamil, and their combination (trandolapril–verapamil), on arterial mechanical properties, arterial wall hypertrophy and extracellular matrix proteins. Four-week-old SHR were randomly allocated to oral treatment with verapamil (50 mg kg^{-1} day^{-1}), trandolapril (0.3 mg kg^{-1} day^{-1}), the combination of verapamil (50 mg kg^{-1} day^{-1}) plus trandolapril (0.3 mg kg^{-1} day^{-1}), or placebo for 4 months. A group of Wistar Kyoto (WKY) control rats received placebo for the same period of time. At the end of the treatment, mean blood pressure was lower in verapamil–trandolapril than in trandolapril SHR, but remained higher than in WKY. Verapamil had no effects on blood pressure. Equivalent reduction in aortic wall hypertrophy was obtained in all treated SHR. Trandolapril and verapamil–trandolapril combination produced a significant reduction of aortic collagen density compared with placebo SHR. Carotid total fibronectin, EIIIA fibronectin isoform and $\alpha 5\beta 1$ integrin, were higher in the media of placebo SHR than in WKY. EIIIA fibronectin isoform and $\alpha 5\beta 1$ integrin were reduced in verapamil-SHR compared with placebo-SHR and normalized in trandolapril and verapamil–trandolapril-SHR compared with WKY. SHR-placebo and SHR treated with either verapamil or trandolapril as single-drug treatment showed a 4-fold increase in total fibronectin compared to the WKY. Only SHR treated with verapamil–trandolapril combination had total fibronectin not significantly different from that of WKY. Carotid arterial distensibility increased only in verapamil–trandolapril treated rats. Multivariate analysis showed arterial distensibility to be negatively correlated to mean blood pressure ($P < 0.0001$) and total fibronectin ($P < 0.01$). In conclusion, chronic treatment with the verapamil–trandolapril combination significantly improved in vivo arterial distensibility in SHR. The most important effects of the combination on arterial mechanics compared to those of verapamil or trandolapril alone may have been the consequence of its stronger action on arterial pressure, arterial wall hypertrophy and total fibronectin density. However we suggest that, in addition to the structural effects, complete normalization of blood pressure is necessary to obtain normal arterial distensibility.

Keywords: Arterial distensibility; Collagen; Fibronectin; Hypertension; Ca^{2+} channel antagonist

1. Introduction

Chronic hypertension is associated with large artery alterations including wall hypertrophy, an increase in extracellular matrix and, reduction in compliance and distensibility. Although these alterations are partly related to elevated blood pressure, other factors have been found to stimulate hypertrophy, collagen accumulation and arterial stiffness in hypertension. Experimental results have implicated humoral factors in the development of these arterial alterations. Angiotensin II increases collagen synthesis by acting directly on vascular smooth muscle cells (Kato et al., 1991) and promotes cardiovascular growth (Wolf et al., 1992; Schunkert et al., 1995). A pressure-independent role of angiotensin AT_1 activity in arterial stiffness has also been shown in clinical genetic studies; a significant association between aortic stiffness and the angiotensin AT_1 receptor A/C polymorphism was observed despite the absence of any difference in blood pressure levels accord-

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ing to the genotypes (Benetos et al., 1996). Chronic treatment with Ca^{2+} channel antagonists and angiotensin converting enzyme inhibitors can prevent some of these alterations, presumably independently of the antihypertensive action (Linz et al., 1989; Albaladejo et al., 1994; Lacolley et al., 1995a; Benetos et al., 1997). Clinical pharmacological studies have shown that, for an equipotent blood pressure reduction, the increase in arterial distensibility is more pronounced with the angiotensin converting enzyme inhibitors and the Ca^{2+} channel antagonists than with other antihypertensive drugs such as diuretics and some β-adrenoceptor blockers (Safar et al., 1983; Simon et al., 1984; Van Merode et al., 1990). Since aortic stiffness is strongly related to the organization of the extracellular matrix, the question arises as to whether treatment with angiotensin converting enzyme inhibitor, Ca^{2+} channel antagonists and their combination is able to prevent arterial stiffness by acting on the extracellular matrix composition and organization.

Indeed, the elastic properties of the arterial wall material depend not only on the smooth muscle cells, elastin and collagen contents, but also on the way these components are spatially organized within the media (Cox, 1989). In disease situations, as in vascular wall remodeling in genetic hypertension, cell–matrix interactions may play an important role in adaptation of the mechanical properties of vascular smooth muscle cells to the higher level of wall stress. Fibronectin, by interacting with specific cellular integrin receptors, plays an important role in cell–matrix interactions (Hynes, 1992). The interaction of specific extracellular matrix proteins with their integrin receptors has been shown to play a central role in the transmission, of mechanical forces to vascular smooth muscle cells (Ingber, 1991). Fibronectin expression in the rat large arteries increases with age and hypertension (Takasaki et al., 1990, 1992). Angiotensin converting enzyme inhibitors such as trandolapril have been shown to reduce aortic fibronectin expression, and this effect is not dependent solely on blood pressure. We hypothesized that drug-induced changes in fibronectin, collagen and elastin may play a role in arterial mechanical properties (Himeno et al., 1994).

