Plzeň. lék. Sborn., 79, 2013: 43–48

POSSIBLE PROOXIDANT EFFECT OF ANGIOTENSIN CONVERTING ENZYME INHIBITORS AND ANGIOTENSIN II TYPE 1 RECEPTOR (AT1) ANTAGONISTS IN NEURAL TISSUE

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Angiotensin converting enzyme (ACE) inhibitors and angiotensin II type 1 receptor (AT_1) antagonists (ARBs, sartans) are widely used for treatment of cardiovascular diseases such as essential hypertension and chronic heart failure. Many studies show they also have a potent ability to protect tissues against damage associated with oxidative stress such as ischemia/reperfusion injury, metal intoxication, inflammation, intracerebral haemorrhage or neurotoxin induced oxidative stress (4, 9, 12, 13, 17, 20). As the important role of angiotensin in brain (in ischemic stroke, neurogenesis or cognition) became obvious and because many ACE inhibitors and angiotensin II receptor blockers can cross the blood-brain barrier, their possible central effects have been widely studied (3, 5, 16, 25). Some of the protective effects of ACE inhibitors and angiotensin II receptor blockers can be mediated by their antioxidant effects. These effects were observed not only in ACE inhibitors containing sulfhydryl group (-SH) such as captopril (Fig. 1) but also in other ACE inhibitors and angiotensin II receptor blockers (1, 14, 15, 18). This can be explained partially by the role of reactive oxygen species in signaling by angiotensin II as was proven in vascular cells and in neural tissue (6, 24). Angiotensin II activates NADPH-dependent oxidases and so stimulates intracellular production of superoxide (7, 24).

The present study was designed to investigate the effects of short term oral administration of ACE inhibitor captopril and angiotensin II type 1 receptor (AT_1) antagonists losartan and telmisartan on parameters of liver and brain oxidative state of adult male rats.

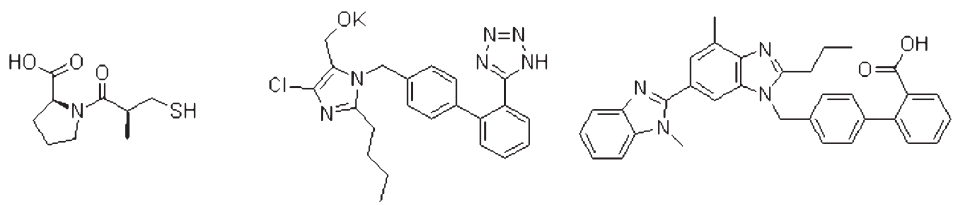

Fig. 1 Molecular structures of captopril, losartan potassium and telmisartan. Freely accessible on http://www.chemblink.com

MATERIAL AND METHODS

Chemicals

The chemicals used in the experiment were of analytical grade. Captopril and telmisartan were purchased from Sigma, losartan potassium from Fluka.

Animals and experimental design

Male Wistar rats (190 ± 10 g Anlab Prague, CZ) were used for the experiment. The animals were kept at 22–24 °C with 12 h light/dark cycle and free access to standard pellet diet and drinking water. After being acclimatized for 7 days, animals were divided into four groups of 8 rats each. Substances were given dissolved/suspended in 0.5% methylcellulose via gastric gavage (0.5 ml / 100 g body weight) once daily for three days. The test groups received: captopril 50 mg/kg, losartan 20 mg/kg or telmisartan 10 mg/kg body weight per day. The control group received an equivalent volume of the vehicle. Twenty-four hours after the last dose, rats were sacrificed and liver and brain samples were excised, rinsed in ice-cold saline and used immediately or stored frozen at −70 °C until analysed.

