The goal of the study was to monitor the antioxidative effect of stobadine derivative in the conditions of ischemia-reperfusion of laboratory rat kidney tissue. The animals were divided by random selection into 5 groups (n = 10). The treated groups were given stobadine derivative in peroral doses of 5, 10 and 20 mg/kg in 0.5% solution of Avicel once a day; the placebo group was given only the solution of Avicel. The last group was an intact group (without ischemia-reperfusion and without treatment). After conclusion of medication on the 15th day all animals were subjected to kidney tissue ischemia (60 min.) followed by reperfusion (10 min.). All animals were subsequently exsanguined and single identification of superoxide dismutase, glutathion peroxidase, total antioxidative capacity, and malondialdehyde level in the blood were determined. Kidneys were recovered for histopathological examination. A statistically significant decrease of the superoxide dismutase and statistically significant increase of the glutathione peroxidase catalytic activity in the treated groups compared to the groups of placebo and intact was discovered. There was also a statistically highly significant increase of total antioxidative capacity in the treated groups compared to the groups of placebo and intact. A statistically significant decrease of malondialdehyde level was identified in the treated groups compared to the groups of placebo and intact. The results of biochemical examination show a protective antioxidative effect of stobadine derivative. The results of histopathological examination support this assumption.

INTRODUCTION

Stobadine, (-) cis-2,8-dimethyl-2,3,4,4a,5,9b-hexahydro-1H-pyrido[4,3b]indole was developed in the Institute of Experimental Pharmacology of the Slovak Academy of Sciences (Bratislava) and synthesized in cooperation with the Institute of Organic Chemistry and Biochemistry of the former Czechoslovak Academy of Sciences. It is a pyridoindole derivative with a cardioprotective, anti-hypoxic and anti-arrythmical effect.

Acylderivative of stobadine – (heptanoyl) stobadine – was prepared by the reaction of stobadine base with heptanoyl chloride. The applied substance was in an oxalate form. In the preceding in vitro studies the stobadine acyl-derivate proved a remarkable antioxidant activity.

The objective of the study was to analyse the dependence of the antioxidative effect of the applied dose of stobadine derivative during prophylactic administration in the conditions of ischemia-reperfusion of kidney in the laboratory rat.
and watered ad libitum. After 10 days of acclimation, the animals were randomly divided into 5 groups. The tested compound was administered to three groups of treated animals (n = 10) at concentrations of 5 mg/kg, 10 mg/kg, and 20 mg/kg in 0.5 % Avicel solution perorally once a day. The fourth group (n = 10) – the placebo group – was given only 0.5 % Avicel solution in the quantity and by the mode of administration used in the treated groups. The fifth group of animals, the intact one (n = 10), was without any medication. After the discontinuation of medication on day 15, laparotomy in general anaesthesia (2 % Rometar 0.5 ml + 1 % Narkamon 10 ml, dose 0.5 ml solution/100 g of the rat body mass) was performed, renal ischemia was induced by clamping the left renal artery with a vascular clamp for 60 min with subsequent 10 min renal reperfusion. After the termination of reperfusion, the animals were exsanguinated by blood collection from the left ventricle and selected laboratory parameters were analysed – superoxide dismutase (SOD), glutathion peroxidase (GSHPx), total antioxidative capacity (AOC) using RANDOX testing kits (Dublin, Ireland), in COBAS MIRA S automatic analyser, and malondialdehyde (MDA) was analysed spectrophotometrically using the TBARs method. The samples of the reperfused kidney tissue were employed for histopathological examination. After the fixation in neutral 10 % formaldehyde, they were stained with hematoxylin-eosin and examined under an optical microscope. Evaluation principle: all samples in the material were evaluated and scored separately in 3 kidney topicalities, the result was added up and in the end the average score of each medicated group was stated. Scoring schedule: 1st topicality – kidney medulla – the grade of tissue destruction through bleeding (according to the extent) and presence of inflammatory infiltrate (max. ++) were evaluated. 2nd topicality – cortex and glomerules – both extravasates in the glomerule (max. ++) and presence of hemorrhages and increased cellularity and extravasates in the glomerule (max. ++) were evaluated. 3rd topicality – kidney channels – presence of regressive changes of epithelia from edema to necrosis was evaluated (+ in the case of necrosis and ++ in the case of regression not reaching the grade of necrosis). In addition, the channel content was evaluated (protein and hyaline cylinders +). The highest possible (worst) result per one sample was 7 (7 times +).