The aim of the present study was to evaluate the chronic effects of the angiotensin converting enzyme inhibitor, trandolapril, the Ca^{2+} channel antagonist, verapamil, and the combination of verapamil–trandolapril on large artery structural parameters and mechanical properties in SHR (spontaneously hypertensive rat). We focused our study on the extracellular matrix proteins and adhesion molecules of the media since their dynamic role is seen only in the presence of vascular smooth muscle cells. We used low doses of trandolapril and verapamil to determine whether the changes in extracellular matrix proteins were dependent solely on reduction in blood pressure or on a direct mechanism in the vessel wall. It was also attempted to relate the structural changes to the elastic properties assessed in vivo by using a high-resolution echo-tracking system.

2. Materials and methods

2.1. Animals

Male, 4-week-old SHR and WKY (Wistar Kyoto) rats were obtained from Iffa Credo France, and housed in our animal room (temperature, 20–22°C; humidity, 55–65%; 12-h light/12-h dark cycle). They were fed a standard diet (0.130 mEq/g Na^+ and 0.205 mEq/g K^+), and given free access to tap water.

2.2. Treatments

The rats were randomly allocated to five groups and were treated from 4 weeks of age to 20 weeks of age. Two groups (SHR = 8; WKY = 8) received a standard diet and served as placebo groups. The others groups received either verapamil 50 mg kg^{-1} day^{-1} (SHR = 15), trandolapril 0.3 mg kg^{-1} day^{-1} (SHR = 15), or a combination of the two drugs (verapamil 50 mg kg^{-1} day^{-1} + trandolapril 0.3 mg kg^{-1} day^{-1}) (SHR = 15) All additions were incorporated in the food (prepared by Iffa Credo). The daily doses of the drugs were calculated on the basis of a mean consumption of 20 g food day^{-1} rat^{-1}.

In a preliminary study, we treated SHR under the same conditions for a period of 4 weeks from the fourth to the eighth week of age. At the end of this time blood pressure was measured intra-arterially in anesthetized rats. We found that verapamil at the dose of 50 mg kg^{-1} day^{-1}, trandolapril at the dose of 0.3 mg kg^{-1} day^{-1}, and their combination exerted a mild and non-significant hypotensive effect. The results of this preliminary study (see Table 1a) showed that the development of hypertension in SHR was not blocked by these doses of the two drugs or their combination.

2.3. Arterial pressure and heart rate in conscious rats

Following the preliminary experiments the doses chosen were used for the main experiments for a period of 16 weeks. At the end of the treatment period, the animals were anesthetized with pentobarbital (60 mg kg^{-1} i.p.) and a catheter (PE-50 fused to PE-10; Guerbet-Biomedical, France) was placed in the lower abdominal aorta via the femoral artery. The catheter was filled with heparinized saline (50 U/ml) and was tunneled under the skin of the back and exited between the scapula. At 20 weeks of age (24 h after anesthesia) the arterial pressure was measured in conscious freely moving rats after at least 30 min of rest. The blood pressure was recorded continuously over 30 min with a Statham P23 ID pressure Transducer (Gould Instruments, Cleveland, OH) connected to a Gould Brush recorder G4133.

2.4. Carotid artery mechanical properties evaluation

The common carotid diameter–pressure relationship was established from the simultaneous recording of arterial diameter and blood pressure, in pentobarbital-anesthetized rats. The technique of arterial diameter measurement, using an ultrasonic echo-tracking device (NIUS-01, Asulab, Neuchâtel, Switzerland) in rats has been earlier described (Hayoz et al., 1992; Lacolley et al., 1995a,b). The relationship between the pressure (P) and the lumen cross-sectional area (LCSA) was fitted to the model of Langewouters using an arc-tangent function and three optimal fit parameters (α, β and γ) (Tardy et al., 1991).

Local arterial cross-sectional compliance (C), in the case of a cylindrical vessel, is defined by the change in lumen cross-sectional area for a given change in intravascular pressure. Local arterial cross-sectional distensibility (D) is defined by the relative change in lumen cross-sectional area for a given change in intravascular pressure:

$$\mathrm{LCSA} = \alpha\{\pi/2 + \tan^{-1}((P-\beta)/\gamma)\} \quad (1)$$

$$C(P) = \frac{\partial \mathrm{LCSA}}{\partial P} = \frac{\alpha}{\gamma} \times \frac{1}{1 + ((P-\beta)/\gamma)^2} \quad (2)$$

$$D(P) = \frac{1}{\mathrm{LCSA}} \times \frac{\partial LCSA}{\partial P} \quad (3)$$

