Acta Biochimica Polonica

Vol. 55 No. 1/2008, 97–105

on-line at: www.actabp.pl

Regular paper

Tenocyclidine treatment in soman-poisoned rats — intriguing results on genotoxicity *versus* protection

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Received: 24 November, 2007; revised: 04 February, 2008; accepted: 26 February, 2008
available on-line: 07 March, 2008

This study aimed to evaluate the antidotal potency of tenocyclidine (TCP) that probably might protect acetylcholinesterase (AChE) in the case of organophosphate poisoning. TCP was tested alone as a pretreatment or in combination with atropine as a therapy in rats poisoned with ¼ and ½ of LD_{50} of soman. Possible genotoxic effects of TCP in white blood cells and brain tissue were also studied. Results were compared with previous findings on the adamantyl tenocyclidine derivative TAMORF. TCP given alone as pretreatment, 5 min before soman, seems to be superior in the protection of cholinesterase (ChE) catalytic activity in the plasma than in brain, especially after administration of the lower dose of soman. Plasma activities of the enzyme after a joint treatment with TCP and soman were significantly increased at 30 min ($P < 0.001$) and 24 h ($P = 0.0043$), as compared to soman alone. TCP and atropine, given as therapy, were more effective than TCP administered alone as a pretreatment. The above therapy significantly increased activities of the enzyme at 30 min ($P = 0.046$) and 24 h ($P < 0.001$), as compared to controls treated with ¼ LD_{50} of soman alone. Using the alkaline comet assay, acceptable genotoxicity of TCP was observed. However, the controversial role of TCP in brain protection of soman-poisoned rats should be studied further.

Keywords: tenocyclidine, soman, cholinesterase activity, rat, plasma, brain, genotoxicity, comet assay

INTRODUCTION

Organophosphates are highly toxic compounds comprising the largest number of chemicals that are used worldwide as insecticides in agriculture, in industry, and around the home. Some organophosphorus compounds (OPc) have been recommended as therapeutic agents in human and veterinary medicine, whereas the strongest of them have been used as nerve agents in chemical warfare and terrorist attacks. Using *in vivo* and *in vitro* experimental models, these compounds have been found to produce a variety of toxicological effects on the central and peripheral nervous systems, and cardiovascular, ocular, neurobehavioral, immunological, reproductive, placental, and other body systems, in addition to endocrine disruption, oxidative stress and carcinogenesis. Since organophosphorus poisoning is generally rare, there has been limited research interest by pharmaceutical companies to develop new antidotes, but also to confirm the efficacy of those that are currently available. On the other hand, the increased threat of the misuse of nerve agents during military conflicts (MacIlwain, 1993) and by terrorists (Nagao *et al*., 1997) prompted us to search for new, more effective treatment regimen against these

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Abbreviations: AChE, acetylcholinesterase; ChE, cholinesterase; DTNB, 5,5′-dithio-bis(2-nitrobenzoic acid); HI-6, [(1-(2-hydroxyiminomethylpyridinium)-3-(4-carbamoylpyridinium)-2-oxapropane dichloride)]; i.p., intraperitoneally; LD_{50}, acute toxicity based on 24 h mortality rates; LMP, low melting point; NMDA, *N*-methyl-D-aspartate; NMP, normal melting point; OPc, organophosphorus compounds; PCP, phencyclidine; s.c., subcutaneously; TAMORF, 1-[2-(2-thienyl)-2-adamantyl] morpholine; TCP, tenocyclidine.

poisons. For ethical reasons, the efficacy of nerve agent antidotes cannot be investigated in humans *in vivo*. Therefore, testing is primarily performed with experimental animals (Dawson, 1994).

The current standard treatment for poisoning by OPc includes the combined administration of cholinesterase reactivators (oximes), a muscarinic cholinergic receptor antagonist (atropine sulfate) and pre-treatment with a reversible carbamate AChE-inhibitor, such as physostigmine. However, none of the usually used oximes can be regarded as a universally suitable reactivator of AChE that has been inhibited by various classes of OP pesticides and nerve agents (Worek *et al*., 1997). In theory, oximes may be ineffective in OP poisoning for the following reasons: re-inhibition of AChE is faster than reactivation, and oxime therapy is ineffective due to the "ageing" of the enzyme, which makes reactivation impossible. Soman is probably one of the most dangerous agents, and its harmful effects are especially difficult to counteract. Some studies have demonstrated that high plasma concentrations of oxime could be a consequence of unwanted side effects (Vale, 1995; Worek *et al*., 1997).

