

RENIN-ANGIOTENSIN SYSTEM AND LIPID PEROXIDATION

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Summary

Uzunova, V. V., A. N. Tolekova, G. S. Ilieva, K. I. Trifonova & A. P. Logofetov, 2005. Renin-angiotensin system and lipid peroxidation. *Bulg. J. Vet. Med.*, **8**, No 1, 69–75.

The state of renin-angiotensin system (RAS) activation influences the production of reactive oxygen species (ROS). This study aimed to investigate the correlation between plasma renin activity (PRA) and malondialdehyde (MDA) as a marker of lipid peroxidation. The experiment was carried out on male Wistar rats, treated with nifedipine, nicardipine and captopril. After application of all drugs, PRA was significantly increased compared to controls ($P < 0.05$) from the 1st to the 7th hour. Significant differences ($P < 0.05$) between MDA levels in controls and treated groups were found at hours 1, 3 and 5 for all three drugs. Different degrees of positive correlation among studied parameters were established in the groups. All three drugs led to a marked increase in lipid peroxidation. Nifedipine and nicardipine were expected to have such an effect because they cause accumulation of angiotensin II (Ang II), which is known to have a prooxidative action. Captopril however is supposed to have antioxidant effect because it lowers angiotensin II. The increase in MDA observed in this study could be linked to the "effect of escape" of the blockade.

Key words: captopril, lipid peroxidation, MDA, nicardipine, nifedipine, RAS

INTRODUCTION

Angiotensin II (Ang II), the major effector of renin-angiotensin system (RAS) is an important mediator of endothelial and vascular smooth muscle cells (De Gasparo, 2002). There is increasing evidence that Ang II increases the generation of reactive oxygen species (ROS) in the vessel walls and in the blood through activation of angiotensin I receptors, mainly via membrane bound NADH/NADPH oxidase (Seshiah *et al.*, 2002). Treatment of vascular smooth muscle cells with Ang II for 4 hours increases ROS production through membrane bound NADH and NADPH-driven oxidase (Ishiki *et al.*,

2001). Furthermore, Ang II increases redox-sensitive and proinflammatory genes such as vascular cell adhesion molecule-1 and intercellular adhesion molecule, which play a critical central role in the initiation and progression of atherosclerosis (Diep *et al.*, 2002). The overproduction of ROS plays an important role and contributes to the pathogenesis of hypertension, endothelial dysfunction, cardiovascular and renal diseases (Schnackenberg *et al.*, 1998; Gadjeva *et al.*, 2000).

The main groups of antihypertensive drugs, acting via different mechanisms, also influence the redox state of the orga-

nism. The reported data about their effects are however insufficient.

This study aimed to investigate the correlation of the state of activity of RAS, influenced by some of the antihypertensive drugs and the oxidative status. In order to do this we evaluated malondialdehyde (MDA) as a major product of lipid peroxidation in blood. The tested drugs were two calcium-channel blockers (CCBs): nifedipine and nicardipine and the angiotensin-converting enzyme (ACE) inhibitor captopril.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 250–300 g were maintained in groups under a 12-hour light/dark cycle (lights on from 7 AM to 7 PM) and temperature-controlled conditions (22 ± 2 °C). One animal was housed in each metabolic cage. The animals were fed standard laboratory animal chow. Rats had free access to tap water. The experiments started after a 3 days adaptation period and were performed at the same time of the day: at 8.00 AM.

Drugs

Nifedipine and nicardipine (St. Louis, MO) were dissolved in polyethylene glycol M400 (Fluka Chemie, AG, Switzerland). Captopril (St. Louis, MO) was dissolved in water. The drugs were introduced orally through a gastric tube once at 8.00 AM.

Experimental design

The experiment was performed on 157 rats. They were divided into 3 experimental and one control groups. The experimental groups were given nifedipine at 20 mg/kg (Carmines *et al.*, 1992) (group I; $n=69$); nicardipine at 20 mg/kg (Kaw-

shima *et al.*, 1991) (group II; $n=35$) or captopril at 30 mg/kg (Katayama *et al.*, 1989) (group III; $n=35$). The control group ($n=18$) was subdivided into 2 subgroups: 10 intact animals and 8 – treated with polyethylene glycol at 2 mL/kg. Because of the lack of significant differences between the results of the two types of controls, they were combined into one group.

