ORIGINAL ARTICLE

Tacrine is implicated in oxidative stress in the laboratory guinea pig model

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Abstract

OBJECTIVES: Tacrine was the first acetylcholinesterase inhibitor approved for the treatment of Alzheimer disease. The compound is not available for therapeutic purposes as it was withdrawn due to hepatotoxicity of its metabolites. The hepatotoxicity can be decreased by alternative ways of drug administration avoiding thus the first pass effect. The present study is aimed to investigate the influence of intramuscularly administrated tacrine on oxidative stress.

METHODS: Laboratory guinea pigs were exposed to tacrine at doses of 0–800 μg/kg. The animals were euthanized 1 and 24 hours after the exposure. Parameters such as ferric reducing antioxidant power (FRAP), thiobarbituric acid reactive substances (TBARS), carbonylated proteins, caspase 3 activity, superoxide dismutase activity and glutathione reductase activity were assessed in the frontal, temporal and occipital lobe, cerebellum, liver, spleen, heart, and kidney. Moreover, levels of glucose, total and HDL cholesterol forms, triglycerides, blood urea nitrogen, creatinine, total bilirubin, total protein, albumin and activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and lactate dehydrogenase were assessed in plasma samples.

RESULTS: Activities of the enzymatic markers, level of carbonylated proteins in organs and levels of biochemical markers in plasma were only slightly influenced by tacrine. Dose-dependent elevation of the FRAP value was recognized in the brain tissues and the liver. The TBARS value was increased in the kidney and heart 1 and 24 hours, respectively, after exposure.

CONCLUSION: In the study, the effect of tacrine on markers of oxidative stress was proved. Possible positive effects of tacrine on the antioxidant defence in the brain tissue were discussed.

Abbreviations:

AD	- Alzheimer disease
ALP	- alkaline phosphatase
ALT	- alanine aminotransferase
AST	- aspartate aminotransferase
BUN	- blood urea nitrogen
CASP3	- caspase 3
CRE	- creatinine
FRAP	- ferric reducing antioxidant power
GR	- glutathione reductase
LDH	- lactate dehydrogenase
SOD	- superoxide dismutase
TBARS	- thiobarbituric acid reactive substances
TG	- triglycerides

INTRODUCTION

The exact pathogenesis of Alzheimer disease (AD) is not yet understood; however, a link between reduction of the cholinergic activity and its pathogenesis is well known. Currently, the symptomatic treatment of AD widely used is based on inhibition of acetylcholinesterase as a part of the cholinergic nervous system. Cholinesterase inhibitors resolve deficiency in the cholinergic system which also means improvement of the altered cognitive function.

Tacrine, (1,2,3,4-tetrahydro-9-aminoacridine), a reverse and nonselective cholinesterase inhibitor, was the first drug approved by the US Food and Drug Administration for the treatment of AD (Madden *et al.* 1995; Pohanka 2011; Pohanka 2012). The original studies on tacrine summarizing clinical trials were documented by e.g. Crismon (1994) and Blank *et al.* (1994). The oral bioavailability of tacrine is quite low because of its extensive first pass metabolism. Three major metabolites of tacrine, which are generated in liver by cytochrome P450 1A2, are 1-hydroxytacrine, 2-hydroxytacrine and 4-hydroxytacrine (Marques *et al.* 2010; Qian *et al.* 2012). Probably due to the cytochrome P450 1A2 activity, hepatotoxicity of tacrine was revealed (Meng *et al.* 2007). The dose-dependent hepatoxicity along with gastrointestinal side effects were the major reasons of tacrine withdrawal. The adverse effects can be attenuated by alternative ways of drug administration, such as transdermal or nasal routes, avoiding the first pass metabolism (Luppi *et al.* 2011).

Oxidative stress is a factor implicated in the both degeneration of cholinergic neurons (Saxena *et al.* 2008) and hepatotoxicity of tacrine (Meng *et al.* 2007). Furthermore, different ways of tacrine`s impact on the oxidative stress were contemplated. For example, Xiao *et al.* (2000) observed protective effects of tacrine against oxidative injury of neurons induced by amyloid beta-peptide. On the other hand, exposure of liver cells to tacrine leads to an increase in production of reactive oxygen species and to a decline of intracellular reduced glutathione (Osseni *et al.* 1999). Unfortunately, no complex experiment devoted to scaling of the oxidative homeostasis after tacrine application has been done to date. The present experiment is aimed to investigate the influence of tacrine on biochemical markers of oxidative stress in selected organs including the central nervous system and liver. We try to answer whether tacrine can be implicated significantly in the homeostasis and alter levels of endogenous low molecular weight antioxidants. For the experiment purposes, we chose two times intervals: one hour and one day. The selection was purposeful as it covers the both crucial intervals. Firstly, the interval when tacrine is around its half time and time when it is eliminated (Lou *et al.* 1996, Pool *et al.* 1997, Quian *et al.* 2012, Goh *et al.* 2011).

MATERIALS AND METHODS

Experimental animals

We chose the female guinea pig *(Cavia porcellus)* as a model species, because, similar to humans, guinea pigs are not able to produce vitamin C and they are considered to be a representative model of oxidative stress (Kaplan *et al.* 2010).

The animals were purchased from the Velaz Company (Prague, Czech Republic). They were 3 months old with body weight of 250±10 g. The animals were kept in the experimental facility (22±2 °C, 50±10% humidity, 12 hours light per day) and food and water was provided *ad libitum*.