Determination of the circumferential wall stress (σ) and the incremental elastic modulus (E_{inc}) required the value of arterial medial cross-sectional area (MCSA) and were calculated from the following equations (Fung, 1993; Matsumoto and Hayashi, 1994; Bezie et al., 1998):

$$\sigma = \frac{2\mathrm{LCSA} \times P}{\mathrm{MCSA}} \quad (4)$$

$$E_{\mathrm{inc}} = \frac{3}{D}\left(1 + \frac{\mathrm{LCSA}}{\mathrm{MCSA}}\right) \quad (5)$$

The animals were killed at the end of arterial parameter recording. The thoracic aorta was used for histomorphometry after formaldehyde fixation in vitro, and the carotid artery was used for immunohistologic staining in freeze dried tissues. The quality of immunohistologic staining was markedly better of freeze-dried sections than in fixed arteries (Stein et al., 1985). Since tissue samples from the same animal were fixed for MCSA measurement or freeze-dried for immunochemistry, it was not possible to fix the arterial tissue at mean arterial pressure.

2.5. Morphological study of the thoracic aorta

The rat aorta was fixed in saline with 4% formaldehyde solution. The different structures of the aortic media were studied in a segment of thoracic aorta longitudinally imbedded in paraffin. Three successive sagittal sections of 5 μm thickness were stained specifically staining to obtain a monochromatic color associated with the various structures studied in the aortic media. Sirius red was used for collagen staining, orcein for elastin, and hematoxylin after

Table 1

(a)Results of the preliminary experiments showing the mean values for intracarotid blood pressure evaluated in anesthetized rats after 1 month of treatment

	Placebo ($n=5$)	Ver ($n=5$)	Trand ($n=5$)	Ver + trand ($n=5$)	WKY ($n=5$)
SBP (mm Hg)	194 ± 6	188 ± 7	186 ± 8	181 ± 9	138 ± 5*
DBP (mm Hg)	135 ± 4	125 ± 6	127 ± 7	121 ± 6	96 ± 4*
MBP (mm Hg)	154 ± 4	146 ± 6	147 ± 7	141 ± 7	110 ± 5*
PP (mm Hg)	59 ± 4	63 ± 2	58 ± 2	59 ± 4	42 ± 2*
HR (bpm)	372 ± 7	363 ± 24	396 ± 14	384 ± 12	336 ± 10

(b) Body weight, blood pressure and heart rate in SHR and WKY rats after 16 weeks of treatment

	Placebo ($n=8$)	Ver ($n=13$)	Trand ($n=12$)	Ver + trand ($n=12$)	WKY ($n=8$)
Body weight (g)	398 ± 14	353 ± 7[a,c]	347 ± 7[a,c]	331 ± 4[a,c]	408 ± 5
Conscious rats					
MBP (mm Hg)	169 ± 6	164 ± 2	142 ± 3[a,b]	134 ± 3[a,b]	119 ± 2[d]
HR (bpm)	335 ± 5	331 ± 5	336 ± 10	341 ± 18	289 ± 5[d]
Anesthetized rats					
SBP (mm Hg)	200 ± 14	193 ± 6	177 ± 5[a,b]	165 ± 6[a,b]	121 ± 2[d]
DBP (mm Hg)	149 ± 8	153 ± 4	136 ± 4[a,b]	132 ± 3[a,b]	89 ± 5[d]
MBP (mm Hg)	173 ± 10	172 ± 5	155 ± 4[a,b]	147 ± 4[a,b]	104 ± 5[d]
PP (mm Hg)	51 ± 9	39 ± 3	41 ± 3	33 ± 4[a]	32 ± 4[a]
HR (bpm)	354 ± 14	346 ± 9	341 ± 11	349 ± 15	288 ± 11[d]

Values are means ± S.E.M. Ver = verapamil at 50 mg kg^{-1} day^{-1}; Trand = trandolapril at 0.3 mg kg^{-1} day^{-1}.
SBP = systolic blood pressure; DBP = diastolic blood pressure; MBP = mean blood pressure; PP = pulse pressure (SBP − DBP), HR = heart rate.
* $P < 0.001$ vs. others.
[a] $P < 0.001$ vs. SHR Placebo, [b] $P < 0.001$ vs. Ver, [c] $P < 0.005$ vs. WKY, [d] $P < 0.001$ vs. others.

Table 2
Histomorphometric parameters of the thoracic aorta in SHR after 16 weeks of treatment

	Placebo ($n = 8$)	Ver ($n = 5$)	Trand ($n = 12$)	Ver + Trand ($n = 14$)	WKY ($n = 8$)
Media thickness (μm)	120.4 ± 3.3	103.0 ± 1.6[a]	95.4 ± 1.32[a,b]	89.9 ± 1.4[a,b,c]	98.5 ± 2.5[a]
MCSA (μm^2)	1640 ± 206	1320 ± 75[a]	1117 ± 86[a]	1113 ± 57[a]	1230 ± 169[a]
Elastin density (%)	29.1 ± 1.5	32.3 ± 1.0	32.6 ± 1.0	32.0 ± 0.7	30.6 ± 0.9
Elastin content (mm^2/mm aorta)	3500 ± 182	3314 ± 88	3105 ± 107[a]	2900 ± 74[a]	3037 ± 132[a]
Collagen density (%)	14.5 ± 0.2	14.3 ± 0.2	12.9 ± 0.2[a,b]	12.8 ± 0.2[a,b]	13.6 ± 0.2[a]
Collagen content (mm^2/mm aorta)	1742 ± 57	1478 ± [a]	1228 ± 28[a,b,c]	1150 ± 24[a,b,c]	1381 ± 59[a]