Blood samples were collected from the heart by post treatment hours 0.5, 1, 3, 5, 7, 9 and 11 under ether anaesthesia in tubes, containing Na_2 -ethylenediamine tetraacetic acid. The samples were kept according to the requirements of appropriate method of plasma renin activity (PRA) determination. It was assayed radioimmunologically with commercial kits (Sorin Biomedica, Italy). PRA was calculated as nanogrammes of angiotensin I generated per mL per hour (ng/mL/h) (Malvano *et al.*, 1972).

Lipid peroxidation was assessed by a simple and sensitive method, based on the reaction of the thiobarbituric acid with MDA. The concentration of total thiobarbituric acid reactive substances (TBARS) in whole blood was estimated by a spectrophotometric assay (Plaser & Cushman, 1966, Stoyanova & Alexiev, 1997; Georgieva & Gadjeva, 2002).

Calculations

The total areas under the PRA (AUC_{PRA}) and MDA (AUC_{MDA}) curves were obtained by integration of values time courses from hours 0 to 11 hour. The effects of drugs were compared by means of a coefficient (C) which represents the $\text{AUC}_{\text{PRA}}/\text{AUC}_{\text{MDA}}$ ratio. The reached maximum PRA values were obtained by interpolation of experimental data using the computer program KORELIA-DYNAMICS (Yankov, 1998a; 1998b).

Statistical analyses

All data were expressed as mean $\pm$ SD. Unpaired comparisons using Student's *t* test were used to determine the significant differences among groups. Differences at $P<0.05$ were considered as statistically significant. The correlation analysis was done by the Pearson's *r* coefficient.

RESULTS

Effect of captopril on PRA and MDA

After application of captopril, blood PRA showed a significant increase vs the control group by the 1st ($P<0.05$), 3rd ($P<0.001$), 5th ($P<0.001$) and 7th ($P<0.01$) hour (Fig. 1A,a). The calculated highest value was reached by hour 3.3 (25.4 ± 2.4 ng/mL/h). The changes in MDA were less significant. Significant differences were found by the 1st ($P<0.001$), 3rd ($P<0.001$) and 5th ($P<0.05$) hour (Fig. 1A,b). The correlation between PRA and MDA was very high ($r=0.92$, $P<0.05$). The C coefficient was found to be 9.31.

Effect of nifedipine on PRA and MDA

The blockade of L-type calcium channels produced a significant ($P<0.05$) increase in PRA vs controls from the 30th min to 5th ($P<0.001$) and at the 7th ($P<0.01$) hour (Fig. 1B,a). The calculated maximum response to nifedipine was reached by hour 1.32 (51.9 ± 8.5 ng/mL/h). The respective differences in MDA values were significant at the 1st ($P<0.001$), 3rd ($P<0.001$), and 5th ($P<0.01$) hour (Fig. 1B,b). The degree of correlation between both studied parameters was high ($r=0.77$, $P<0.05$). The C coefficient was 14.6.

Effect of nicardipine on PRA and MDA

The treatment with nicardipine induced elevation in PRA depending on the inter-

val between the application and blood collection. The differences with the control group were significant between the 30th minute and the 7th hour ($P<0.001$) (Fig. 1C,a). The calculated highest value was reached by hour 2.31 (47.8 ± 8.1 ng/mL/h). The tendency of MDA changes was the same as in the two other groups. Significant differences were detected at the 1st ($P<0.05$), 3rd ($P<0.01$) and 5th ($P<0.05$) hour (Fig. 1C,b). The correlation between PRA and MDA was high ($r=0.83$, $P<0.05$). The C coefficient was 16.7.

DISCUSSION

All three used drugs are expected to cause a rise in PRA. The mechanisms underlying the stimulation of renin secretion by CCB are probably multifactorial. They involve the direct effects on the level of the renin-secreting juxtaglomerular cells and systemic effects such as fall of blood pressure (Churchill, 1990; Epstein & Loutzenhiser, 1992; Schriker *et al.*, 1996). Captopril causes elevation in PRA preventing generation of Ang II and its inhibitory effect on renin production.