The experiment was permitted and supervised by the Ethical Committee of the Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic.

Animal Exposure and sample collection

The animals were divided into twelve groups of eight specimens: 1 – 6 A and 1 – 6 B. Tacrine or saline solution (for controls – group 1A and 1B) in the amount of 100 μl per 100 g of body weight was administrated by intramuscular injection into the pelvic limb. Animals in groups 2A and 2B received tacrine in the dose of 50 μg/kg. Doses of tacrine administrated to animals in groups 3A, B and 4A, B corresponded approximately to the reported human therapeutic doses, i.e., 100 and 200 μg/kg (Davis *et al.* 1992). The animals in the rest of groups 5A, B and 6A, B received 400 and 800 μg of tacrine per kilogram of body weight.

The animals from the groups 1 – 6A were sacrificed in CO_2 atmosphere 1 hour after the exposure and the animals from the groups 1 – 6B 24 hours after the exposure. The animals were bloodedby cutting carotid artery and the fresh blood was collected into heparinized tubes. Frontal, temporal and occipital lobes, the cerebellum, liver, spleen, heart, and kidney were collected from each animal.

Tissue processing

Fresh heparinized blood was centrifuged at 1,000 ×g. Plasma was separated into a new tube and frozen at –80 °C until assayed.

100 mg of tissue from a freshly collected organ was mixed with 1 ml of phosphate buffered saline (Sigma-Aldrich, Saint Louis, USA – 1 tablet dissolved in 200 ml deionized water), then mechanically homogenized by an Ultra-Turrax mill (Ika Werke, Staufen, Germany) for one minute. The suspension was stored at –80 °C like plasma.

Markers of oxidative stress and plasma biochemistry

Ferric reducing antioxidant power (FRAP), thiobarbituric acid reactive substances (TBARS), carbonylated proteins, caspase 3 activity (CASP3), superoxide dismutase activity (SOD) and glutathione reductase activity (GR) were measured in tissue homogenates.

Low molecular weight antioxidants were assessed using the spectrophotometric method FRAP, based on reduction of ferric to ferrous ions, introduced by Benzie and Strain (1996). The assay was performed as previously described (Pohanka *et al.* 2009, Pohanka *et al.* 2010b).

Malondialdehyde, a marker of lipid peroxidation, was assayed as TBARS using standard spectrophotometry and disposable cuvettes. The protocol was based on the original work of Kobayashi *et al.* (1983). The method was modified as described in the previously published protocol (Pohanka *et al.* 2010a,b).

For assay of carbonylated proteins, another marker of increased oxidative stress, the colorimetric method based on carbonyl substitution by hydrazine from 2,4-dinitrophenylhydrazine, was used. The assay described by Cao & Cutler (1995) was used with minor modifications (Pohanka *et al.* 2010b).

GR activity was assayed using the Wartburg optical test in a way as mentioned in references (Pohanka *et al.* 2010a, Pohanka *et al.* 2011b). Activities of CASP3 and SOD were measured using Sigma caspase 3 assay kit and the SOD assay kit (Sigma-Aldrich, Saint Louis, USA) in compliance with protocols provided by manufacturer. For the SOD assay, it was necessary to dissolve the samples in phosphate buffered saline at a tenfold ratio because of its high enzymatic activity.

Levels of glucose, total and HDL cholesterol forms, triglycerides (TG), blood urea nitrogen (BUN), creatinine (CRE), total bilirubin, total protein, albumin and activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were measured in plasma samples using an automated analyzer SPOTCHEM™ EZ SP-4430 (Arkray, Japan).

Statistical analysis

Experimental data were processed using the statistical software Origin 8 SR2 (OriginLab Corporation, Northampton, USA). Comparison of the obtained results was done using one-way ANOVA with Scheffe`s test.

RESULTS

The FRAP levels increased significantly in the liver, cerebellum and the frontal, occipital and temporal lobes one hour after tacrine exposure ($0.1<p\leq0.5$). Twenty four hours after exposure, almost all FRAP levels returned to the levels similar to control. No significant alterations in FRAP values in other organs, including the spleen, heart and kidney, were found (Table 1).

The TBARS values in the liver, spleen and brain were not markedly influenced neither one nor 24 hours after tacrine administration. The TBARS levels showed only some minor effects in the heart and kidney (Table 2).

While the production of malondialdehyde in the heart decreased significantly one hour after the exposure, there was an evident increase of TBARS levels

Tab. 1. Ferric reducing antioxidant power (FRAP) in tissue samples ± standard errors of mean (μmol/g).

