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Comparison of Valsartan With Candesartan on Their Possible Protection From Atherosclerosis

A. O. Mueck, H. Seeger, W. Heuberger, D. Wallwiener

It is well appreciated that AT_1-antagonists do diminish long-term effects of angiotensin on the blood pressure which are regarded as detrimental. In the present *in vitro* experiments we compared the efficacies of valsartan and candesartan in preventing negative outcomes of angiotensin effect on markers of endothelial function and on proliferation of smooth muscle cells.

Angiotensin II (10 μM) induced a decrease in the concentration of endothelial-derived nitric oxide synthase and increases the concentration of the vasoconstrictor endothelin, the procoagulatory substance plasminogen-activator-inhibitor-1 (PAI-1) and of the precursor of the matrix-metalloproteinase 1 (MMP-1) in endothelial cell cultures from human coronary arteries. These changes were completely prevented by the addition of 10 μM of valsartan or candesartan and partially by the addition of lower concentrations of the sartans, ie 1 μM and 0.1 μM. No significant difference was observed in the effect of the two sartans. The angiotensin II-induced increase of coronary artery smooth muscle cell proliferation was also completely prevented by the addition of 10 μM of the sartans.

These results suggest that sartans may class-specifically inhibit negative actions of angiotensin II on endothelial function and smooth muscle cell proliferation. Thus sartans may be able to prevent the initiation and progression of atherosclerosis. *J Clin Basic Cardiol 2001; 4: 297–9.*

Key words: angiotensin II, valsartan, candesartan, atherogenesis

The peptide angiotensin II elicits several important physiologic and pathophysiologic effects including vasoconstriction, cell proliferation, stimulation of aldosterone release and anti-natriuretic and anti-diuretic effects [1]. These actions are most likely mediated by the angiotensin receptor of the type 1 (AT_1). Since angiotensin II is mainly involved in the pathogenesis of hypertension, the blockade of its synthesis by ACE-inhibitors or blockade of its action by AT_1-antagonists are currently the medications of choice. Several AT_1-antagonists with different pharmacokinetic properties are available. It is still uncertain whether there are class-specific differences in the inhibitory effects on angiotensin II-induced atherosclerosis. Therefore, we conducted several *in vitro* experiments comparing valsartan with candesartan with respect to their effects on angiotensin II-induced negative changes in vessel homeostasis. The synthesis of different endothelial-derived substances as well as proliferation of coronary artery smooth muscle cells were investigated since they are regarded as being a good indication for initiation and progression of atherosclerosis.

Material and Methods

Angiotensin II was purchased from Sigma Chemical Company, Munich, valsartan and candesartan were kindly provided by Novartis, Switzerland, and Takeda, Japan, respectively. The substances were dissolved in water (angiotensin II) and ethanol (AT_1-antagonists). The sartans were tested alone at the concentrations of 0.1, 1 and 10 μM. The influence of angiotensin was tested at the concentration 10 μM, the sartans were added at the concentrations of 0.1, 1 and 10 μM. The most effective angiotensin concentration was determined in pre-experiments by testing the concentration range from 0.01 μM to 10 μM.

Endothelial cells from human male coronary arteries were purchased from PromoCell, Germany, and the experiments were conducted using passages 3–4. The cells were placed in culture dishes and allowed to replicate to confluence in MCDB 131 Medium containing 10 % fetal calf serum, 20 μg/ml endothelial growth factor, 50 μg/ml heparin, 50 μg/ml penicillin, 100 μg/ml streptomycin and 2.5 μg/ml amphotericin B. Cell cultures were maintained in an atmosphere of 5 % CO_2 in air.

The cells were transferred to 24-well dishes and maintained in growth medium. After confluence, the medium was changed to a serum-free one, the test substances were added and the cells incubated for 24 h. Control values were determined by addition of water or ethanol alone; the final ethanol concentration per well was < 1 %.

Concentrations of endothelial-derived nitric oxide synthase (eNOS) were measured by enzyme immunoassay (R&D Systemy) after lysing of the cells. The sensitivity of the assay was 20 pg/ml. Inter- and intraassay variation coefficients were 9.3 % and 6.4 %, respectively.

Endothelin was measured directly in the cell supernatant by enzyme immunoassay (BioTrend). The sensitivity of the assay was 10 pg/ml. Inter- and intraassay variation coefficients were 9.9 % and 7.1 %, respectively.

PAI-1 was measured directly in the cell medium by enzyme immunoassay (American Diagnostics). The sensitivity of the assay was 50 pg/ml. Inter- and intraassay variation coefficients were 9.5 % and 7.4 %, respectively.

