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J. Pharm. Pharmacol. 1992, 44: 277–278
Communicated June 4, 1991

Inhibition by diltiazem of left ventricle collagen proliferation during renovascular hypertension development in rats

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Abstract—Diltiazem administered in drinking water (0·7 mg mL^{-1}) to Goldblatt two kidney-one clip rats over 16 weeks did not prevent the development of hypertension and left ventricular hypertrophy (LVH). When the collagen content of the left ventricles was assayed (as hydroxyproline), it was found that the fibrosis, characteristic of LVH, was inhibited by diltiazem treatment, despite the fact that hypertension and LVH had developed. This study provides some indirect evidence for the notion that the collagen and myocyte compartments of the myocardium are under separate influences during LVH development in renovascular hypertension.

It seems likely that the altered mechanical properties of the hypertrophied myocardium in hypertension and the subsequent development of heart failure are due, at least in part, to a progressive perivascular and interstitial fibrosis (Weber et al 1990). Several laboratory and clinical studies have shown that antihypertensive treatment with the calcium antagonist diltiazem can induce regression of established left ventricular hypertrophy (LVH) (Grellet et al 1988; Szlachcic et al 1989). Furthermore, it has been reported recently that LVH regression with diltiazem in a salt model of hypertension involves parallel regression of the myocyte and collagen compartments of the myocardium (Baxter 1991).

Brilla et al (1990) have suggested that during LVH development, myocytes and fibroblasts are under separate haemodynamic and humoral controls. If this is the case, it may be possible to modify the response of each compartment to hypertension. In the present study, the effects of diltiazem treatment on hypertrophy development were investigated in a rat model of renovascular hypertension (the Goldblatt two kidney-one clip model) (Robertson et al 1986). The influence of this treatment on blood pressure elevation, left ventricle mass and left ventricle collagen content (assayed as hydroxyproline) was assessed.

Materials and methods

Animals. Renovascular hypertension was induced in male Sprague-Dawley rats, 6–8 weeks old, by placing a silver clip (internal gap width, 0·2 mm) on the left renal artery. Sham operated animals underwent the same surgical procedure but no clip was placed on the renal artery.

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Drug treatment. Two days after surgery, a sham operated (i.e. normotensive) group and a renal artery-clipped group were assigned to receive diltiazem hydrochloride (Sigma, Poole, Dorset, UK) in distilled water as drinking fluid. The concentration of the drug was increased gradually over one week to 0·7 mg mL^{-1} and maintained at this concentration for a further 15 weeks. Fluid intake was monitored daily and the dose taken by each animal was estimated. During the first three weeks after the concentration was increased to 0·7 mg mL^{-1}, the mean dose ($\pm$s.e.m.) was 89 ± 3 mg kg^{-1} day^{-1} and during the final three weeks it was 72 ± 3 mg kg^{-1} day^{-1}. Corresponding normotensive and clipped control groups received distilled water as drinking fluid.

Blood pressure measurement. Systolic blood pressure was measured by tail-cuff plethysmography (PE-300 programmed electrosphygmomanometer; Narco Biosystems, Houston, TX) after 15 min pre-warming at 37°C.

Tissue processing and hydroxyproline assay. Sixteen weeks after surgery, hearts were excised under pentobarbitone sodium anaesthesia. Each heart was dissected into right ventricle free wall (right ventricle) and left ventricle free wall plus interventricular septum (left ventricle). The tissues were washed, blotted and weighed to give wet weight and then dried to constant weight at 45°C to give the dry weight and water content. The left ventricle samples were hydrolysed by heating with 5 M HCl for 3 h at 130°C in sealed Pyrex ampoules. The resulting tissue digests were neutralized with NaOH, filtered and diluted. Each solution was assayed in duplicate for hydroxyproline by a modified method of Woessner (1961). In brief, after oxidation with chloramine-T, the solution was treated with *p*-dimethylaminobenzaldehyde and the absorbance of the chromophore was measured at 557 nm. The concentration of hydroxyproline in the left ventricle samples (mg g^{-1} dry weight) was calculated.

Differences between group means were evaluated using Student's unpaired *t*-test with Dunnett's modification for multiple comparisons, and they were considered significant when $P < 0.05$.

Results and discussion

The data are presented in Table 1. The large oral dose of

Table 1. Effects of 16 weeks of diltiazem treatment on body weight, blood pressure and left ventricle mass, water content and collagen concentration in normotensive and renal artery-clipped rats.