Values are means ± S.E.M. MCSA = medial cross-sectional area.
[a] $P < 0.0001$ vs. SHR Placebo, [b] $P < 0.001$ vs. Ver, [c] $P < 0.005$ vs. WKY.
Values for arterial thickness, MCSA and elastin and collagen contents were adjusted for body weight.

periodic acid oxidation for nucleus staining. Aortic thickness and composition were quantified with an automated image processor based upon morphological principles described in previous studies (Lacolley et al., 1995a; Benetos et al., 1997).

2.6. Fibronectin and α5 subunit of integrin immunoperoxidase staining of carotid artery

2.6.1. Tissue freeze drying

Immunohistochemical study of fibronectin and integrin α5 subunit in rats was performed using a technique in which fresh unfixed tissue is freeze-dried and then embedded directly in paraffin wax (Stein et al., 1985). The freeze-dried paraffin-embedded sections that we used in the present study retain tissue morphology and reactivity of tissue antigens better than do cryostat sections or classical paraffin-embedded tissues after ethanol fixation. Briefly, the carotid artery was deep-frozen in liquid nitrogen and stored at −70°C until sectioning. On the day of use, the frozen tissues were rapidly transferred to a precooled plate of an Edwards–Pearce tissue dryer and dried at −45°C, 10^{-2} Torr in the presence of phosphorus pentoxide for 12 h. The tissues were then allowed to warm to room temperature and were embedded in paraffin wax at 55 to 56°C for 24 h. Serial sections of 6-μm thickness were cut and the paraffin blocks were stored at room temperature. Immediately before staining, the sections were dewaxed by 1-min incubation with xylene followed by 10 min of incubation in acetone.

2.6.2. Fibronectin and integrin α5 subunit immunoperoxydase staining

The antibodies used were monoclonal mouse antibody reactive with an alternatively spliced form of fibronectin, EIIIA (clone IST-9, Sera-Lab) and polyclonal antibody (rabbit Anti-Human Fibronectin, Dako Laboratories) directed against epitopes present in both rat plasma and cellular fibronectin, referred to as a total fibronectin and a rabbit anti-integrin α5 subunit polyclonal antibody (Valbiotech). Antibodies were directed against human fibronectin and α5 subunit, and their specificity toward rat fibronectin and α5 subunit has been described (Borsi et al., 1987; Plantefaber and Hynes, 1989). Fibronectin monoclonal antibody and α5 polyclonal antibody were diluted 1:200 in TBST (Tris-buffered saline + 0.05% Tween 20); fibronectin polyclonal antibody was diluted 1:500 in TBST. The indirect immunoperoxidase technique was then used. Briefly, samples were treated with the antibodies to be tested, then incubated with a biotinylated anti-mouse or anti-rabbit antibody (Kit LSAB2, Dako Laboratories). After three extensive washes in TBST, the samples were incubated with streptavidin–peroxidase complex. The presence of peroxidase was revealed after incubation with diaminobenzidine (Sigma). The controls were as follows, with the first or second antibody omitted. Tissue sections were counterstained with hematoxylin. The distribution and quantification of fibronectin and α5 subunit was then determined by computer-directed color analysis, using the Quant'Image software (Quancoul, Talence, France). The number of positive pixels was determined as already described, with minor modifications (Daniel Lamaziere et al., 1993).

2.7. Statistical analysis

Values are expressed as means ± S.E.M.. Statistical analysis was done using the analysis of variance. When F was less than 0.05, a Bonferroni/Dunn test was used for intergroup comparisons. Multiple regression analysis was used to assess the contribution of blood pressure levels and

Fig. 1. Immunostaining of sections of the common carotid artery from control WKY (right panel), placebo SHR (middle panel), and SHR treated with the verapamil–trandolapril combination (Ver–Trand) (left panel). Monoclonal mouse antibody raised against EIIIA fibronectin isoform and polyclonal antibody directed against epitopes present in both rat plasma and cellular fibronectin, referred to as a total fibronectin. Quantitative evaluation showed that EIIIA fibronectin and total fibronectin-positive staining was increased in the media from placebo SHR as compared to WKY rat. In Ver–Trand treated SHR, EIIIA fibronectin and total fibronectin staining was different from that of WKY.