Biochemical Assays

Lipid peroxidation was measured as malondialdehyde (MDA) production formed in the thiobarbituric acid reaction in tissue homogenates (22). Reduced glutathione (GSH) level was estimated in the homogenates using 5,5′-dithiobis(2-nitrobenzoic acid) (19). Glutathione peroxidase (GPx) activity was estimated in tissue homogenates by a coupled test system, in which glutathione reductase is employed for the regeneration of GSH and butylhydroperoxide is used as the acceptor substrate (8). Glutathione reductase (GR) activity was measured using a commercial kit (Glutathione reductase Assay Kit, Sigma®).

Statistical Analysis

The statistical significance of the differences between the groups was determined by unpaired Student's *t*-test after ascertaining the homogeneity of variance between treatment groups. The data are presented as mean ± SD values. Significant difference: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

RESULTS

Administration of captopril as well as losartan and telmisartan significantly increased lipid peroxidation in brain tissue ($P < 0.01$, Fig. 2).

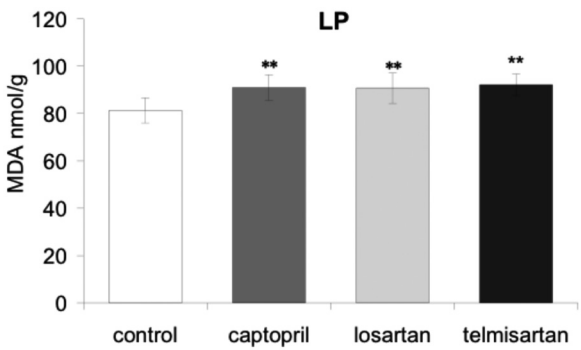

Fig. 2 The effect of captopril, losartan and telmisartan on the level of lipid peroxidation in rat brain

There were no significant changes of brain GSH content or glutathione peroxidase and glutathione reductase activities (Tab. 1). Losartan decreased the activity of glutathione peroxidase ($P < 0.01$) and both losartan and telmisartan decreased glutathione reductase activity ($P < 0.001$; $P < 0.05$) in liver tissue (Tab. 2). There were no significant changes in liver lipid peroxidation after administration of captopril or any of the AT_1 receptor blockers. Telmisartan slightly increased the content of liver glutathione ($P < 0.05$) and the activity of glutathione peroxidase in brain ($P < 0.05$), but neither captopril nor losartan had such effect (Tab. 1, 2).

Tab. 1 The effect of captopril, losartan and telmisartan on the level of reduced glutathione and the activity of glutathione peroxidase and glutathione reductase in rat brain

BRAIN	GSH µmol/g	GPx µmol/g/min	GR U/g
control	1.25 ± 0.18	1.02 ± 0.07	0.82 ± 0.06
captopril	1.13 ± 0.13	1.07 ± 0.14	0.81 ± 0.04
losartan	1.23 ± 0.04	1.04 ± 0.14	0.78 ± 0.03
telmisartan	1.32 ± 0.06	1.13 ± 0.12*	0.84 ± 0.05

Tab. 2 The effect of captopril, losartan and telmisartan on the level of lipid peroxidation, reduced glutathione and the activity of glutathione peroxidase and glutathione reductase in rat liver

LIVER	LP MDA nmol/g	GSH µmol/g	GPx µmol/g/min	GR U/g
control	34.7 ± 6,0	5.03 ± 0.51	20.1 ± 1.4	6.53 ± 0.41
captopril	37.1 ± 3,7	5.07 ± 0.38	19.9 ± 2.0	6.38 ± 0.73
losartan	31.5 ± 6.1	5.10 ± 0.25	18.1 ± 0.9**	5.64 ± 0.23***
telmisartan	36.6 ± 4.1	5.59 ± 0.21*	18.8 ± 2.1	5.98 ± 0.45*

DISCUSSION

Contrary to previous findings of other authors, our experiments show that ACE inhibitors and AT_1 receptor blockers may have some prooxidant activity, mainly in neural tissue. The prooxidant effect can be partly caused by the dosage we used, as some other antioxidants we have previously used also showed biphasic antioxidant and prooxidant effects at low and high concentrations (unpublished results). The fact, that the antioxidant effect of AT_1 receptor blockers can be closely related to the chosen dose, was seen in a study with telmisartan in mice: A high dose of telmisartan showed an attenuated antioxidative effect in brain in comparison to a low dose of telmisartan (23).

