Department of Biochemistry[1] and Department of Cardiac Surgery[2], University of Medicine and Pharmacy, Timisoara, Romania

Renin – angiotensin system inhibitors – implications in the metabolism of the reactive oxygen species

M. Ianosi-Irimie[1], P. Deutsch[2] and G. Deutsch[1]

Renin-angiotensin system (RAS) inhibitors, such as the angiotensin-converting enzyme inhibitors (ACEI) and the selective antagonists of the angiotensin II-1 (AT1) receptor, are used for the treatment of hypertension. Advances in research have shown that their mode of action is complex. It has become clear that angiotensin II induces cellular hypertrophy in vascular smooth muscle cells (VSMC) and cardiac myocytes. [1]. It has also been demonstrated that angiotensin II-induced hypertrophy is associated with intracellular release of reactive oxygen species (ROS) [1]. The RAS has also an important role in the induction of atherosclerosis, process intermediate by an increased ROS production in the intima [2], or by an increased expression of vascular inflammatory genes such as vascular cell adhesion molecule-1 [3].

The importance of the RAS in the modulation of the renal growth is also well known [4]. In a previous study we have argued for the role of RAS in the control of the compensatory kidney hypertrophy development (at the rat) and we have demonstrated that the two ACEI (ramipril and enalapril) and the selective antagonist of the angiotensin II-1 (AT1) receptor (losartan) have an antihypertrophic effect [5]. Our present goal was to determine if the ROS are involved in this process and if the RAS inhibitors may influence the concentration of ROS in the kidney tissue. Therefore, we searched to determine the influence of the ACEI (ramipril and enalapril) and of the selective antagonist of the angiotensin II-1 (AT1) receptor (losartan), on the catalase activity in tissues from kidneys with compensatory hypertrophy (CKHG), compared with the catalase activity in tissues from normal kidneys (at the rat). We made our experiments on a model for the investigation of the kidney hypertrophy developed after the ablation of the opposite kidney [5]. The experiments were made on Sprague-Dowley white rats (males and females) having a standard lab feeding (160 to 240 g weight).

We investigated the kidney mass obtained trough the extirpation made at the occasion of the unilateral nephrectomy (control group – CG –, $n = 11$) and the kidney mass of the restant kidney at 7 days after the unilateral nephrectomy (the group with compensatory kidney hypertrophy – CKHG –, $n = 11$). After the unilateral nephrectomy a first group of animals was treated with losartan (1 mg/kg body weight– LKHG –, $n = 16$), the second group was treated with enalapril (25 mg/kg body weight – EKHG –, $n = 18$) and the third group with ramipril (5 mg/kg body weight RKHG –, $n = 9$).

The experimental surgery was done using Nembutal (35 mg/kg body weight i.p.) for anesthesia. The harvested kidneys were weight, homogenized at 0 °C and the tissues used for the determination of the catalase activity. The catalase activity in the kidney was measured using the Sinha technique, with potassium bichromate [6]. The results were statistically evaluated, using the Student's t-test.

Our results show that the catalase activity was 19.19 ± 3.5 μM H_2O_2/min · g · tis (mean ± S.D.) in the CG and, 28.03 ± 7.18 μM H_2O_2/min · g · tis (mean ± S.D.) – In the CKHG; in the LKHG the catalase activity was 19.67 ± 5.25 μM H_2O_2/min · g · tis (mean ± S.D.); while in the EKHG the catalase activity was 21.7 ± 3.5 μM H_2O_2/min · g · tis (mean ± S.D.) – and in the RKHG 14.8 μM $\pm$ 2.2 H_2O_2/min · g · tis (mean ± S.D.) (Fig.).

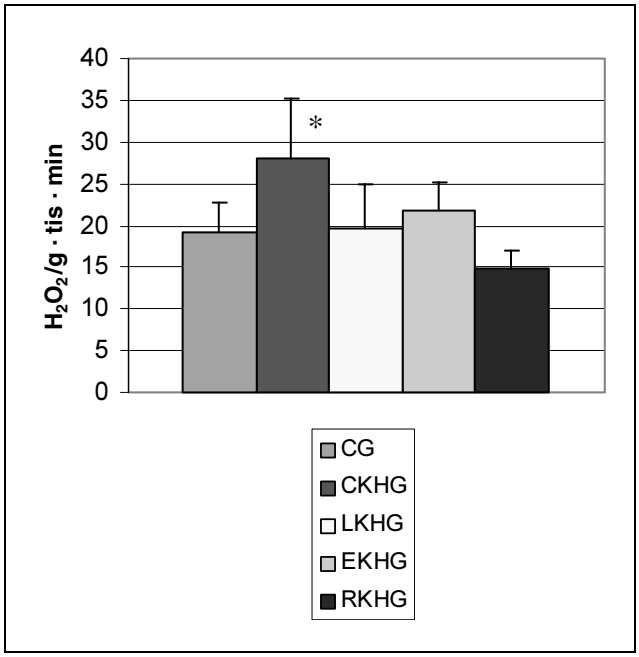

Fig.: The influence of losartan, enalapril and ramipril on the H_2O_2 production in the hypertrophied kydneys at the rat, *$p < 0.01$