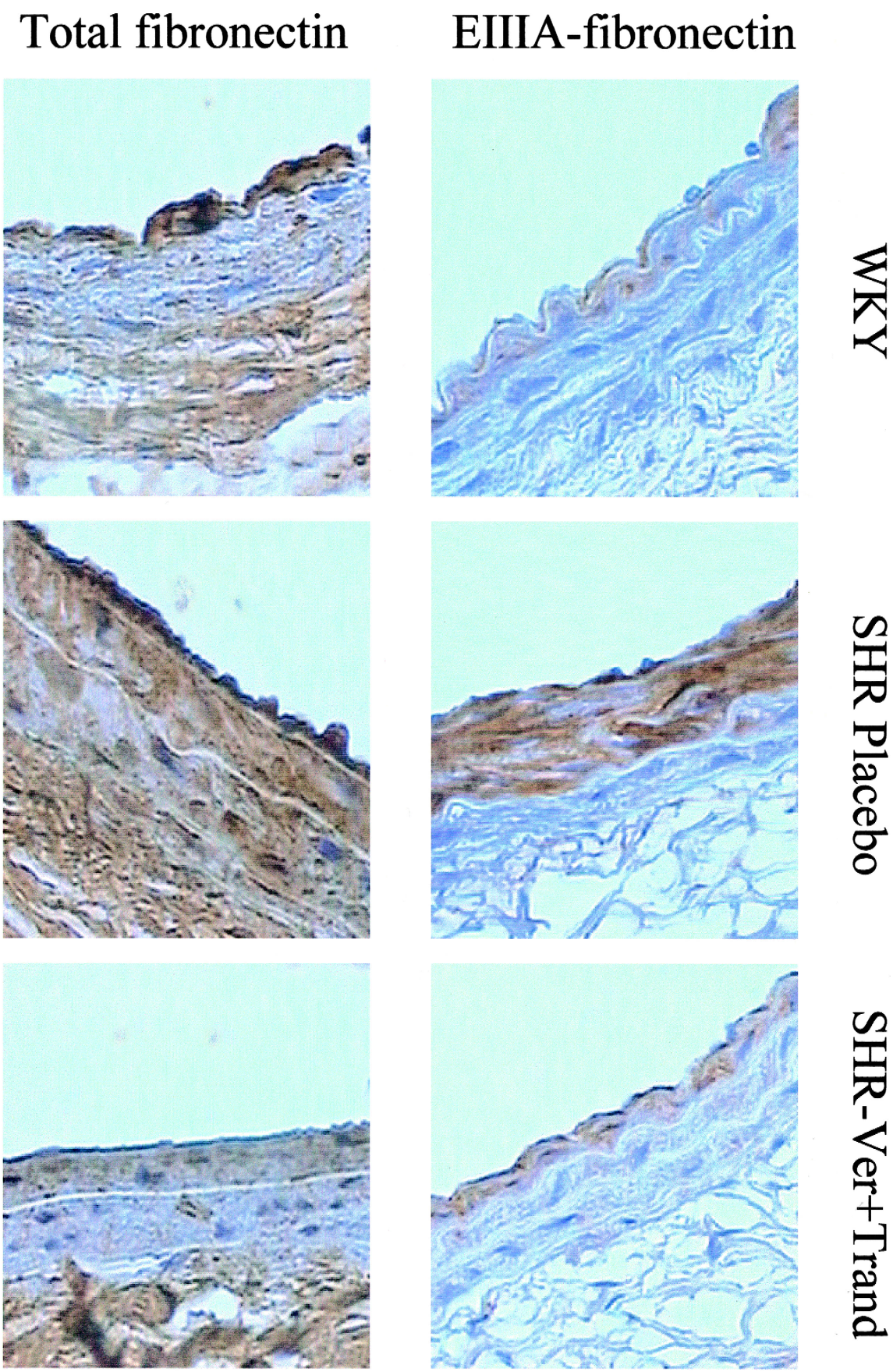
Total fibronectin
EIIIA-fibronectin
WKY
SHR Placebo
SHR-Ver+Trand

Table 3
Immunostaining for carotid artery EIIIA-fibronectin, total fibronectin and α5 integrin in SHR after 16 weeks of treatment

	Placebo ($n = 6$)	Ver ($n = 9$)	Trand ($n = 7$)	Ver + Trand ($n = 7$)	WKY ($n = 6$)
Total fibronectin					
Labeled carotid wall (%)	22.2 ± 2.6	25.9 ± 8.0	24.9 ± 6.0	13.2 ± 5.8	6.5 ± 2.8[c]
EIIIA fibronectin					
Labeled carotid wall (%)	13.44 ± 0.94	7.87 ± 1.59[a]	2.66 ± 0.23[a,b]	2.13 ± 0.39[a,b]	1.80 ± 0.30[a,b]
α5-Integrin					
Labeled carotid wall (%)	3.04 ± 0.83	3.84 ± 1.45	1.56 ± 0.43[a,b]	1.28 ± 0.22[a,b]	1.73 ± 0.29[a,b]

Values are means ± S.E.M.
[a] $P < 0.05$ vs. SHR placebo, [b] $P < 0.05$ vs. Ver, [c] $P < 0.05$ vs. placebo, Ver and Trand.

structural parameters to the determination of arterial mechanical properties.