ACE-inhibitors and CCBs have different mechanisms of action which explains the difference in obtained responses. Captopril influences the last segment of RAS disturbing the negative feedback that exists between Ang II and renin production. The maximal increase in PRA after application of captopril is about three times the control value and it is reached at the 3.3 hour. Nifedipine and nicardipine on the other hand act upon the first component of RAS – the renin producing cell. The rise in PRA is therefore greater and faster.

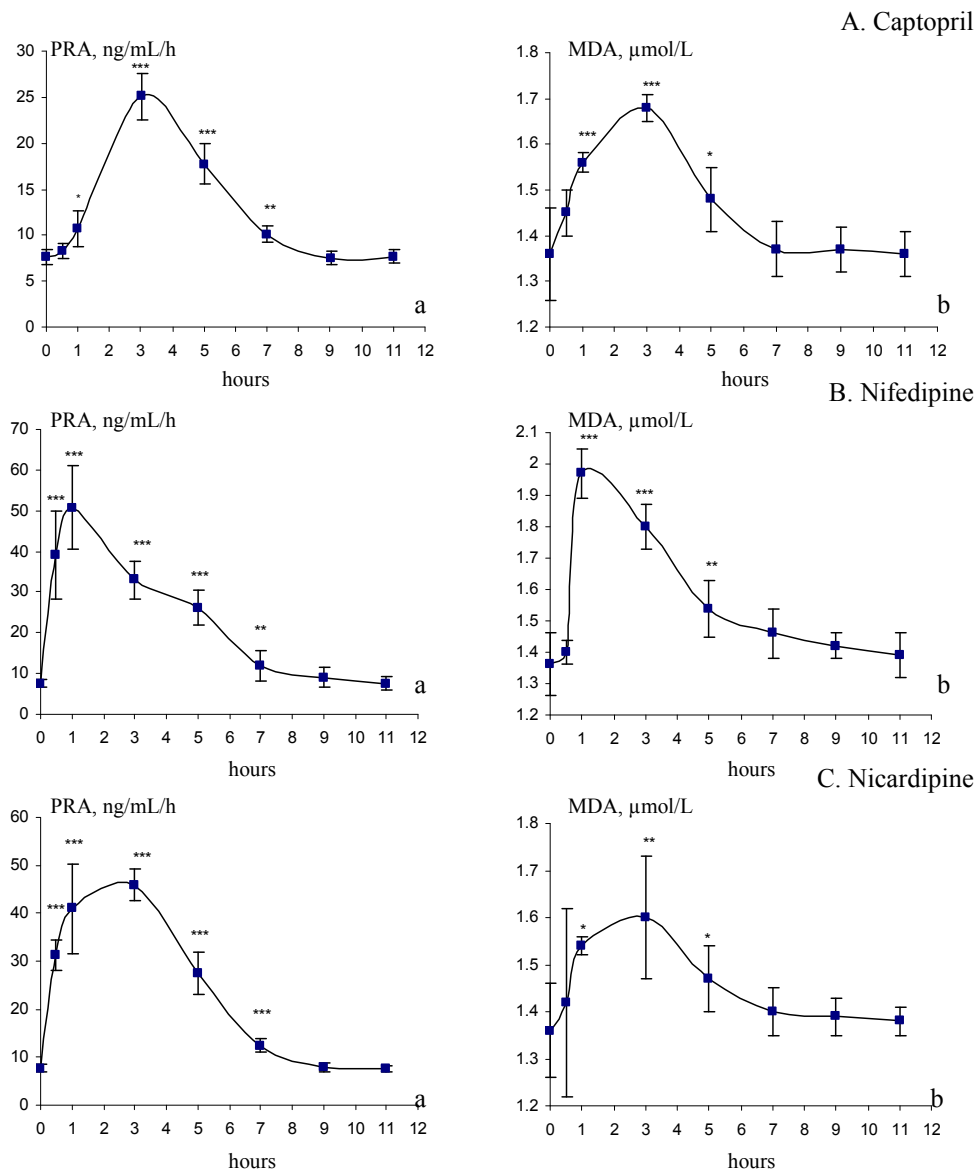


Fig. 1. The effects of treatment with captopril at 30 mg/kg (A); nifedipine at 20 mg/kg (B) and nicardipine at 20 mg/kg (C) on PRA [ng/mL/h] (a) and MDA [μ mol/L] (b) in rats. The values are presented as mean $\pm$ SD. *P<0.05, **P<0.01, ***P<0.001 vs controls.