Our results demonstrated that the catalase activity was significantly increased in the kidneys with compensatory hypertrophy compared with the control group. Under the ACEI and under losartan the catalase activity decreases; the compensatory kidney growth is also decreased under treatment.

It has recently been demonstrated that in VSMC and cardiac myocytes the angiotensin II – induced hypertrophy is mediated by intracellularly produced ROS, derived at least in part from a membrane-associated NAD(P)H oxidase [1, 7, 8]. ROS are regulating several classes of genes, including the genes for the antioxidant enzymes, such as catalase [1]. Therefore the catalase activity may be regulated by the ROS levels [9].

We conclude that in the compensatory kidney hypertrophy the mechanism of cellular growth could be based on the production of ROS, induced by angiotensin II. The increased catalase activity, we have found, is an answer to the elevated levels of these. In atherosclerosis [2] and in certain forms of hypertension, ROS probably have a similar effect [1], the ACEI and the AT1-receptor antagonists having the same positive influence.

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Received March 14, 2001
Accepted May 3, 2001

Dr. G. Deutsch, Ph.D.
Department of Biochemistry
University of Medicine
and Pharmacy Timisoara
1900 Timisoara, str. Buftea nr.2A,
Romania
gdeutsch28@hotmail.com

Biological Sciences Group, Birla Institute of Technology and Science, Pilani, India

Antioxidant activity of rhamnazin-4′-*O*-β-[apiosyl(1→2)] glucoside in the brain of aged rats

V. PANDE

Rhamnazin-4′-*O*-β-[apiosyl(1→2)] glucoside is a flavonoid drug [1]. We have recently shown its antioxidative activity against tetrachloromethane-induced hepatotoxicity in rats [2]. Ageing of the central nervous system might, partly, be a result of the damage caused by oxygen free radicals and their intermediates [3, 4]. During ageing free-radical generation increases and production of small molecular weight antioxidants becomes insufficient leading to defective disposal of reactive oxygen species (ROS) [5, 6]. This leads to increased oxidative stress on cells and causes a large amount of oxidative damage to vital biomolecules [6, 7]. Also, Alzheimer's disease (AD), which is linked to ageing, may be, in part, due to oxidative stress in the brain [8]. Memory deficits induced by the neurotoxins – colchicines and ibotenic acid, proposed as animal models in AD are, at least, partly due to neurodegeneration induced by oxidative stress injury [9, 10].

Antioxidants are useful for preventing the formation free radicals and the deleterious actions of ROS that damage lipids, DNA and proteins [11].

The present study investigated the effect of rhamnazin-4′-*O*-β-[apiosyl(1→2)] glucoside on the oxidative free radical scavenging enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and on lipid peroxidation (LPO), in order to assess the antioxidant effect of the drug in the brain of aged rats. The study was undertaken to develop the novel drug as an antioxidative therapy against ageing and related disorders like AD.

The Table shows LPO and antioxidant status in the brains of young and aged rats. A significant increase in LPO and significant reductions in the activities of SOD, CAT and GPx were observed in aged rats. These values returned to almost normal on 15 days administration of the drug in aged rats. No significant changes were observed in the brain of young rats.

There are certain merits of brain tissue in investigations of free-radical metabolism in relation to ageing: brain tissue has a relatively high lipid content, consumption of oxygen by the tissue is high and the brain has a relatively weak antioxidant defense system [17].

The high lipid content of neural tissue comprises large amounts of polyunsaturated fatty acids, which are highly susceptible to oxidative attack and this leads to changes in membrane fluidity, permeability and cellular metabolic function [18].

Though, cells can destroy free radicals by coordinated expression and functioning of an array of free-radical-scavenging enzymes including SOD, CAT and GPx, these antioxidant defense systems are not perfect and are subject to alteration during ageing [19]. As a result there is an age-dependant increase in the function of ROS and free radicals escaping these cellular defense systems, which eventually leads to the damage of cellular constituents [5]. So an effective antioxidant agent should be capable of augmenting intracellular concentrations of SOD, CAT and GPx, in finally reducing the lipid peroxidation [20].

The results of the present study show that rhamnazin-4′-*O*-β-[apiosyl(1→2)] glucoside increased SOD, CAT and