changed to fresh serum free medium without phenol red and containing UA (200 μM), UA with Captopril (10−4 M) and UA with Irbesartan (10−5 M) (n=4 for each group). After 24 hours incubation, the medium was collected and the concentration of NO was measured by modified Griess method and the protein content quantified by the BCA method. The concentration of 8-Isoprostane (8IP) was measured by an enzymatic immunoassay. Baseline NO produced in control cells was 0.17 ± 0.033 nmoles/μg protein. Addition of UA decreased NO production to 0.13 ± 0.031 nmoles/μg protein (p<0.01). In the cells incubated with UA in the presence of captopril the NO production was 0.21 ± 0.032 nmoles/μg protein, and 0.23 ± 0.034 nmoles/μg protein in cells incubated with UA in the presence of irbesartan (p<0.01 versus UA alone for both agents). In control cells, baseline concentration of 8IP was 0.18 ± 0.046 pg/μg protein. In the presence of UA, 8IP increased to 0.24 ± 0.018 pg/μg protein (p<0.01). Addition of captopril decreased 8IP to 0.15 ± 0.002 pg/μg protein and irbesartan addition decreased 8IP to 0.19 ± 0.037 pg/μg protein (p<0.01 versus UA alone for both agents). Our data suggests that in VSMC, UA causes Ang II-induced oxidative stress and reduced NO availability which are prevented by modulators of the renin angiotensin system. Thus, UA is not only a marker for cardiovascular disease but is playing a pivotal role in the pathophysiology of vascular diseases.

Key Words: Angiotensin II, Nitric Oxide, Uric Acid

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MALIGNANT HYPERTENSION (MHT) : A SYSTEMATIC THERAPEUTIC APPROACH THROUGH BLOCKADE OF RENIN ANGIOTENSIN SYSTEM

Despite MHT is a potentially fatal form of hypertension (HT), no study has shown superiority of one therapeutic strategy compared to another one. The pathogenesis of MHT seems to be based on a failure in the usual feedback mechanisms of renin-angiotensin-aldosterone system (RAAs) whereby aldosterone usually causes sodium retention which in turn inhibits renin secretion. ACE-inhibitors, Beta blockers (BB), and angiotensin II receptor blockers (ARBs) inhibit the RAAs at different levels. The place of these drugs in this indication has not been well established. The aims of this study were to assess a standard therapeutic strategy based on the physiopathology of MHT and study evolution of clinical and paraclinical characteristics of a cohort of patients admitted for MHT.

All patients admitted in the unit with diagnosis of MHT (severe HT, hypertensive retinopathy grade III or IV Keith-Wagener-Barker) were treated with the same protocol: 1/ lisinopril 5 mg per os, progressively increased up to 40 mg in the following 72 hours if blood pressure (BP) was not controlled 2/ In case of failure, a BB per os was administered 3/ in third line, we added an ARBs. Central antihypertensive drugs, calcium channel antagonists could be used in fourth line. Diuretics were excluded as possible. Biological, clinical, electrocardiographic characteristics of patients were recorded at admission, and at distance during following-up.

A total of 42 patients were admitted for MHT between 1995 and 2002. 27 men and 15 women, the mean age was 43 ± 11 years. 29 patients were followed up in the unit for a period of 22 ± 21 months. The mean systolic BP decreased from 196 ± 35 to 136 ± 21 mmHg, and the mean diastolic BP decreased from 113 ± 22 to 83 ± 10 mmHg. The serum creatinine levels decreased from 176 ± 120 to 153 ± 64 m mol/L. The mean Sokolow score was 36 ± 13 mm at admission, and 26 ± 10 mm at follow-up. One patient died and one progressed to renal haemodialysis.

The present study shows that the therapeutic strategy applied to treat MHT based on the physiopathology of MHT is well tolerated, permits a decrease in BP, a preservation of renal function, and a reduction of electrocardiographic signs of left ventricular hypertrophy. This strategy seems to be effective in both acute and long-term management of MHT.

Key Words: Malignant Hypertension, Antihypertensive Treatment, Renin-Angiotensin System

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ANGIOTENSIN II POTENTIATES VEGF-INDUCED PROLIFERATION AND NETWORK FORMATION OF ENDOTHELIAL PROGENITOR CELLS
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Background Bone marrow-derived endothelial progenitor cells (EPCs) in the peripheral blood of adult animals and adult humans have been shown to play a role in neovascularization. On the other hand, Angiotensin II (Ang II) plays a role in the development of many vascular diseases.

Purpose: To investigate whether Ang II affects human vascular endothelial growth factor (VEGF)-induced EPCs proliferation and network formation.