The zymogen pro-MMP-1 was measured directly in the supernatant by enzyme immunoassay (R&D Diagnostics). The sensitivity of the assay was 20 pg/ml. Inter- and intraassay variation coefficients were 10.5 % and 6.9 %, respectively.

Human male smooth muscle cells from coronary artery were purchased from PromoCell, Germany, and the experiments were conducted using passages 5–8. The cells were cultured in MDCB 131, 10 % FCS, 1 % amphotericin B and 1 % penicillin/streptomycin (all from Gibco, Eggenstein, Germany). After confluence, 30000–50000 cells were transferred in standard medium to 6-well plates. Twenty-four hours later the medium was changed to a medium containing only 5 % FCS and the test substances were added. Both the test solutions and the control solutions contained ethanol in a final

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concentration of 1 %. Medium and test substances were changed every 48 h. After incubation for 7 days the cells were lysed by trypsination and counted using a Coulter counter ZM1.

Statistical analysis was performed by ANOVA and Dunnett's test from triplicates of two different experiments.

Results

Figure 1 shows the changes in eNOS concentrations after addition of angiotensin II (A II) and combinations of A II with valsartan and candesartan. A II (10 μM) triggered a decrease in eNOS concentration by 25.8 % (CI 22; 31). This reduction was dose-dependently inhibited by valsartan and by candesartan. No statistically significant difference was observed between the two sartans.

Figure 2 depicts the changes in endothelin production observed after the addition of A II and combinations of A II with valsartan and candesartan. A II (10 μM) increased endothelin synthesis by 70 % (CI 74; 65). This increase was reduced to basal values by addition of 10 μM valsartan or candesartan. At the lower concentrations of 1 and 0.1 μM the increase was partially inhibited. There were no significant differences between the effects of the two sartans.

The changes in PAI-concentration after addition of A II and combinations of A II with valsartan and candesartan are depicted in Figure 3. A II (10 μM) increased PAI-1 synthesis by 20 % (CI 23; 16). Again, this increase was completely reduced to basal values by the addition of 10 μM of valsartan or candesartan and partially inhibited by the addition of 1 or 0.1 μM. No differences between the effects of the two sartans were found, although valsartan exhibited a tendency toward a stronger inhibitory effect.

Figure 4 shows the changes in pro-MMP-1 concentration after addition of A II and combinations of A II with valsartan and candesartan. A II (10 μM) increased pro-MMP-1 synthesis by 44 % (CI 47; 41). Both antagonists exhibited a concentration-dependent inhibitory effect. With 10 μM valsartan or candesartan the angiotensin-induced effect was even reduced beyond the control value, yet lacking statistical significance. No significant difference was found between the two sartans.

Figure 5 illustrates the changes in cell numbers of human coronary artery smooth muscle cells after addition of A II and

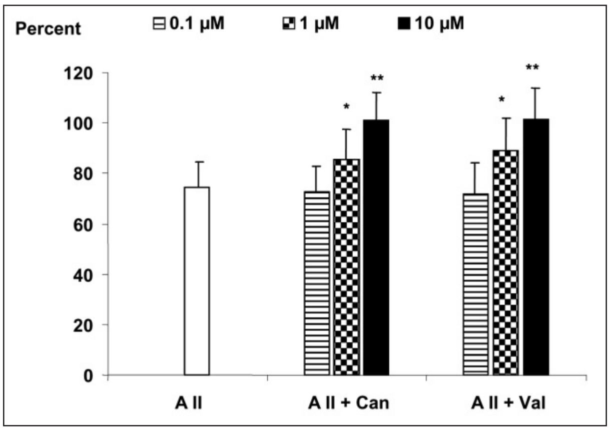

Figure 1. Changes in eNOS concentrations in endothelial cell cultures from human coronary artery after addition of angiotensin II alone (10 μM), angiotensin (10 μM) + candesartan, and angiotensin (10 μM) + valsartan as percentage of control value (= 100 %). (means ± SD, triplicates from 2 different experiments, * $p < 0.05$, ** $p < 0.01$ vs A II; A II = angiotensin II; Can = candesartan; Val = valsartan)