Tacrine dose	0 μg/kg	50 μg/kg	100 μg/kg	200 μg/kg	400 μg/kg	800 μg/kg
after 1 hour						
Frontal lobe	0.336±0.007	0.425±0.006 (**)	0.510±0.018 (**)	0.447±0.010 (**)	0.463±0.007 (**)	0.596±0.014 (**)
Temporal lobe	0.273±0.013	0.383±0.013 (*)	0.484±0.018 (**)	0.430±0.010 (**)	0.449±0.005 (**)	0.596±0.016 (**)
Occipital lobe	0.215±0.025	0.390±0.010 (**)	0.407±0.010 (**)	0.517±0.046 (**)	0.416±0.016 (**)	0.575±0.011 (**)
Cerebellum	0.252±0.005	0.295±0.010	0.362±0.010 (**)	0.412±0.025 (**)	0.430±0.012 (**)	0.470±0.014 (**)
Liver	1.86±0.03	3.63±0.27 (**)	3.51±0.12 (**)	3.76±0.20 (**)	2.79±0.22	3.37±0.22 (**)
Kidney	1.84±0.20	1.33±0.08	1.30±0.09	1.28±0.05	1.51±0.05	1.53±0.08
Spleen	2.45±0.19	2.21±0.14	2.11±0.18	2.67±0.23	2.78±0.12	2.73±0.16
Heart	0.484±0.062	0.367±0.015	0.348±0.007	0.365±0.021	0.407±0.023	0.332±0.017
after 24 hours						
Frontal lobe	0.262±0.015	0.218±0.014	0.346±0.010 (*)	0.292±0.010	0.295±0.008	0.213±0.012
Temporal lobe	0.260±0.016	0.246±0.010	0.370±0.015 (*)	0.299±0.010	0.322±0.031	0.301±0.007
Occipital lobe	0.225±0.011	0.227±0.010	0.269±0.009	0.246±0.010	0.288±0.010	0.262±0.008
Cerebellum	0.184±0.013	0.168±0.007	0.377±0.015 (**)	0.216±0.018	0.227±0.004	0.163±0.007
Liver	2.58±0.14	2.71±0.27	2.08±0.14	2.50±0.14	2.18±0.18	2.95±0.14
Kidney	1.96±0.14	1.35±0.17	1.67±0.09	2.08±0.10	2.07±0.14	1.61±0.05
Spleen	1.90±0.12	1.13±0.06	1.56±0.07	1.88±0.15	2.52±0.18	2.50±0.22
Heart	0.376±0.017	0.353±0.017	0.292±0.016	0.405±0.044	0.428±0.020	0.414±0.015

* $p<0.05$, ** $p<0.01$, n=8 specimens in each group

Tab. 2. Thiobarbituric acid reactive substances (TBARS) in tissue samples ± standard errors of mean (μmol/g).

Tacrine dose	0 μg/kg	50 μg/kg	100 μg/kg	200 μg/kg	400 μg/kg	800 μg/kg
after 1 hour						
Frontal lobe	0.291±0.007	0.301±0.013	0.326±0.014	0.352±0.017	0.274±0.008	0.247±0.009
Temporal lobe	0.323±0.017	0.319±0.009	0.387±0.026	0.365±0.016	0.297±0.039	0.285±0.012
Occipital lobe	0.261±0.011	0.294±0.010	0.333±0.014	0.336±0.014	0.285±0.013	0.227±0.007
Cerebellum	0.292±0.027	0.312±0.007	0.349±0.010	0.326±0.013	0.274±0.009	0.241±0.007
Liver	0.185±0.017	0.185±0.012	0.168±0.005	0.174±0.007	0.159±0.010	0.186±0.014
Kidney	0.205±0.007	0.231±0.010	0.239±0.008	0.239±0.014	0.247±0.004	0.270±0.008 (*)
Spleen	0.0732±0.0085	0.0742±0.0089	0.0920±0.0095	0.0722±0.0120	0.0420±0.0045	0.0694±0.0130
Heart	0.189±0.008	0.172±0.006	0.155±0.009	0.169±0.013	0.126±0.005 (*)	0.102±0.010 (**)
after 24 hours						
Frontal lobe	0.295±0.013	0.336±0.013	0.311±0.016	0.313±0.007	0.237±0.010	0.221±0.009
Temporal lobe	0.305±0.015	0.314±0.010	0.327±0.011	0.324±0.010	0.256±0.011	0.241±0.009
Occipital lobe	0.253±0.012	0.309±0.009 (**)	0.345±0.022	0.275±0.009	0.220±0.013	0.216±0.009
Cerebellum	0.273±0.013	0.313±0.018	0.270±0.010	0.293±0.013	0.239±0.010	0.218±0.007
Liver	0.177±0.007	0.174±0.006	0.190±0.011	0.171±0.010	0.178±0.012	0.169±0.010
Kidney	0.240±0.018	0.214±0.006	0.223±0.010	0.198±0.007	0.175±0.013 (*)	0.184±0.004
Spleen	0.102±0.012	0.190±0.013 (**)	0.159±0.006	0.101±0.017	0.058±0.005	0.048±0.002
Heart	0.0738±0.0036	0.0841±0.0060	0.109±0.018	0.148±0.007 (**)	0.137±0.005 (*)	0.156±0.009 (**)

* $p<0.05$, ** $p<0.01$, n=8 specimens in each group

Tab. 3. Carbonylated proteins in tissue samples ± standard errors of mean (μmol/g).