Methods and Result: RT-PCR analysis demonstrated that Ang II induced a significant increase of VEGF receptor kinase domain-containing receptor (KDR) mRNA in a dose- and time-dependent manner; the maximal increase, which was 3-fold the control value, occurred after a 4-h stimulation. In addition, flow cytometric analysis revealed that Ang II up-regulated KDR protein expression in human EPCs. Both the angiotensin type 1 (AT1) receptor antagonist (Valsartan: 200 nmol/L) and the PKC inhibitor, bisindolylmaleimide (GFX: 10 μmol/L) reduced Ang II-induced KDR mRNA expression to almost the control level. The culture assay showed that Ang II dose-dependently enhanced VEGF-induced EPC proliferation by activating AT1 receptors, which was also confirmed by the colorimetric MTS assay with the electron coupling reagent mithanisulfate. Finally, a Matrigel assay, EPCs treated with both Ang II and VEGF were shown to be more likely to integrate into the network formation than those treated with VEGF alone.

Conclusions: Our data indicate that Ang II potentiates VEGF-induced human EPCs proliferation and network formation through the up-regulation of KDR.

Key Words: Angiotensin II, Endothelial Progenitor Cell, Vasculogenesis formation

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EFFECT OF ANGIOTENSIN-RECEPTOR BLOCKADE ON REDUCED CEREBROVASCULAR RESERVE AND NEURONAL MASS IN DIABETIC HYPERTENSIVES
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Background. Diabetes and hypertension are both potent risk factors for cerebrovascular disease. We studied the impact of diabetes and the effects of an angiotensin II receptor blockade (ARB) on the functional properties of the cerebral artery and metabolism in hypertensives with or without diabetes.

Subjects. We studied cerebral hemodynamics and metabolism before and 3-4 months after candesartan therapy (12mg/day) in 20 previously untreated asymptomatic hypertensive patients with type II diabetes (DM group: mean age: 69 years; male: 35%) and 20 age- and gender-matched hypertensives without diabetes (non-DM group). Quantitative volume flow in the internal carotid arteries (ICAs) and the middle cerebral arteries (MCAs) was assessed by phase-contrast MR angiography. Cerebrovascular reserve (CVR) was assessed as an increase in volume flow of ICAs and MCAs 10 min after administration of acetazolamide (an
arteriolar dilator). Cerebral metabolites in white matter were measured by proton MR spectroscopy. We also studied the 12 age- and gender-matched normotensives.

Results. Multiple silent cerebral infarcts were more common in the DM group than the non-DM group (50 vs 25%). Baseline ICA and MCA volume flow were comparable between the 2 groups, while CVR in ICAs (25 vs 35%, p=0.03) and MCA’s (20 vs 31%, p=0.01) were lower in the DM group than the non-DM group. Cerebral N-acetylaspartate (NAA: an indicator of functional neuronal mass) was decreased in DM group than in non-DM group (8.35 vs 9.58 mmol/L, p=0.007). These baseline CVR and NAA values of the DM group were lower than those of the normotensive group (CVR: 44% for ICA, 41% for MCA; NAA: 10.5 mmol/L, all p<0.005). After candesartan therapy, CVR in ICAs and MCAs were significantly increased compared with the baseline (p=0.001), while cerebral NAA level did not changed. The CVR increase in ICAs (15 vs 5.7%, p=0.03) and that in MCAs (20 vs 7.3%, p=0.01) were higher in the DM group than in the non-DM group.

Conclusion. In hypertensive patients, silent cerebrovascular disease with reduced CVR and neuronal mass occurs when accompanied by diabetes. ARB partly improved this impaired CVR, indicating that the renin-angiotensin-aldosterone system may play a role in regulating cerebrovascular microcirculation.

Key Words: Diabetic Hypertension, Cerebral Circulation, Angiotensin Receptor Blocker

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ARGinine-AMINopeptidase IN RAT CARDiac FIBroblasts Participates IN Angiotensin TUNOVER


The aim of this study was to elucidate the presence in rat cardiac fibroblasts of Arginine-aminopeptidase and its involvement in the hydrolysis of angiotensin peptides. Peptidase activity was measured as hydrolysis of synthetic substrates, argyl-p-nitroanilides, and the appearance of aryl-p-nitroanilides and the appearance of the peptides in the media. Experiments performed with antibodies to aminopeptidase B. Arg-aminopeptidase found in fibroblasts was arginine- and lysine-specific, sensitive to various aminopeptidase inhibitors and to the inhibitor of metalloproteases. 1,10-phenantroline. Experiments with arphamenine A, a specific inhibitor of arginine-aminopeptidase B, have shown the presence of two Arg-aminopeptidase activities: arphamenine-sensitive: chloride-stimulated aminopeptidase and arphamenine-insensitive: chloride-insensitive aminopeptidase. TGF-beta1 (400 pmol/L) stimulated both Arg-aminopeptidase activities by 3-fold. Immunoblot with an antibody specific to rat aminopeptidase B has revealed that arphamenine-sensitive: chloride-stimulated aminopeptidase is arphamenine B. Arginine-p-nitroanilide hydrolysis was significantly inhibited by angiotensin peptides such as angiotensin (1–10), (1–8), (1–7), (1–4), (5–8), (4–8), (3–8) and (2–8) at a concentration of 50 micromol/L, which was 4-fold less than the Arginine-p-nitroanilide concentration. Our data suggest that chloride-insensitive Arginine-aminopeptidase could contribute to the hydrolysis of all studied angiotensin peptides in concert with other peptidases present in fibroblasts. Some of the peptides could probably not be hydrolyzed by Arginine-aminopeptidase.Instead, they could be firstly hydrolyzed by another peptidase present in fibroblasts and the product of this hydrolysis could be a substrate for Arginine-aminopeptidase.