Treatment group	n	Body weight (g)	SBP (mm Hg)	LV/BW (g/100 g) (wet weight)	LV water content (% w/w)	LV hydroxyproline concn (mg g^{-1}) (dry weight)
Control normotensive	6	469 ± 27	127 ± 4	0·225 ± 0·005	81·46 ± 0·16	3·29 ± 0·02
Diltiazem-treated normotensive	6	479 ± 10	131 ± 4	0·252 ± 0·002**	81·39 ± 0·29	3·15 ± 0·10
Control clipped	6	471 ± 20	194 ± 13**	0·314 ± 0·019**	81·38 ± 0·17	3·97 ± 0·25*
Diltiazem-treated clipped	5	473 ± 20	182 ± 8**	0·304 ± 0·027*	81·35 ± 0·11	3·18 ± 0·15§

Results are expressed as mean ± s.e.m. SBP—systolic blood pressure; LV/BW—left ventricle to body weight ratio.
* $P < 0·05$ compared with control normotensive group; ** $P < 0·01$ compared with control normotensive group; § $P < 0·05$ compared with control clipped group; other differences not significant (unpaired t-test with Dunnett's modification).

diltiazem employed in this study failed to prevent the gradual development of hypertension in the clipped rats. Sixteen weeks after surgery, systolic pressure in the diltiazem-treated clipped group was comparable with that of the control clipped group (difference not significant) and considerably higher than in the control normotensive group ($P < 0·01$). Diltiazem did not influence the blood pressure of normotensive animals. The rise in blood pressure in both clipped groups was associated with the development of LVH. The left ventricle to body weight ratio in the diltiazem-treated clipped group was slightly lower than that in the control clipped group but the difference was not significant. There was no increase in the relative right ventricle mass in either of the clipped groups (data not shown). However, there was an unexpected 9% increase in left ventricle mass in the diltiazem-treated normotensive group ($P < 0·01$ compared with control normotensive group). This observation cannot be explained. Water content of the left ventricle was similar in all groups.

Myocardial hydroxyproline concentration was significantly increased in the hypertrophied left ventricles of the control clipped group ($P < 0·05$). This fibrosis is characteristic of experimental renovascular hypertension (Weber et al 1990) and occurs in other models of hypertensive LVH (Sen et al 1976; Baxter 1991). However, no increase in hydroxyproline concentration occurred in the diltiazem-treated clipped group despite similar increases in blood pressure and left ventricle mass. It is also notable that the unexpected increase in ventricle mass observed in the diltiazem-treated normotensive group was not associated with elevated hydroxyproline concentration. Although many studies have reported regression of established LVH with various antihypertensive treatments, there are few reports of non-antihypertensive therapy affecting collagen proliferation in the heart during LVH development. Ritter & Forster (1976) described the action of the coronary vasodilators oxyfedrine, dipyridamole and prenylamine on hypertrophy progression during hypertension development. Chronic administration of these drugs during the development of hypertension inhibited the proliferation of collagen despite large increases in left ventricle mass, an effect identical to that observed with diltiazem in the present study. In contrast to these results, Jalil et al (1989) demonstrated inhibition of the development of renovascular hypertension by the angiotensin converting enzyme inhibitor captopril. This treatment prevented the development of LVH but collagen proliferation, although attenuated, was not prevented.

This work provides no information on the mechanisms involved in the inhibition of collagen proliferation, although it would seem that mechanical overload of the left ventricle is not the sole stimulus for increased collagen synthesis in LVH. Several humoral factors have been implicated in myocyte growth, including angiotensin II, catecholamines and epidermal growth factors (Devereux 1990) but less is known about the factors affecting fibroblast activity in LVH. Weber et al (1990) have proposed that circulating angiotensin II affects fibroblast proliferation in renal hypertension. It is known that the maximum rate of collagen synthesis by fibroblasts in-vitro is achieved within a narrow range of extracellular calcium concentrations (Rokosova & Bentley 1986). One might speculate that diltiazem, and possibly other calcium antagonists, can modify an abnormal fibroblast response in hypertension, whilst not affecting normal fibroblast activity in the normotensive heart. Whether prevention of gross fibrotic changes in the heart undergoing hypertrophic changes is associated with functional benefits, such as preservation of diastolic function and a reduction in the risk of congestive failure, remains to be determined. However, there is growing evidence for a dissociation between blood pressure and the complex processes involved in LVH development. Further studies are warranted to explore this intriguing phenomenon and the mechanisms involved.

The author is supported by a Science and Engineering Research Council award.

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