The obtained values of the studied laboratory parameters were processed by the Microsoft Excel table processor and statistically interpreted using a non-pair T-test. The value p ≤ 0.05 was considered significant.

**RESULTS**

The results of laboratory analysis are given in Table 1. A statistically highly significant decrease of SOD values (p ≤ 0.05, p ≤ 0.01) was detected in the groups treated with stobadine derivative at the doses of 5, and 10 mg/kg, compared with the placebo group. Further, a statistically highly significant decrease of SOD values (p ≤ 0.05, p ≤ 0.01) was found in the group treated with stobadine derivative at 20 mg/kg compared with the intact animal group. Mutual comparison of the SOD values received from the animal groups treated with stobadine derivative at the doses of 10 and 20 mg/kg showed a significant difference (p ≤ 0.05).

A statistically significant increase in GSHPx values (p ≤ 0.05, p ≤ 0.01) was detected in the groups treated with stobadine derivative at the doses of 5 and 10 mg/kg, compared with the placebo group. Further, a statistically significant difference in GSHPx values (p ≤ 0.01) was found in the group treated with stobadine derivative at

<table>
<thead>
<tr>
<th>Group of animals (n = 10)</th>
<th>SOD (U/ml)</th>
<th>GSHPx (µkat/l)</th>
<th>AOC (mmol/l)</th>
<th>MDA (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated (5 mg/kg of heptanoylstobadine)</td>
<td>210.50 ± 35.36 *</td>
<td>1045.87 ± 84.07 * ++</td>
<td>0.75 ± 0.22 *</td>
<td>4.17 ± 0.97 ** ++</td>
</tr>
<tr>
<td>Treated (10 mg/kg of heptanoylstobadine)</td>
<td>212.67 ± 16.79 ** +</td>
<td>1035.63 ± 36.67 ** ++</td>
<td>0.62 ± 0.04 ** +</td>
<td>4.19 ± 0.84 ** ++</td>
</tr>
<tr>
<td>Treated (20 mg/kg of heptanoylstobadine)</td>
<td>245.77 ± 29.56 ++</td>
<td>989.49 ± 60.68 ++</td>
<td>0.71 ± 0.12 **</td>
<td>4.07 ± 0.93 ** ++</td>
</tr>
<tr>
<td>Placebo group</td>
<td>253.54 ± 20.33 • •</td>
<td>962.43 ± 36.15 • •</td>
<td>0.45 ± 0.07 • •</td>
<td>14.06 ± 1.07 • •</td>
</tr>
<tr>
<td>Intact group</td>
<td>182.94 ± 19.81</td>
<td>829.10 ± 75.69</td>
<td>0.68 ± 0.04</td>
<td>2.58 ± 0.33</td>
</tr>
</tbody>
</table>

* p ≤ 0.05 treated vs placebo group, ** p ≤ 0.01 treated vs placebo group, + p ≤ 0.05 treated vs intact group, ++ p ≤ 0.01 treated vs intact group, • p ≤ 0.05 placebo vs intact group, •• p ≤ 0.01 placebo vs intact group

Influence of antioxidant effect of stobadine derivative in condition of kidney ischemia-reperfusion
in a pre-clinical experiment (effect in prophylaxis)

the doses of 5, 10 and 20 mg/kg in comparison with
the intact animal group. Mutual comparison of the GSHPx
values received from the animal groups treated with dif-
ferent doses of stobadine derivative showed no significant
difference.

A statistically significant increase of AOC values
(p ≤ 0.05, p ≤ 0.01) was detected in the groups treated
with stobadine derivative at the doses of 5, 10, and
20 mg/kg, compared with the placebo group. Further, a
statistically significant change of AOC values (p ≤ 0.05)
was found in the groups treated with stobadine derivative
at the dose 10 mg/kg, compared with the intact animal
group. Mutual comparison of the AOC values received
from the animal groups treated with different doses of
stobadine derivative showed no significant difference.