3. Results

3.1. One-month treatment: effects on body weight, blood pressure and heart rate (Table 1a)

One month of treatment (from the fourth to the eighth week of age) with verapamil (50 mg kg^{-1} day^{-1}), trandolapril (0.3 mg kg^{-1} day^{-1}) and their combination did not significantly decrease blood pressure and heart rate in SHR compared to the placebo treatment. The same doses of each drug were used for the four-month treatments.

3.2. Four-month treatment: effects on body weight, blood pressure and heart rate (Table 1b)

The body weight of placebo SHR was similar to that of WKY placebo rats. Trandolapril, verapamil or their combination, significantly decreased body weight in SHR. In conscious animals, the mean blood pressure was significantly reduced in SHR treated with trandolapril alone or with its combination with verapamil but did not reach the WKY level. The verapamil–trandolapril combination was more efficacious than was trandolapril alone on mean blood pressure. Verapamil used alone as single-drug therapy had no effect. Anesthesia did not change the effects of any treatment on blood pressure. When compared to that of WKY, the pulse pressure was only normalized in the verapamil–trandolapril group. A higher value for heart rate was observed in all SHR groups than in the WKY.

3.3. Histomorphometry parameters of thoracic aorta (Table 2)

Compared to the effect in the SHR placebo group, chronic treatment with verapamil, trandolapril and their combination significantly decreased the aortic medial cross-sectional area (−29%, −37%, −37% respectively), so that all the values for treated rats were similar to the WKY values. Aortic elastin density was similar in SHR controls and WKY rats and was not affected by the various treatments. The decrease in elastin content in the trandolapril and verapamil–trandolapril groups was due to the lower medial cross-sectional area values.

Aortic collagen density and content of SHR placebo rats were significantly greater than those of WKY. Trandolapril and verapamil and their combination reduced collagen content in the aortic media of SHR. Moreover, a decrease in collagen density was only observed in animals receiving trandolapril alone or combined with verapamil (−12%).

3.4. Carotid artery fibronectin and integrin α5 subunit

In the carotid artery of WKY rats, there was cellular fibronectin staining for the isoform EIIIA in the intimal and the internal part of the media (Fig. 1). In SHR treated with placebo, EIIIA fibronectin was diffuse in the media

Table 4
Effects of treatment on geometry and distensibility of the carotid artery in SHR

	Placebo ($n = 8$)	Ver ($n = 13$)	Trand ($n = 10$)	Ver + Trand ($n = 8$)	WKY ($n = 7$)
Ds (μm)	1485 ± 89	1296 ± 36[a]	1196 ± 42[b]	1255 ± 56[b]	1251 ± 85[b]
Dd (μm)	1391 ± 87	1223 ± 36[a]	1098 ± 48[b]	1163 ± 58[b]	1148 ± 81[b]
Dm (μm)	1430 ± 87	1254 ± 36[a]	1144 ± 46[a]	1208 ± 57[a]	1199 ± 83[a]
Ds − Dd (μm)	94 ± 22	73 ± 4	98 ± 9	92 ± 12	103 ± 8
(Ds − Dd)/Dd (%)	6.3 ± 1.4	5.7 ± 0.3	8.4 ± 1.0	7.5 ± 1.0	8.3 ± 0.6
Distensibility (mm $Hg^{-1} \times 10^{-1}$)	2.1 ± 0.5	2.9 ± 0.4	3.5 ± 0.5	3.8 ± 0.5[a]	5.0 ± 0.5[a,d]
E_{inc} (kPa)	2500 ± 481	1809 ± 207	1369 ± 147[a]	1480 ± 291[a]	914 ± 75[a]
σ (kPa)	440 ± 26	425 ± 13	400 ± 15	371 ± 7[a,c]	281 ± 14[e]

Values are means ± S.E.M. Ds = systolic diameter; Dd = diastolic diameter; Dm = diameter.
[a] $P < 0.05$; [b] $P < 0.01$ vs. SHR Placebo, [c] $P < 0.05$; [d] $P < 0.01$ vs. Ver, [e] $P < 0.05$ vs. others.

and EIIIA fibronectin staining showed a 7-fold increase compared to WKY rats (Table 3). A similar increase in total fibronectin was also observed in SHR compared to WKY rats. In SHR, α5 subunit staining was 2-fold higher than in WKY rats.

In the verapamil group, EIIIA fibronectin was significantly reduced by approximately 50%. However we observed marked heterogeneity in the responses, as indicated by the S.E.M. of the mean in this group. Treatment with trandolapril completely abolished the accumulation of fibronectin in the media, so that all trandolapril-treated rats had a fibronectin content similar to that of WKY rats (Table 3). The verapamil–trandolapril combination had no additional effect on fibronectin accumulation. Total fibronectin was not affected in SHR treated with verapamil or trandolapril alone. For the verapamil–trandolapril combination, the value for total fibronectin was intermediate between SHR and WKY (Table 3). In treated SHR, the α5 subunit-positive staining with trandolapril and verapamil–trandolapril was normalized compared with that of WKY rats. Treatment with verapamil induced a significant, though partial, reduction of α5 subunit staining in the media of carotid artery.