Tacrine dose	0 μg/kg	50 μg/kg	100 μg/kg	200 μg/kg	400 μg/kg	800 μg/kg
after 1 hour						
Frontal lobe	0.269±0.010	0.258±0.019	0.260±0.013	0.213±0.012	0.199±0.012	0.193±0.007
Temporal lobe	0.228±0.022	0.232±0.005	0.279±0.011	0.226±0.010	0.229±0.014	0.232±0.011
Occipital lobe	0.257±0.015	0.293±0.013	0.292±0.021	0.250±0.024	0.208±0.010	0.226±0.044
Cerebellum	0.265±0.015	0.221±0.016	0.198±0.022	0.270±0.020	0.156±0.018 (*)	0.182±0.020
Liver	0.362±0.033	0.358±0.0080	0.359±0.014	0.371±0.011	0.315±0.009	0.362±0.024
Kidney	0.327±0.006	0.371±0.013	0.309±0.009	0.329±0.022	0.342±0.008	0.336±0.007
Spleen	0.235±0.018	0.244±0.030	0.278±0.037	0.289±0.045	0.316±0.024	0.318±0.027
Heart	0.249±0.020	0.275±0.016	0.243±0.010	0.201±0.026	0.214±0.012	0.215±0.011
after 24 hours						
Frontal lobe	0.249±0.017	0.275±0.013	0.282±0.017	0.335±0.010 (**)	0.278±0.009	0.280±0.005
Temporal lobe	0.284±0.018	0.257±0.009	0.289±0.012	0.275±0.007	0.269±0.008	0.248±0.011
Occipital lobe	0.253±0.011	0.275±0.012	0.306±0.012	0.289±0.014	0.252±0.012	0.241±0.008
Cerebellum	0.257±0.015	0.230±0.014	0.215±0.015	0.263±0.013	0.233±0.008	0.208±0.010
Liver	0.323±0.008	0.397±0.015	0.390±0.018	0.368±0.018	0.341±0.017	0.362±0.011
Kidney	0.259±0.015	0.300±0.016	0.283±0.007	0.323±0.014	0.333±0.008	0.301±0.010
Spleen	0.249±0.020	0.430±0.032	0.388±0.068	0.294±0.014	0.344±0.015	0.281±0.019
Heart	0.266±0.019	0.236±0.017	0.241±0.024	0.261±0.027	0.227±0.012	0.216±0.012

* $p<0.05$, ** $p<0.01$, n=8 specimens in each group

after 24 hours. The increase was significant not only in the groups treated with high tacrine dose but also in the group receiving a lower dose (200 μg/kg). In the kidney, there was a dose-dependent increase of the TBARS value one hour after administration, but only the group of animals that received the highest dose of tacrine differed significantly from the control group. In contrast to the shorter interval, the TBARS value in the kidney slightly decreased after 24 hours and the decrease was dose-dependent. There was no or only a slight correlation between the FRAP and TBARS values in individual organs evaluated.

The level of carbonylated proteins was quite stable in all of the examined organs (Table 3). Even in the heart and kidney, where dose-dependent alteration in the TBARS levels was observed, individual groups showed no differences in the carbonylated proteins. Tacrine had also no impact on the GR activity measured in organs. As shown in Table 4, only moderate, dose-independent and mostly non-significant changes of the GR activity were detected.

The impact of tacrine on the SOD activity was detectable one hour after administration of the drug (Table 5). After 24 hours, the only proved alteration was the increased SOD activity in the liver of animals who received the highest dose of tacrine (i.e., 800 μg/kg). In the temporal and occipital lobes and in the cerebellum, the highest SOD activity was observed in the groups of animals that received the mean dosages of tacrine, from 100 to 200 μg/kg. However, the SOD activity in the brains of the animals treated with tacrine in the dose of 800 μg/kg just mildly differed from the control group. There were no significant alterations in the kidney, spleen and heart.

The CASP3 activity was undetectable by the used assay in all of the examined brain compartments and in the heart. In the liver, kidney and spleen only slight, non-significant alterations in the CASP3 activity were detected. Similarly to the SOD activity in the brain, the largest CASP3 activity was noticed in the groups of animals treated by the mean doses of tacrine (Table 6).

In the plasma samples, there were undetectable levels of total and HDL cholesterol forms (which means levels below 1.3 and 0.26 mmol/l, respectively) and creatinine (bellow 27 μmol/l). Markers of liver injury, i.e., total bilirubin, total protein, albumin, AST, ALT, and ALP, were not altered exceedingly. One hour after the drug administration we observed a moderate increase in albumin concentration corresponding to the administrated dose of tacrine (correlation coefficient r=0.656), but the difference between albumin concentration in the individual groups was not significant. In a similar way, the ALP activity was mildly, non-significantly increased corresponding to the dosage both one hour (r=0.574) and twenty four hours (r=0.619) after the drug administration. One hour after tacrine adminis-

Tab. 4. Glutathione reductase (GR) in tissue samples ± standard errors of mean (nkat/g)

Tacrine dose	0 μg/kg	50 μg/kg	100 μg/kg	200 μg/kg	400 μg/kg	800 μg/kg
after 1 hour						
Frontal lobe	66.2±5.4	66.6±3.8	77.5±6.0	61.3±3.5	66.9±4.2	62.7±3.2
Temporal lobe	62.5±4.1	76.0±5.9	52.2±4.7	56.0±3.0	56.3±1.9	63.5±4.4
Occipital lobe	57.8±4.6	77.5±4.6	69.0±8.7	52.3±5.2	71.3±4.1	69.5±7.0
Cerebellum	62.0±7.8	37.8±6.7	40.2±6.8	56.0±2.2	39.0±3.4	37.8±4.0
Liver	126±4	112±7	96.4±5.5	112±2	112±6	110±8
Kidney	116±3	128±3	128±3	131±3	95.9±3.8	106±3
Spleen	76.2±3.8	70.4±5.1	57.9±4.6	82.1±8.7	54.5±3.6	55.1±1.5
Heart	17.9±2.2	17.4±1.7	18.5±1.8	18.7±1.1	23.8±1.9	21.2±1.5
after 24 hours						
Frontal lobe	63.4±2.8	59.8±3.6	62.5±4.1	60.7±4.0	52.5±5.7	74.1±4.5
Temporal lobe	62.4±7.8	59.5±4.3	54.2±6.2	68.1±5.9	67.8±6.3	67.1±2.9
Occipital lobe	68.7±8.9	65.4±2.6	65.6±4.3	60.6±4.7	60.4±4.4	96.7±1.5
Cerebellum	42.0±5.9	45.0±3.9	45.9±6.1	43.6±4.7	43.4±4.8	28.2±1.4
Liver	103±7	58.6±11.3 (*)	93.5±4.3	112±4	109±4.0	99.8±4.1
Kidney	115±5	128±5	118±3	115±3	116±3	117±5
Spleen	53.5±4.8	72.3±12.2	49.3±2.1	59.0±4.1	55.1±1.8	70.5±3.4
Heart	18.0±0.9	16.9±1.5	15.9±1.0	16.1±1.0	21.6±1.0	17.1±3.4