There are reports on the adjuvant therapeutic effects of phencyclidine (PCP), for example its derivative ketamine (Cohen *et al*., 1974; Schuh, 1975; Clinton *et al*., 1988; Mion *et al*., 2003), thienyl phencyclidine (TCP, tenocyclidine) and some of their derivatives in medical treatment of soman poisoning (McDonough, 1993; Lallement *et al*., 1994; 1998a; Carpentier *et al*., 1994; Gabrielewitz *et al*., 1980). The structure-activity of PCP has been discussed in terms of its 3-ring system: an aromatic ring producing an area of negative charge, a basic heterocyclic nitrogen ring bearing a localized positive charge, and a cycloalkyl ring which is lipophilic in character (Vaupel *et al*., 1984). It seems that the curative efficiency of PCP and TCP is related to the excitatory amino acid glutamate neurotransmitter system and especially to *N*-methyl-D-aspartate (NMDA) receptors involved in the process of soman-induced seizures and brain damage that follows (McDonough & Shih, 1993; Carpentier *et al*., 1994; Lallement *et al*., 1998b). We therefore reasoned that other adamantane derivatives may exhibit an even greater biological effect and that an evaluation of such compounds would give an insight into the chemical features responsible for the activity. Many investigators consider adamantane as a highly promising candidate in drug design (Donath *et al*., 1987; Kornhuber *et al*., 1991; Tsuzuki *et al*., 1994). For example, aminoadamantane derivatives memantine (1-amino-3,5-dimethyladamantane) and amantadine (1-aminoadamantane) are uncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonists that have been used clinically in the treatment of dementia and Parkinson's disease for several years without serious side effects (Donath *et al*., 1987; Kornhuber *et al*., 1991). Also, an earlier experiment performed in our laboratory confirmed that TCP and its newly synthesized adamantane derivatives could be used as adjuvant therapy to atropine and HI-6 therapy in soman poisoning (Škare *et al*., 2002; Lucić Vrdoljak *et al*., 2006). These compounds combined with standard therapy effectively antagonize the acute toxicity of soman in mice. According to the preliminary investigations, TCP and its derivatives also possess attractive pharmacological activities such as radioprotective and anticancer effects (Ferle-Vidović *et al*., 1993; 1995; Wang *et al*., 2004a; 2004b).

In order to evaluate the usefulness of TCP as an antagonist of NMDA receptors that potentially might protect AChE in the case of OP poisoning, we studied its therapeutic efficacy on rats poisoned with two different sub-lethal doses of soman (¼ and ½ of LD_{50}). Furthermore, to investigate its possible genotoxic effects, especially primary DNA damage in white blood cells and brain tissue, the alkaline comet assay was also performed as a sensitive technique for detecting DNA lesions (Singh, 2000; Tice *et al*., 2000). Results were compared with the findings of our previous *in vitro* and *in vivo* studies with the adamanyl tenocyclidine derivative TAMORF (1-[2-(2-thienyl)-2-adamantyl] morpholine).

MATERIALS AND METHODS

Chemicals. Soman was purchased from NC-Laboratory Spiez (Spiez, Switzerland). Stock solution of 1×10^{-6} mol/l (M) soman was prepared in propylene glycol. Further dilutions were made in water, shortly before use. Rats were poisoned subcutaneously (s.c.) with ¼ or ½ of the LD_{50} dose. TCP, Fig. 1, was purchased from Institute Ruđer Bošković (Zagreb, Croatia). TCP was dissolved in water; the solution for animal treatment was prepared in the concentration of 1.25 mg/ml, and administered intraperitoneally (i.p.) (2.5 mg/kg body mass). Atropine sulfate (Kemika, Zagreb, Croatia) was dissolved in water (5.0 mg/ml) and also administered i.p. (10.0 mg/kg body mass).

Animals. Male adult Wistar rats were obtained from the Institute for Medical Research and Occupational Health (Zagreb, Croatia) and selected by mass (240–280 g). The animals were kept in macrolone cages under controlled conditions (room temperature 21°C, 12 : 12 h light : dark cycle), received a standard diet for laboratory rodents (Sljeme, Zagreb, Croatia) and had free access to water. The rats were randomly distributed in groups of four and were deprived of food for 24 h before the experiment.

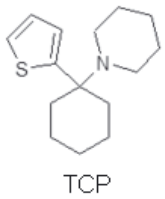

Figure 1. Structural formula of TCP.

The study was carried out according to the NIH Guide for the Care and Use of Laboratory Animals. Experiments received necessary approvals, both from the Local Ethical Committee of the Institute for Medical Research and Occupational Health and from the Ministry of Science, Education and Sports of the Republic of Croatia.

Poisoning and treatment. For the purpose of biochemical experiments, rats received a subcutaneous (s.c.) sub-lethal dose of soman (either ¼ or ½ LD_{50}; LD_{50} = 135.0 μg/kg body mass) (Lucić Vrdoljak *et al*., 2006). The therapeutic efficacy of TCP (2.5 mg/kg body mass, i.p.) was tested as follows: a) as pretreatment, given alone five minutes before soman poisoning, and b) as therapy, given in combination with atropine one minute after soman poisoning. TCP was selected as an antidote in the dose of 2.5 mg/kg body mass, i.p., because our preliminary experiments (unpublished) as well as the results of other studies (Tricklebank *et al*., 1989; Carpentier *et al*., 1994) demonstrated that the increase in dose from 2.5 mg/kg to ¼ of the respective LD_{50} dose did not correlate with an increase in therapeutic efficiency. All animals were killed with coal gas 10, 30, 60 min or 24 h after the treatment. The controls were given saline using the same experimental design.