3.5. Mechanical properties of the carotid artery

Compared to that of the WKY rats, the placebo SHR had a greater arterial diameter and lower mean distensibility. Carotid systolic, diastolic and mean diameters were significantly decreased by all three treatments (Table 4). The verapamil–trandolapril combination produced a significant increase in arterial distensibility (+80%), and a similar trend was observed in rats treated with trandolapril ($P = 0.09$).

Multiple regression analysis showed that mean blood pressure and total fibronectin density were negatively correlated with operating distensibility (at mean blood pressure), explaining 71.6% of the variability of this parameter (see Table 5). This analysis showed that other aortic structural parameters including medial cross-sectional area, collagen and elastin densities were not significant determinants of carotid distensibility.

Table 5
Multiple regression analysis of the determinants of carotid artery distensibility

Independent variables	Partial R^2	Significance
Mean blood pressure	0.587	0.0001
Total fibronectin density	0.130	0.01
MCSA	–	0.09
Collagen density	–	ns
Elastin density	–	ns
Model R^2	0.717	0.0001

MCSA = medial cross-sectional area.

4. Discussion

We had shown that angiotensin-converting enzyme inhibition prevents the increase in aortic collagen in SHR, independently of blood pressure reduction (Albaladejo et al., 1994). In addition, we recently reported an increase in aortic fibronectin and α5β1 integrin in SHR, and suggested their involvement in arterial mechanical properties (Bezie et al., 1998). Our results showed that chronic treatment of SHR with the combination of low doses of trandolapril and verapamil prevented hypertension-related structural and mechanical alterations of large arteries. A similar reduction of media hypertrophy and a reduced extracellular matrix were also observed with trandolapril alone, whereas blood pressure reduction and arterial stiffness improvement were more pronounced with the combination.

4.1. Chronic anti-hypertensive effects

The present study aimed to evaluate the effects of low doses of verapamil, trandolapril or their combination on arterial parameters. As detailed in Section 2 the choice of the doses was based upon previously published data (Kirchengast, 1997) and on the results of our preliminary study obtained after one month of treatment. The preliminary study showed that, in SHR, the doses of 50 mg kg^{-1} of verapamil or 0.3 mg kg^{-1} of trandolapril had no significant antihypertensive effects. Surprisingly, the results after 4 months of treatment were somewhat different: although verapamil remained without any antihypertensive action at the doses used, trandolapril 0.3 mg kg^{-1} had a significant (though mild) antihypertensive effect. We cannot yet fully explain this discrepancy between the 1 and 4 month antihypertensive actions. It is possible that the late antihypertensive effect of low doses of trandolapril is related to a greater inhibition of angiotensin converting enzyme. Although several studies have shown that, after chronic administration of angiotensin converting enzyme inhibitors, inhibition of plasma angiotensin-converting enzyme decreases and plasma angiotensin II increases, we cannot rule out the possibility that tissue angiotensin converting enzyme blockade increases with time. Moreover, trandolapril is known to have a very long duration of action and therefore we also cannot rule out accumulation of the drug during long-term use. Another explanation is that the observed decrease in blood pressure after 4 months of treatment could have been the result of arterial structural changes following trandolapril or the combination treatment, leading to a long-term decrease in blood pressure in these groups. The more pronounced effects on systolic than on diastolic pressure could suggest that the blood pressure decrease was at least partially related to the effects of the drug on large artery structure and mechanics. However, verapamil, while having significant structural effects had no such blood pressure effects. These results

demonstrate the complex relationships between blood pressure levels and large artery structure and mechanics. Indeed large artery alterations not only are the result of increased blood pressure but also can actively participate in the development of high blood pressure, especially systolic hypertension.

4.2. Effects of drugs on arterial hypertrophy and extracellular matrix

Arterial hypertrophy, collagen and cellular fibronectin accumulation were completely prevented by trandolapril and by the combination of the two drugs, so that the mean values for these parameters were similar to those of WKY controls. Verapamil was less efficient than trandolapril or the combination. These effects were observed despite the absence of blood pressure lowering with verapamil and a mild antihypertensive action of trandolapril.

Aortic collagen accumulation is prevented by angiotensin converting enzyme inhibitors, and this effect is obtained with both antihypertensive and non-antihypertensive doses (Linz et al., 1989; Albaladejo et al., 1994). The effect was mainly related to the blockade of the effects of angiotensin II on angiotensin AT_1 receptors (Benetos et al., 1997). In the present study the three active treatments significantly prevented the increase in collagen in SHR. To our knowledge, the present experiments are the first to show that non-hypotensive doses of a Ca^{2+} channel antagonist can prevent structural alterations in SHR. Previous studies have shown that antihypertensive doses of the dihydropyridine, isradipine, could reverse aortic hypertrophy and collagen accumulation when this drug was used at fully antihypertensive doses (Levy et al., 1994; Lacolley et al., 1995a). However in other studies, Ca^{2+} channel antagonists were less effective than angiotensin converting enzyme inhibitors to prevent large artery alterations in hypertension (Shimamoto and Shimamoto, 1995). Dihydropyridines Ca^{2+} channel antagonists were used in most of these studies, and the authors suggested that the lack of significant prevention of hypertension-induced hypertrophy was due to stimulation of the renin–angiotensin or sympathetic systems in response to chronic administration of these drugs (Nyborg and Mulvany, 1985; Aalkjaer et al., 1988; Lindqvist et al., 1994). In our study, the arterial effects with verapamil were observed in the absence of any blood pressure changes as evaluated after 1 and 4 months of treatment. However the effects on collagen and hypertrophy regression were more pronounced in the trandolapril and in the combination groups. The differences observed among treatments were proportional to the blood pressure levels, showing that the structural effects of the treatments are related to both the endogenous actions of the drugs on the vascular wall and to the reduction in blood pressure.