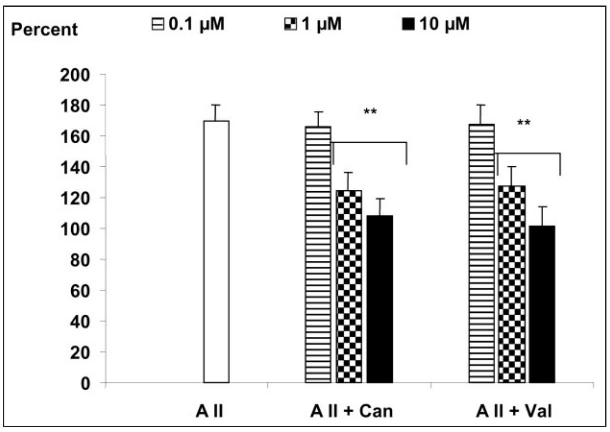

Figure 2. Changes in endothelin concentration in endothelial cell cultures from human coronary artery after addition of angiotensin II alone (10 μM), angiotensin (10 μM) + candesartan, and angiotensin (10 μM) + valsartan as percentage of control value = (100 %). (means ± SD, triplicates from 2 different experiments, ** $p < 0.01$ vs A II; A II = angiotensin II; Can = candesartan; Val = valsartan)

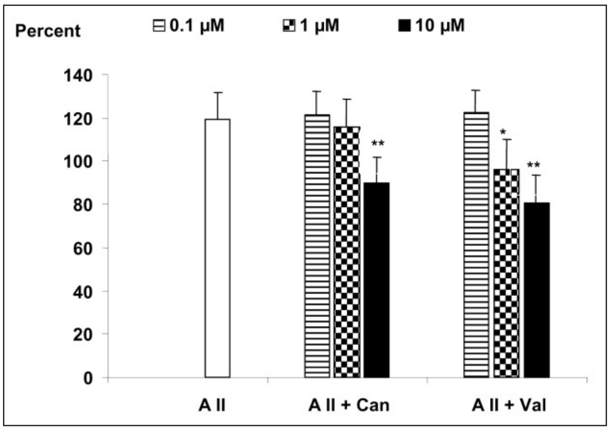

Figure 3. Changes in PAI-1 concentration in endothelial cell cultures from human coronary artery after addition of angiotensin II alone (10 μM), angiotensin (10 μM) + candesartan, and angiotensin (10 μM) + valsartan as percentage of control value = (100 %). (means ± SD, triplicates from 2 different experiments, * $p < 0.05$, ** $p < 0.01$ vs A II; A II = angiotensin II; Can = candesartan; Val = valsartan)

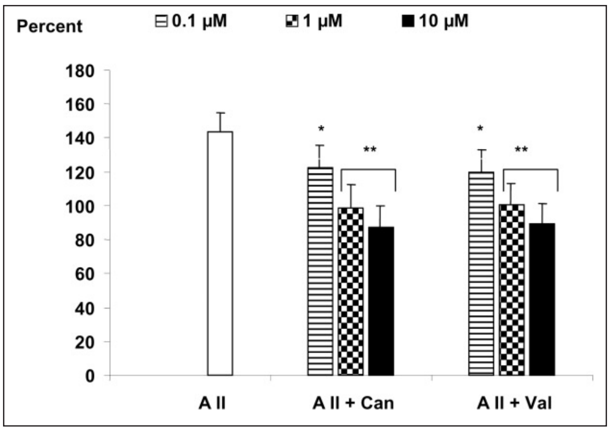

Figure 4. Changes in pro-MMP-1 concentration in endothelial cell cultures from human coronary artery after addition of angiotensin II alone (10 μM), angiotensin (10 μM) + candesartan, and angiotensin (10 μM) + valsartan as percentage of control value = (100 %). (means ± SD, triplicates from 2 different experiments, * $p < 0.05$, ** $p < 0.01$ vs A II; A II = angiotensin II; Can = candesartan; Val = valsartan)

combinations of A II with valsartan and candesartan. Angiotensin II was able to increase the cell number by 45.3 % (CI 38; 51). This increase was reduced dose-dependently by valsartan and candesartan. At the highest dosage of the AT_1-blockers even a reduction of the cell numbers compared to the control values were observed. No statistically significant difference was found between valsartan and candesartan.

Discussion

Disturbance of endothelial function as well as proliferation and migration of smooth muscle cells are considered early events in the initiation of atherosclerotic lesions. Angiotensin II may be involved in the initiation and progression of atherosclerosis by triggering endothelial dysfunction and proliferation of smooth muscle cells [1]. We have investigated the influence of angiotensin on several endothelial-derived substances which are involved in different stages of atherosclerosis. As markers we chose eNOS, endothelin, PAI-1 and pro-MMP-1.

The synthase of endothelial-derived nitric oxide is responsible for converting L-arginine into L-citrulline and thus generating nitric oxide (NO), a potent vasodilative, anti-aggregatory and anti-atherosclerotic compound [2]. This synthase is constitutively expressed in endothelial cells and can be up- or down-regulated by several humoral substances [2].