Key Words: Transforming Growth Factor-beta1, Cardiac Fibroblasts, Angiotensin Turnover

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REVERSAL OF ANGIOTENSIN II-STIMULATED COLLAGEN GEL CONTRACTION IN CARDiac FIBROblasts BY AMINopeptidase-INHIBITION


The purpose of this investigation was to determine whether aminopeptidase (AP)-inhibition could affect the angiotensin II (Ang II)-stimulated collagen gel contraction in basal (control) and TGF-beta1-treated cardiac fibroblasts (or myofibroblasts). The tested AP-inhibitors were the broad range AP-inhibitor benzamil, the specific inhibitor of Alanine-AP leuhistin and the specific inhibitor of Arginine-AP arphamenine A. Cardiac fibroblasts (from normal male adult rats) from passage 2 were cultured to confluence and incubated with(out) 400 pmol/L TGF-beta1 in Dulbecco’s Modified Eagle’s Medium (DMEM) with 10% foetal bovine serum (FBS). These fibroblasts were further incubated in a floating collagen gel lattice with the tested products for 3 days in DMEM without FBS. The contraction of the collagen gel lattice by cardiac fibroblasts was determined by measuring the gel volume with titrator water. Ang II (0.1 microM) reduced the gel volume in control and TGF-beta1-treated fibroblasts. The Ang II-stimulated collagen gel contraction in control and TGF-beta1-treated fibroblasts was completely reversed by leuhistin and arphamenine A (100 microM). Bestatin (100 microM) only partially inhibited the Ang II-stimulated collagen gel contraction in control fibroblasts while it did not affect the Ang II-induced contraction in TGF-beta1-treated fibroblasts. In control and TGF-beta1-treated fibroblasts, 100 microM leuhistin or arphamenine A only partially inhibited Alanine-AP activity, while bestatin (100 microM) completely inhibited the Alanine-AP activity. Arginine-AP activity was only partially inhibited by leuhistin and arphamenine A at 100 microM in control and TGF-beta1-treated fibroblasts. Bestatin (100 microM), however, completely blocked the Arginine-AP activity in control fibroblasts and only partially in TGF-beta1-treated fibroblasts. Our data suggest that both Alanine- and Arginine-AP are involved in the reversal of the Ang II-stimulated collagen gel contraction in control and TGF-beta1-treated cardiac fibroblasts or myofibroblasts.

Key Words: Angiotensin II, Cardiac Fibroblasts, Aminopeptidases

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COLLAGEN PRODUCTION IN CARDiac FIBROblasts DURING INHIBITION OF ANGIOTENSIN CONVERTING ENZYME AND AMINopeptidases


The objective of this study was to determine whether the angiotensin converting enzyme (ACE) inhibitor lisinopril and the aminopeptidase inhibitor with a broad specificity bestatin could affect the collagen production in basal and TGF-beta1-treated cardiac fibroblasts. Cardiac fibroblasts (from normal male adult rats) from passage 2 were cultured to confluence, incubated without) 600 pmol/L TGF-beta2 for 2 days in serum-free Dulbecco’s Modified Eagle’s Medium and further incubated with the tested products (lisinopril or bestatin) for 1 day in this serum-free medium with ascorbic acid, beta-aminopropionitrile and taurine, aminoproprionitrile. Soluble collagen was measured in the conditioned medium and nonsoluble collagen in the cell layer. Lisinopril dose-dependently reduced ACE-activity in basal and TGF-beta1-treated cardiac fibroblasts. Bestatin inhibited the basal and TGF-beta1-stimulated aminopeptidase activity in a concentration dependent manner. Lisinopril (10 microM) decreased (p<0.05) the soluble collagen production from 882±160 (mean±SEM) to 658±142 pmol/mg DNA and the nonsoluble collagen production from 267±65 to 193±45 pmol/mg DNA in basal cardiac