The opinions about the effects of these drugs on lipid peroxidation are less definite. Captopril has a widely known anti-oxidative effect (Pepine & Handberg, 2001). Some authors believe that nifedipine

has no effect on ROS formation (Cominacini *et al.*, 2003). Others have found that nifedipine directly interacts with the polymorphonuclear leucocytes by inducing a marked decrease in plasma membrane

fluidity and an inhibition of the oxidative burst (Grassi *et al.*, 1995). It is known that nifedipine, can inhibit phagocyte oxidative burst in mice (Mohan *et al.*, 1993). Nicardipine is rarely discussed.

The rise in MDA observed by us shows that all three drugs cause a marked increase in lipid peroxidation. CCB were expected to have such an effect because they cause accumulation of Ang II as a result of increased secretion of renin (Churchill, 1990; Epstein & Loutzenhiser, 1992; Schricker *et al.*, 1996). It is known that Ang II has a prooxidative action (Seshian *et al.*, 2002). Captopril however is supposed to have an antioxidant effect because it lowers Ang II (Omata *et al.*, 1996). The increase in MDA found by us could be attributed to the "effect of escape" of the blockade that occurs in the first few hours after the application (Borghi *et al.*, 1993).

Our experiment has shown that after application of both types of drugs, the increased PRA correlated strongly with the increase of MDA and thus, with increased lipid peroxidation in erythrocytes.

We propose the following explanation of the relationship between the rises in PRA and MDA. The origin of MDA could be attributed to Ang II. The elevation of PRA leads to increased formation of Ang II in blood. Human leukocytes and endothelial cells have Ang II generating activities and angiotensin I receptors (Richards *et al.*, 1998; Fukuda *et al.*, 2003). Stimulation of Ang II formation in blood and into the endothelial cells and macrophages activate angiotensin I receptors (Singh & Mehta, 2001.). This leads to the generation of ROS through activation of membrane bound NADH/NADPH oxidase and/or redox-sensitive and proinflammatory genes. Increased quantities of ROS lead to

augmentation of lipid peroxidation. MDA in plasma is indicator of this event.

Besides their role in oxygen transport and buffering systems, erythrocytes have been lately shown to play a significant role in systemic defense against oxidative damage. It is found that erythrocytes are very extensive and efficient systems for the removal and detoxification of free radicals. As a consequence of this function the liable components of individual erythrocytes suffer oxidative damage (Richards *et al.*, 1998). This way erythrocytes could also be a source of MDA regardless of the fact that Ang II does not directly influence them.

The AUC of each parameter represents the overall change it undergoes in the course of the experiment. AUC_{MDA} is relatively the same for all drugs. AUC_{PRA} however shows considerable differences among groups. It can be deduced that the same disturbance in MDA appears simultaneously with very different changes in PRA when different agents are applied.

The bigger the value of C, the better response of the system to oxidative stress is. It indicates that for the particular drug the numerator (the overall change in PRA) can reach greater values producing minimal change in MDA. C had the biggest value after application of nicardipine. It was very closely followed by the C of nifedipine. The coefficient C of captopril however was significantly lower.

A possible explanation could again be found in the mechanism of action of both types of drugs. They increase Ang II through different mechanisms (Churchill, 1990; Borghi *et al.*, 1993). The second messenger of Ang II is increase in intracellular calcium. However, in the case of CCB, there is a concomitant lowering of intracellular calcium. The net change in calcium concentration should therefore be

lesser and its effects – less pronounced. The anticipated rise in lipid peroxidation in a cell which is simultaneously influenced by Ang II and a CCB should be smaller.

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Paper received 15.05.2003; accepted for publication 23.11. 2004

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