Endothelin is a more potent vasoconstrictor than angiotensin II itself and may be involved in the pathogenesis of hypertension [3]. Plasma levels of endothelin are enhanced in congestive heart failure and correlate with the severity of the disease [3]. In addition, endothelin is involved in the development of atherosclerosis due to its proliferative action on smooth muscle cells [3]. The observed A II-induced increase of endothelin was prevented by the addition of valsartan or candesartan.

PAI-1 is a procoagulatory substance, and a reduced fibrinolytic activity induced by enhanced serum concentrations of PAI-1 is considered an independent risk factor for cardiovascular diseases [4].

MMP-1, a collagenase, is a member of the group of proteinases which have been detected in atherosclerotic plaques [5]. An enhanced synthesis of MMP-1 may contribute to plaque instability during the acute coronary syndrome which can lead to fatal outcomes such as unstable angina pectoris, myocardial infarct and sudden death.

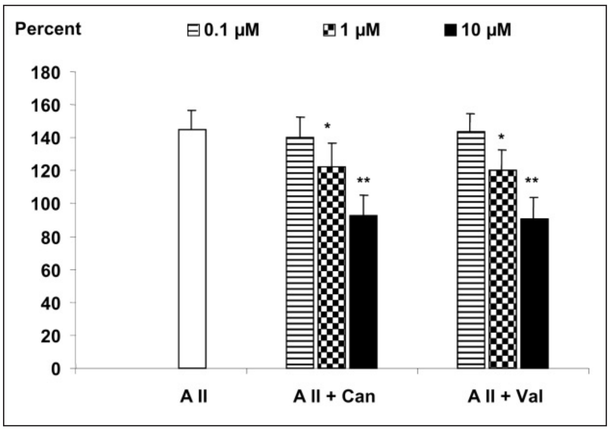

Figure 5. Changes in cell number of human coronary artery smooth muscle cells after addition of angiotensin II alone (10 μM), angiotensin (10 μM) + candesartan, and angiotensin (10 μM) + valsartan as percentage of control value = (100 %). (means ± SD, triplicates from 2 different experiments, * $p < 0.05$, ** $p < 0.01$ vs A II; A II: angiotensin II; Can: candesartan; Val: valsartan)

In our experiments, A II down-regulated the concentrations of eNOS and up-regulated those of endothelin, PAI-1 and pro-MMP-1. These A II-induced negative effects were dose-dependently prevented by the addition of valsartan and candesartan. For all parameters investigated no significant differences in the effects of valsartan and candesartan were observed.

Proliferation of vascular smooth muscle cells represents a crucial step in the pathogenesis of atherosclerosis [6]. In the present study, angiotensin II stimulated the proliferation of smooth muscle cells from human coronary artery indicating that this substance may play a significant role in the development of coronary artery disease in humans. This deteriorating effect was completly suspended by the addition of valsartan or candesartan with no significant difference between these two sartans.

It is noteworthy that the concentrations used in the present study are in the upper pharmacological dosage range. For an angiotensin dosage lower than 10 μM we observed only small effects on the markers investigated. Thus, for the angiotensin antagonists higher concentrations are also required to antagonize the angiotensin-induced effects. However, higher concentrations may be required in short-time *in vitro* tests in which the reaction threshold can only be achieved with supraphysiological dosages. In addition, higher concentrations may be present *in vivo* in the vessel wall or organs compared to the concentrations which are usually measured in the blood. At the dosage of 10 μM both substances exhibit affinity for the AT_2-receptor, but this receptor is usually only marginally expressed in healthy human tissues. For other receptors highly expressed on the endothelium like α_1-, α_2-, β_1-receptors and others, both substances lacked any affinity at this concentration [7, 8].

The angiotensin-induced activation of these endothelial markers may contribute to vasoconstriction, development of atherosclerosis and destabilization of atherosclerotic plaques. The two AT_1-antagonists valsartan and candesartan were equally effective in inhibiting the negative effects of angiotensin II on these markers of endothelial function. It can be assumed that a class-specific mechanism exists for sartans on angiotensin-induced effects on endothelial function, although the role of other sartans should be examined. This mechanism can contribute to the prevention of the development of atherosclerosis and the prevention of triggering acute coronary syndromes by using sartans.

It can be summarized that valsartan and candesartan are able to prevent negative effects of angiotensin II on markers of endothelial function and on the proliferation of smooth muscle cells. These beneficial sartan effects may contribute to the protection from atherosclerosis. Since no significant difference between the two sartans was found in any of the cases, it may be speculated that a class-specific action exists. However, it might be necessary to investigate other sartans to clarify this point.

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