A statistically highly significant decrease of MDA val-
ues (p ≤ 0.01) was detected in the groups treated with
stobadine derivative at the doses of 5, 10, and 20 mg/kg,
compared with the placebo and intact group. Mutual
comparison of the MDA values received from the animal
groups treated with different doses of stobadine derivative
showed no significant difference.

Comparison of the values obtained from the placebo
group and intact animal group showed significant changes
of the all biochemical parameters.

Results of histopathological examination are as fol-
loows: In the placebo group, massive hemorrhage in
the interstitium was observed in all samples, especially on
the cortex and medulla boundaries. Hemorrhage also occurred
in the glomerular area (Bowman’s capsule and capillary
convolution) as well as in the medulla. The channels had
regressively changed epithelia from the simple edema to
epithelium necrosis with all the above described features.
In the lumina there was mostly a proteinic content with
hyaline cylinder formation. The accompanying edema and
the generally increased cellularity of the glomerules were
inflammatory reactive. The more marked inflammatory in-
filtrate was smaller than in the treated groups in the form
of sporadic lymphocytes with rare polymorphs. The total
average score was oscillating from 5 to 7. In the intact
treatment group, hemorrhage occurred only accidentally,
most probably caused by contusion. In the treated animal
groups, the best protective effect was detected at the dose
of 5 mg/kg (score 6.33).

DISCUSSION

The statistically significant changes of SOD and
GSHPx levels found in the treated groups confirm the
readiness to the destruction of superoxide, disposal of
hydrogen peroxide and other free radicals causing injury
to the reperfusion sustained kidney tissue. It is supposed
that this is a result of the previous preventive supplemen-
tation of the animal group with a compound having the
in vitro evidenced antioxidative effect. The analysed en-
yzmes act intracellularly and their activity mostly follows
one after another. It may be supposed that their activity
can change in accordance with the state of organism, or
in relation to the on-going pathological processes. The
test results demonstrate that the mutual compensation
mechanisms formed by the effect coordination of more
enzymes can be potentiated by the presence of adminis-
tered antioxidants.

Individual authors greatly differ in their views on the
change of SOD and GSHPx activity caused by the declining
function of kidneys. Literature references include both
the increasing SOD activity2 dependent on the declining
function of kidneys and the detection of the reduced SOD
and GSHPx activity3 as well as the normal one4. For bet-
ter assessment of the existing problem it would be more
suitable to determine the erythrocyte catalase activity in
experimental animals, which is sometimes reduced in pa-
ients with declining function of kidneys5, and the level of
selenium, which has antioxidative effects, forms a part of
GSHPx, and the deficit of which is frequently diagnosed
in patients with renal failure6.

Statistically highly significant increase of AOC values
in the treated animal groups was recorded in comparison
with the values of the placebo group and also of the intact
animal group. It is a significant difference and it may be
supposed that this is another logical result of the previ-
ous supplementation with a compound with antioxidative
effect which was first induced by the lowest dose of stoba-
dine derivative. On the other hand, one of the causes of
the AOC increase can also be an increased level of uric
acid7. The uric acid should be considered to be not only
a nitrogenous metabolite of purine compounds but it also
has significant antioxidative effects. The authors differ in
their views on the AOC changes due to possibly declining
function of kidneys8,9.

The results of statistical comparison of the MDA val-
ues demonstrate significant changes at the statistical sig-
ificance level (p ≤ 0.01); in the treated animal groups, a
statistically significantly lower average value of this toxic
by-product of lipid peroxidation was found, compared
with the control placebo group. Comparing the results
of the performed studies, many authors agree on the in-
creased MDA concentration in plasma or in erythrocytes7
in patients with renal failure; however, the cause can be
not only its increased formation from lipid peroxides but
also its reduced renal elimination6. The MDA can subse-
quently modify the proteins and lead to similar changes
which can be observed during their glycation6,9.

The positive effect of the antioxidant administration in
the conditions connected with ischemia and subsequent
reperfusion of kidney tissue in relation to the improve-
ment of the values of the antioxidative system indicators
is also discussed in other studies10,11.

In our study, the potential protective effect of sto-
badine derivative is demonstrated in the prophylaxis of
ischemic-reperfusion injury to kidney in the laboratory
rat. The assumption is supported by the results of the
evaluation of histopathological findings in the examined
kidney samples.
ACKNOWLEDGEMENT

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REFERENCES