* $p<0.05$, n=8 specimens in each group

Tab. 5. Superoxide dismutase (SOD) in tissue samples ± standard errors of mean (nkat/g).

Tacrine dose	0 µg/kg	50 µg/kg	100 µg/kg	200 µg/kg	400 µg/kg	800 µg/kg
after 1 hour						
Frontal lobe	200±4	201±14	221±2	221±2	208±1	202±2
Temporal lobe	196±5	204±3	227±2 (**)	231±2 (**)	221±4 (**)	215±2 (*)
Occipital lobe	188±7	195±2	217±2 (**)	214±3 (**)	219±2 (**)	202±2
Cerebellum	223±2	222±2	253±2 (**)	243±7 (*)	227±2	230±2
Liver	274±0	271±1 (*)	272±0	273±0	276±0	276±0
Kidney	267±2	273±2	271±1	272±1	268±1	268±1
Spleen	208±3	205±3	207±2	205±5	204±3	205±3
Heart	161±5	166±2	161±2	164±3	160±4	160±2
after 24 hours						
Frontal lobe	200±4	201±14	221±2	221±2	208±1	202±2
Temporal lobe	216±4	205±4	206±2	210±3	205±2	199±2
Occipital lobe	205±2	204±2	200±2	203±3	193±3	191±2
Cerebellum	232±2	221±2	227±3	229±2	222±2	222±2
Liver	275±0	276±0	276±0	275±0	274±0	278±1 (**)
Kidney	267±1	266±1	260±2	263±1	269±1	268±1
Spleen	206±3	200±4	206±2	203±7	210±3	221±2
Heart	161±3	165±3	154±3	147±5	154±3	149±6

* $p<0.05$, ** $p<0.01$, n=8 specimens in each group

Tab. 6. Caspase 3 (CASP3) in tissue samples ± standard errors of mean (pkat/g).

Tacrine dose	0 µg/kg	50 µg/kg	100 µg/kg	200 µg/kg	400 µg/kg	800 µg/kg
after 1 hour						
Liver	46.0±4.1	72.3±4.1	72.3±8.1	68.1±5.3	48.1±6.3	49.5±6.9
Kidney	26.8±4.1	38.3±5.8	18.3±5.1	17.4±3.7	21.3±3.7	27.6±3.4
Spleen	62.4±8.8	72.7±10.5	91.8±22.9	182±50	136±37	82.4±13.9
after 24 hours						
Liver	52.8±7.1	50.4±12.0	49.8±5.4	63.8±5.0	64.3±6.3	62.0±7.9
Kidney	28.1±4.1	29.4±3.9	42.4±5.5	6.68±3.94	14.9±7.2	12.3±5.9
Spleen	69.4±14.9	63.8±15.4	149±20	145±17	122±12	105±15

tration, we recognized a significant decline of BUN level in the groups A3 and A5 (dose of tacrine 200 and 800 µg/kg, respectively). In other groups, BUN levels were also lower than in the control group but the decline was non-significant. No effect of tacrine on the level of glucose, TG and the activity of LDH was found (Table 7).

DISCUSSION

The FRAP value informs about the level of low molecular weight antioxidants in the tissue. Low molecular weight antioxidants are responsible for scavenging and defusing of harmful reactive oxygen species in order to minimize tissue damage caused by the oxidative stress (Benzie & Strain 1996). Tacrine administration caused an increase of the FRAP value in the central nervous system without elevating any marker of the oxidative-stress-associated damage in the tissue. This result confirms that the tissue was exposed to the oxidative stress but the antioxidant capacity of the central nervous system was able to deal with it. For this reason, the effect of tacrine may be considered to be positive, as tacrine in doses lower than 800 µg/kg stimulates the antioxidant system without oxidative damage of the brain tissue. This phenomenon should be studied in a greater detail as data about the impact of tacrine on low molecular antioxidants are not currently available. A similar effect on the antioxidant system in the brain was

Tab. 7. Biochemical marker assessment in plasma samples ± standard errors of mean.