The observed prooxidant effect can be also partly caused by the length of the treatment. From our not yet published results we suggest, that with more prolonged treatment

the neural prooxidant effects would diminish. The ability of ACE inhibitors to have opposite effects in brain after chronic administration compared to the effects after acute administration has been already proved. Several studies showed, that acute administration of ACE inhibitors inhibited dopaminergic neurotransmission but the chronic administration induced a significant increase of dopamine content in brain (10, 11, 13, 21). As for the decreased activity of the antioxidant enzymes after the administration of AT_1 receptor blockers, we observed similar effect of an antioxidant on the activity of glutathione peroxidase in our previous experiments with lipoic acid (2).

SUMMARY

Angiotensin converting enzyme (ACE) inhibitors and angiotensin II type 1 receptor (AT_1) antagonists are widely used for treatment of cardiovascular diseases. Many findings suggest they also have a potent ability to protect tissues against the damage associated with oxidative stress. The present study was designed to investigate the effects of short term oral administration of an ACE inhibitor and (AT_1) antagonists on patameters of liver and brain oxidative state of adult male rats.

Male Wistar rats were given captoril (50 mg/kg), losartan (20 mg/kg), or telmisartan (10 mg/kg). orally once daily for 3 days. On the fourth day, the experiment was terminated and liver and brain samples were taken for the estimation of parameters of oxidative state. Contrary to some previous results, our experiments show ACE inhibitors and sartans may have some prooxidant activity, mainly in neural tissue. Administration of captopril as well as losartan and telmisartan significantly increased lipid peroxidation in brain tissue ($P < 0.01$). however there were no significant changes in liver lipid peroxidation.

The ability of ACE inhibitors or AT_1 receptor blockers to increase lipid peroxidation in brain after short term oral administration has not been described in the literature yet and further studies could be useful.

Acknowledgements

Supported by the research grant MSM 0021620819 of the Ministry of Education of the Czech Republic.

Možné prooxidační účinky angiotensin konvertujícího enzymu (ACE) a antagonistů receptoru pro angiotensin II (typu AT_1) v nevové tkáni

SOUHRN

Inhibitory angiotensin konvertujícího enzymu (ACE) a antagonisté receptoru pro angiotensin II (typu AT_1) se široce používají v léčbě kardiovaskulárních onemocnění. Řada studií také ukázala, že mohou mít významnou schopnost chránit tkáně před

poškozením souvisejícím s oxidačním stresem. Tento pokus byl zaměřen na zjištění účinků krátkodobého podávání inhibitoru ACE a blokátorů receptoru AT_1 na parametry oxidačního stresu u dospělých samců potkanů.

Potkani kmene Wistar dostávali kaptoril (50 mg/kg), losartan (20 mg/kg) nebo telmisartan (10 mg/kg) perorálně jedenkrát denně po dobu 3 dnů. Čtvrtý den byl pokus ukončen a byly odebrané vzorky jaterní a mozkové tkáně pro stanovení parametrů oxidačního stresu. V protikladu k některým předchozím zjištěním experiment ukázal, že ACE inhibitory a blokátory receptoru AT_1 mohou mít také prooxidační účinky, a to především v nervové tkáni. Podání jak kaptoprilu, tak losartanu a telmisartanu významně navyšovalo hladinu peroxidace lipidů v mozku ($P < 0,01$), nezaznamenali jsme však změny v peroxidaci lipidů v játrech.

Schopnost ACE inhibitorů nebo AT_1 blokátorů zvyšovat po krátkododém podávání peroxidaci lipidů v mozku nebyla zatím popsána a bylo by vhodné se tímto jevem dále zabývat.

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