Similarly, trandolapril alone or in combination with verapamil was able to suppress the 7-fold increase of cellular EIIIA fibronectin isoform observed in SHR compared to those in normotensive controls. Again, verapamil induced a partial reduction of fibronectin. In contrast, there was no significant effect of the treatments on total fibronectin, with only a trend to a decrease in the combination group (approximately 50% decrease compared to the other SHR). Previous studies have shown that the expression of aortic fibronectin was increased in SHR (Takasaki et al., 1990, 1992) with a most prominent induction of EIIIA fibronectin (Takasaki et al., 1992). EIIIA fibronectin was also increased in the aorta of SHR compared to normotensive rats (Bezie et al., 1998). The in vitro studies (Bardy et al., 1996) have shown that increased transmural pressure interacts with local angiotensin II to enhance EIIIA fibronectin expression in rabbit aortic media. Recent in vitro (Dunn et al., 1997) and in vivo (Sabri et al., 1997) studies have shown that the increase in vascular smooth muscle cells fibronectin expression following angiotensin II was mediated through angiotensin AT_1, but not AT_2 receptors.

Similarly, using an $\alpha 5$ polyclonal antibody, we found that trandolapril alone or in combination with verapamil completely prevented the hypertension-related increase in $\alpha 5$ integrin subunits. The $\alpha 5\beta 1$ integrin is a higher affinity receptor specific for fibronectin (Pytela et al., 1985; Clyman et al., 1990; Hynes, 1992) and $\alpha 5$ integrin subunit is known to bind exclusively to the $\beta 1$ subunit. The concomitant reduction of $\alpha 5\beta 1$ integrins in smooth muscle of trandolapril-treated SHR is consistent with the changes in expression of its ligand.

4.3. Effects of treatment on arterial mechanics

The impairment of mechanical properties increases arterial stiffness, inducing an increase in pulse pressure, and in left ventricular afterload (Boutouyrie et al., 1995). Increased arterial stiffness in human clinical and in experimental hypertension has been reported by several groups (Cox, 1981; Benetos et al., 1993). It is generally accepted that hypertension-induced stiffness is the result of several factors, including an increase in distending pressure, arterial hypertrophy and extracellular matrix accumulation including fibronectin. In the present study, the mechanical properties of the carotid artery were studied in vivo by evaluating arterial distensibility with a high-resolution echo-tracking system (Hayoz et al., 1992; Glaser et al., 1995; Lacolley et al., 1995a). Our results show that single-drug treatment with trandolapril or verapamil has no effect on arterial distensibility despite significant prevention of structural alterations. The combination of the two drugs increased arterial distensibility compared to that in the placebo SHR, but distensibility remained lower than in WKY. The diminution of arterial stiffness in the treated SHR was observed while the treatment normalized collagen, elastin and wall hypertrophy. One of the possible mechanisms to explain the persistence of lower levels of

arterial distensibility in treated rats is the persistence of higher levels of distending blood pressure and total fibronectin density in the vascular wall. The importance of these two factors is suggested by the results of multiple regression analysis, showing that blood pressure, together with total fibronectin, is a represent significant determinant of arterial distensibility. From a mechanical point of view, the increase in total fibronectin may reflect an increase in the number of cell–matrix attachment sites, leading to an increase in the stiffness of the arterial wall material. Moreover, accumulation of fibronectin is also associated with phenotypic changes in the vascular smooth muscle cells, which could also influence the degree of vascular tone. We have suggested that phenotypic changes and the increase in cell matrix attachment sites both contribute to the regulation of arterial distensibility in SHRs (Bezie et al., 1998). Further in vitro studies are necessary to confirm the role of fibronectin and cell matrix interactions in the regulation of the mechanical properties of the vascular wall in SHR.

Compared to the effect of the individual drug, the combination of low doses of the Ca^{2+} channel antagonist verapamil and the angiotensin converting enzyme inhibitor trandolapril, increased arterial distensibility. This effect could be explained by more pronounced effects on the main structural determinants of arterial distensibility, i.e., arterial wall thickness and total fibronectin density. However we suggest that, in addition to the structural effects, complete normalization of blood pressure is necessary to obtain normal arterial distensibility.

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