Tacrine dose	0 µg/kg	50 µg/kg	100 µg/kg	200 µg/kg	400 µg/kg	800 µg/kg
after 1 hour						
GLU (mmol/l)	7.83±0.25	7.50±0.09	7.46±0.20	7.73±0.21	7.18±0.40	7.50±0.39
BUN (mmol/l)	7.92±0.44	6.35±0.44	6.14±0.34	5.41±0.42 (*)	5.91±0.37	5.40±0.37 (*)
T-bil (µmol/l)	5.67±1.28	7.38±1.49	5.75±0.73	5.75±1.08	8.00±1.52	5.13±0.79
AST (µkat/l)	1.31±0.41	1.85±0.42	1.32±0.17	1.85±0.59	2.88±0.64	2.52±0.44
ALT (µkat/l)	0.817±0.061	0.773±0.086	0.745±0.073	0.879±0.131	0.856±0.058	0.735±0.059
ALP (µkat/l)	4.25±0.22	5.66±0.68	4.69±0.30	5.23±0.25	6.11±0.59	6.41±0.76
LDH (µkat/l)	4.88±1.25	9.98±1.83	6.78±0.85	8.04±1.98	16.1±3.9	12.6±2.8
T-pro (g/l)	44.2±2.0	41.9±1.5	41.23±1.1	40.88±1.1	39.6±1.4	37.00±2.3
Alb (g/l)	20.7±1.3	20.3±1.1	19.3±0.6	19.4±0.7	19.9±0.8	17.8±1.6
TG (mmol/l)	0.362±0.041	0.510±0.097	0.471±0.069	0.356±0.032	0.444±0.053	0.467±0.057
after 24 hours						
GLU (mmol/l)	9.02±0.18	8.89±0.23	8.89±0.26	8.16±0.18	9.56±0.42	9.34±0.33
BUN (mmol/l)	4.78±0.29	5.53±0.38	5.23±0.20	5.91±0.46	5.46±0.19	5.31±0.29
T-bil (µmol/l)	5.67±0.33	3.75±0.41	8.25±3.33	6.00±0.76	4.38±0.42	4.38±0.82
AST (µkat/l)	2.03±0.35	1.15±0.22	1.66±0.37	1.39±0.21	1.56±0.27	1.96±0.50
ALT (µkat/l)	0.738±0.064	0.600±0.072	0.690±0.051	0.601±0.055	0.653±0.074	0.664±0.105
ALP (µkat/l)	5.39±0.47	4.25±0.29	4.37±0.27	4.18±0.49	6.06±0.29	7.04±0.75
LDH (µkat/l)	12.1±3.5	5.38±1.48	8.87±2.82	7.10±1.22	9.43±2.12	11.2±3.6
T-pro (g/l)	39.8±0.8	47.8±1.7	45.8±1.4	41.6±1.1	40.6±0.6	40.9±1.5
Alb (g/l)	20.7±0.2	21.6±0.6	22.4±0.7	20.9±0.7	19.6±0.5	21.0±0.8
TG (mmol/l)	0.403±0.081	0.423±0.059	0.465±0.083	0.371±0.049	0.609±0.070	0.654±0.056

* $p<0.05$, n=8 specimens in each group
Abbreviations used in the table: GLU – glucose; BUN – blood urea nitrogen; T-bil – total bilirubin; AST – aspartate aminotransferase; ALT – alanine aminotransferase; ALP – alkaline phosphatase; LDH – lactate dehydrogenase; T-pro – total plasma protein; Alb – albumin; TG – triglycerides.

discussed in connection with another acetylcholinesterase inhibitor – huperzine (Little *et al.* 2008, Pohanka *et al.* 2011a) and huperzine also showed a similar protective effect against amyloid beta-induced oxidative injury as tacrine (Xiao *et al.* 2000), as mentioned in the introduction. These findings are in compliance with the present results.

The TBARS value corresponds to the level of malondialdehyde – a marker of lipid peroxidation which means oxidative damage to membranes (Greggio *et al.* 2009, Salgo & Pryor 1996). We recognized statistically significant elevation in the heart and kidney only. The production of malondialdehyde in the heart decreased significantly one hour after the exposure. We can infer depression of metabolism it the heart after tacrine application. After 24 hours, the oxidative stress-caused injury is manifested by the elevated TBARS level. Increase of the TBARS level in the kidney is probably connected with elimination of the drug at the interval one hour. These results are quite surprising because cardiotoxicity and nephrotoxicity of tacrine were not reported in papers which describe its hepatotoxicity and gastrointestinal adverse effects (Waqstaff & McTavish 1994, Luppi *et al.* 2011). The lower activity of the antioxidant system in the heart is one of the possible explanations. Beside antioxidants and membranes, oxidative stress can exert impact on proteins in an extensive scale. Carbonylation is an irreversible modification of proteins which is linked to various conditions of oxidative stress. The link between increased carbonylated proteins levels in the cerebrospinal fluid or brain tissue and Alzheimer disease has already been reported by some authors (Hartl *et al.* 2012, Korolainen *et al.* 2007). Results of carbonylated proteins assay imply no oxidative alteration of proteins caused by the administration of tacrine in all tissues examined.

The GR and SOD are enzymes involved in the antioxidant defence system (Finaud *et al.* 2006, Kohen *et al.* 2004). The task of GR is to renew the antioxidant capacity of previously oxidized glutathione. The GR activity usually increases with accumulation of the oxidized glutathione. Alterations in the GR activity have

to be interpreted together with other oxidative injury markers like TBARS or carbonylated proteins. As we did not recognize any statistically significant differences in the GR activity between the individual groups, we can conclude that the enzyme part of the antioxidant was not strongly influenced by tacrine. SOD accelerates transformation of superoxide anion, which is one of the most important radicals in pathology of the central nervous system (Shi & Gibson 2007), into hydrogen peroxide (Necas *et al.* 2005). The elevated SOD activity in the frontal, temporal, and occipital lobes and the cerebellum could be evaluated as a marker of increased oxidative stress which was effectively suppressed by the antioxidant system. This result corresponds with data from the FRAP assay, but it is difficult to explain the decline of the activity in groups treated by high-dose of tacrine. Depletion of the SOD capacity is a possible reason, but the elevation of the SOD activity in the groups treated by medium doses was not substantial.

Under normal circumstances, the CASP3 activity in the tissue is low and it increases when the apoptosis occurs (Schroeter *et al.* 2001). As reported in the results for CASP 3 assay, we did not prove any impact of tacrine on the apoptotic activity in the examined organs.

Biochemical assessment of plasma samples did not point at any serious organ injury caused by tacrine. In spite of the anticipated hepatotoxicity, activities of ALP, AST, LDH, and ALP – enzymes signifying liver damage – were not significantly elevated. Just a moderate statistically non-significant increase of ALP does not give the impression of being relevant especially without confirmation by another marker. Metabolism of proteins, lipids and bilirubin was not altered as well. The total protein, triglycerides and bilirubin levels were quite steady and albumin, whose production usually decreases in case of the liver damage, was even mildly elevated. The meaning of the BUN level decrease is not clear. The BUN level is typically reduced because of very serious liver injury, when the liver is not able to synthesize the urea, but the decrease of the markers does not indicate serious damage. The increased glomerular filtration also seems to be quite improbable.

The half time of tacrine in the body is about 1.2 hours (oral administration) or about 1 hour (intravenous administration) and the elimination half time of its metabolites 1-hydroxytacrine and 2-hydroxytacrine is about 2 and 3 h, respectively (Quian *et al.* 2012, Goh *et al.* 2011). In compliance with the experimental data, we did not observe strong influence of tacrine 24 hours after the exposure. The interval 24 hours is highly above half time for tacrine so it is eliminated from the body (Lou *et al.* 1996; Pool *et al.* 1997)..

Tacrine and especially its metabolites were considered to be quite toxic, therefore, alteration of oxidative homeostasis was expected. However, the findings are quite different from the initial expectation in spite of not so wide a scale of administrated doses. Only slight effects of tacrine on oxidative-stress markers were recognized in the present study so the pertinent adverse effects were under distinctiveness of the used methods. It may be explained by the fact that the drug was applied by intramuscular injection. This way of drug administration avoids the extensive first pass metabolism of tacrine and consequentially the genesis of toxic metabolites, taking place in liver, decreases. We infer that tacrine is a potent drug with quite low adverse effects when administered intramuscularly.

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REFERENCES

1 Benzie IFF, Strain JJ (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal Biochem. **239:** 70–76.
2 Blank K, Cheong JA, Cohen GD, GAitz C, Gottlieb G, Katz IR, Leuchter A, Maletta G, Margolin R, Sakauye K, Shamoian C (1994). Impact of tacrine in the care of patients with Alzeimers-disease – what we know one-year after FDA approval. Am J Geriatr Psych. **2**: 285–289.
3 Cao G, Cutler RG (1995). Protein oxidation and aging. Arch Biochem Biophys. **320**: 106–114.
4 Crismon ML (1994). Tacrine – 1st drug approved for Alzheimers-disease. Annals Pharmacother. **28**: 744–751.
5 Davis KL, Thal LJ, Gamzu ER, Davis CS, Woolson RF, Gracon SI, *et al.* (1992). A double-blind placebo-controlled multicenter study of tacrine for Alzheimer`s disease. N Engl J Med. **327**: 1253–1259.
6 Finaud J, Lac G, Filaire E (2006). Oxidative stress: relationship with exercise and training. Sports Med. **36**: 327–358.
7 Goh CW, Aw CC, Lee JH, Chen CP, Browne ER (2011). Pharmacokinetic and pharmacodynamic properties of cholinesterase inhibitors donepezil, tacrine, and galantamine in aged and young lister hooded rats. Drug Metab Dispos. **39**: 402–411.
8 Greggio S, Rosa RM, Dolganov A, de Oliveira IM, Menegat FD, Henriques JAP, et al (2009). NAP prevents hippocampal oxidative damage in neonatal rats subjected to hypoxia-induced seizures. Neurobiol Dis. **36:** 435–444.
9 Hartl D, Schuldt V, Forler S, Zabel C, Klose J, Rohe M (2012). Presymptomatic Alterations in Energy Metabolism and Oxidative Stress in the APP23 Mouse Model of Alzheimer Disease. J Proteome Res. DOI: 10.1021/pr300021e
10 Kaplan B, Cetin F, Elbeg S (2010). The effects of vitamin C administration, acute food deprivation, and acute food intake on vitamin C levels in different brain areas of guinea pigs. Int J Vitam Nutr Res. **80**: 197–204.
11 Kobayashi Y, Yoshimitsu T, Usui T (1983). Evaluation of lipid peroxidation of human erythrocyte hemolysates. J Immunol Methods. **64**: 17–23.
12 Kohen R, Oron M, Zelkowicz A, Kanevsky E, Farfouri S, Wormser U (2004). Low molecular weight antioxidants released from the skin's epidermal layers: an age dependent phenomenon in the rat. Exp Gerontol. **39:** 67–72.
13 Korolainen MA, Nyman TA, Nyyssonen P, Hartikainen ES, Pirttila T (2007). Multiplexed proteomic analysis of oxidation and concentrations of cerebrospinal fluid proteins in Alzheimer disease. Clin Chem. **53**: 657–665.

14 Little JT, Walsh S, Aisen PS (2008). An update on huperzine A as a treatment for Alzheimer's disease. Expert Opin Investig Drugs. **17**: 209–215.
15 Lou GL, Montgomery, Sitar DS (1996). Bioavailability and pharmacokinetic disposition of tacrine in elderly patients with Alzheimer´s disease. J Phychiatr Neurosci. **21**: 334–339.
16 Luppi B, Bigucci F, Corace G, Delucca A, Cerchiara T, Sorrenti M, et al (2011). Albumin nanoparticles carrying cyclodextrins for nasal delivery of the anti-Alzheimer drug tacrine. Eur J Pharm Sci. **44**: 559–565.
17 Madden S, Spaldin V, Park BK (1995). Clinical pharmacokinetics of tacrine. Clin Pharmacokinet. **28**: 449–457.
18 Marques LA, Kool J, de Kanter F, Lingeman H, Niessen W, Irth H (2010). Production and on-line acetylcholinesterase bioactivity profiling of chemical and biological degradation products of tacrine. J Pharm Biomed Anal. **53:** 609–616.
19 Meng Q, Ru J, Zhang G, Shen C, Schmitmeier S, Bader A (2007). Re-evaluation of tacrine hepatotoxicity using gel entrapped hepatocytes. Toxicol Lett. **168:** 140–147.
20 Necas J, Bartosiková L, Benes L, Janostikova E, Bartosik T, Klusakova J, et al(2005). Influence of antioxidant effect of stobadine derivative in condition of kidney ischemia-reperfusion in a preclinical experiment (effect in prophylaxis). Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. **49**: 385–388.
21 Osseni RA, Debbasch C, Christen MO, Rat P, Warnet JM (1999). Tacrine-induced Reactive Oxygen Species in a Human Liver Cell Line: The Role of Anethole Dithiolethione as a Scavenger. Toxicol In Vitro. **13**: 683–688.
22 Pohanka M, Bandouchova H, Sobotka J, Sedlackova J, Soukupova I, Pikula J (2009). Ferric reducing antioxidant power and square wave voltammetry for assay of low molecular weight antioxidants in blood plasma: Performance and comparison of methods. Sensors. **9:** 9094–9103.
23 Pohanka M, Pejchal J, Horackova S, Kuca K, Bandouchova H, Damkova V, Pikula J (2010a). Modulation of ionizing radiation generated oxidative stress by HI-6 (asoxime) in a laboratory rat model. Neuro Endocrinol Lett. **31**: 62–68.
24 Pohanka M, Sobotka J, Jilkova M, Stetina R (2010b). Oxidative stress after sulfur mustard intoxication and its reduction by melatonin: efficacy of antioxidant therapy during serious intoxication. Drug Chem Toxicol. **34**: 85–91.
25 Pohanka M (2011) Cholinesterases, a target of pharmacology and toxicology. Biomed Pap Olomouc **155**: 219–223.
26 Pohanka M, Hrabinova M, Zemek F, Drtinova L, Bandouchova H, Pikula J (2011a). Huperzine induces alteration in oxidative balance and antioxidants in a guinea pig model. Neuro Endcrinol Lett. **32**: 95–100.
27 Pohanka M, Sobotka J, Stetina R (2011a). Sulfur mustard induced oxidative stress and its alteration by epigallocatechin gallate. Toxicol Lett. **201:** 105–109.Pohanka M (2012) Acetylcholinesterase inhibitors; a patent review (2008–present). Expert Opin Ther Pat. **22**: 871–886.
28 Pool WF, Reily MD, Bjorge SM, Woolf TF (1997). Metabolic disposition of the cognition activator tacrine in rats, dogs, and humans – species comparisons. Drug Met Disp. **25**: 590–597.
29 Qian S, Wo SK, Zuo Z (2012). Pharmacokinetics and brain dispositions of tacrine and its major bioactive monohydroxylated metabolites in rats. J Pharm Biomed Anal. **61:** 57– 63.
30 Salgo MG, Pryor WA (1996). Trolox inhibits peroxynitrite-mediated oxidative stress and apoptosis in rat thymocytes. Arch Biochem Biophys. **333:** 482–488.
31 Saxena G, Singh SP, Agrawal R, Nath C (2008). Effect of donepezil and tacrine on oxidative stress in intracerebral streptozotocin-induced model of dementia in mice. Eur J Pharm Sci. **581:** 283–289.
32 Schroeter H, Spencer JPE, Rice-Evans C, Williams RJ (2001). Flavonoids protect neurons from oxidized low-density-lipoprotein-induced apoptosis involving c-Jun N-terminal kinase (JNK), c-Jun and caspase-3. Biochem J. **358:** 547–557.
33 Shi Q, Gibson GE (2007). Oxidative stress and transcriptional regulation in Alzheimer's disease. Alzheimer Dis Assoc Disord. **21**: 276–291.
34 Wagstaff AJ, McTavish D (1994). Tacrine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in Alzheimer's disease. Drugs Aging. **4**: 510–540.
35 Xiao XQ, Wang R, Tang XC (2000). Huperzine A and tacrine attenuate beta-amyloid peptide-induced oxidative injury. J Neurosci Res. **61**: 564–569.