Blood samples were obtained directly from the heart. Brain was also isolated and rinsed with saline. Plasma and brain samples were frozen at –20°C immediately after sampling until further processing. The brain samples were homogenized (200 mg tissue/ml saline), and centrifuged at $2\,800 \times g$ for 15 min to obtain the supernatant.

Enzyme assay. The catalytic activity of ChE in plasma and the catalytic activity of AChE in homogenates of the brain were determined spectrophotometrically 10, 30, 60 min or 24 h after the treatment, using the method of Ellman *et al.* (1961) which is based on the use of 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) as a thiol reagent. DTNB (Sigma ChemCo, St. Louis, USA) was used in the final concentration of 0.33 mmol/l. All experiments were performed in 0.1 mol/l phosphate buffer (pH 7.4) (Kemika, Croatia) and enzyme activities were measured in the presence of 1.0 mmol/l acetylthiocholine iodide (Sigma ChemCo, St. Louis, USA) at 22°C. Relative changes in the enzyme activity in the treated animals are presented as the percentage of activity of the respective control group. All comparisons were made with the enzyme activity in rats treated with soman alone.

The alkaline comet assay. The comet assay was carried out under alkaline conditions, basically as described by Singh *et al*. (1988). The analyses were performed before the application of TCP (1.25 mg/ml) as well as 30 min or 24 h after its application. Negative control was also included, with physiological saline. Samples of whole blood and homogenized brain tissue were used for the comet analysis, taken from four animals per each experimental group. Their tissue was mechanically disintegrated and homogenized in ice-cold buffer (0.075 M NaCl and 0.024 M Na_2EDTA). Preparations were prepared as follows: fully frosted slides were covered with 1% normal melting point (NMP) agarose (Sigma). After solidification, the gel was scraped off from the slide. The slides were then coated with 0.6% NMP agarose. When this layer solidified, a second layer containing the whole blood sample (2 μl) or homogenized tissue sample (7 μl) mixed with 0.5% low melting point (LMP) agarose (Sigma) was placed on the slides. After 10 min of solidification on ice, slides were covered with 0.5% LMP agarose. Afterwards the slides were immersed for one hour in ice-cold, freshly prepared lysis solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris/HCl, 1% Na-sarcosinate (Sigma), pH 10) with 1% Triton X-100 (Sigma) and 10% dimethyl sulfoxide (Kemika) were added fresh to the lysed cells to allow DNA unfolding. The slides were then randomly placed side by side in a horizontal gel-electrophoresis tank, facing the anode. The unit was filled with freshly prepared electrophoretic buffer (300 mM NaOH, 1 mM Na_2EDTA, pH 13.0) and the slides were set in this alkaline buffer for 20 min to allow DNA unwinding and expression of alkali-labile sites. Denaturation and electrophoresis were performed at 4°C under dim light. Electrophoresis was carried out for the next 20 min at 25 V (300 mA). After electrophoresis, the slides were washed gently with a neutralisation buffer (0.4 M Tris/HCl, pH 7.5) three times at five-minute intervals to remove excess alkali and detergents. Each slide was stained with ethidium bromide (20 μg/ml) and covered with a coverslip. Slides were stored at 4°C in humidified sealed containers until analysis. To prevent additional DNA damage, the handling of blood samples and all steps included in the preparation of slides for the comet analysis were conducted under yellow light or in the dark. Slides were examined at 250× magnification in a fluorescence microscope (Zeiss, Germany), equipped with an excitation filter of 515–560 nm and a barrier filter of 590 nm. Through a black-white camera, the microscope was connected

to a computer-based image analysis system (Comet Assay II, Perceptive Instruments Ltd., UK). A total of 50 comets per sample (blood or brain tissue) per animal were scored (25 from each of two replicate slides). Acquired data were then pooled and altogether 200 comets per each experimental group and tissue were measured. Comets were randomly captured at a constant depth of the gel, avoiding the edges of the gel, occasional dead cells and superimposed comets. To avoid the potential variability, one well-trained scorer performed all scorings of comets. Three comet parameters: DNA migration (tail length, expressed in micrometers), tail intensity (DNA %) and tail moment were evaluated.

Statistics. Statistical analyses were carried out with the commercial programme Statistica 5.0 (StatSoft, Tulsa, USA).

Statistical evaluation of data considering the enzyme activity in plasma and brain were made by non-parametric χ^2 test. Statistical decisions were made at a significance level of $P < 0.05$.

Data gathered in the alkaline comet assay were evaluated by means of descriptive statistics and other relevant tests. Each sample was characterized for the extent of DNA damage by considering the mean (±S.D. of the mean), median and range for the comet parameters measured. In order to normalize distribution and to equalize the variances, a logarithmic transformation of data was applied. Multiple comparisons between groups were done by means of ANOVA on log-transformed data. Post-hoc analysis of differences was done by Scheffé test. The level of statistical significance was set at $P < 0.05$.

RESULTS

Enzyme assay

In these experiments, a decrease of ChE catalytic activity in plasma and of AChE catalytic

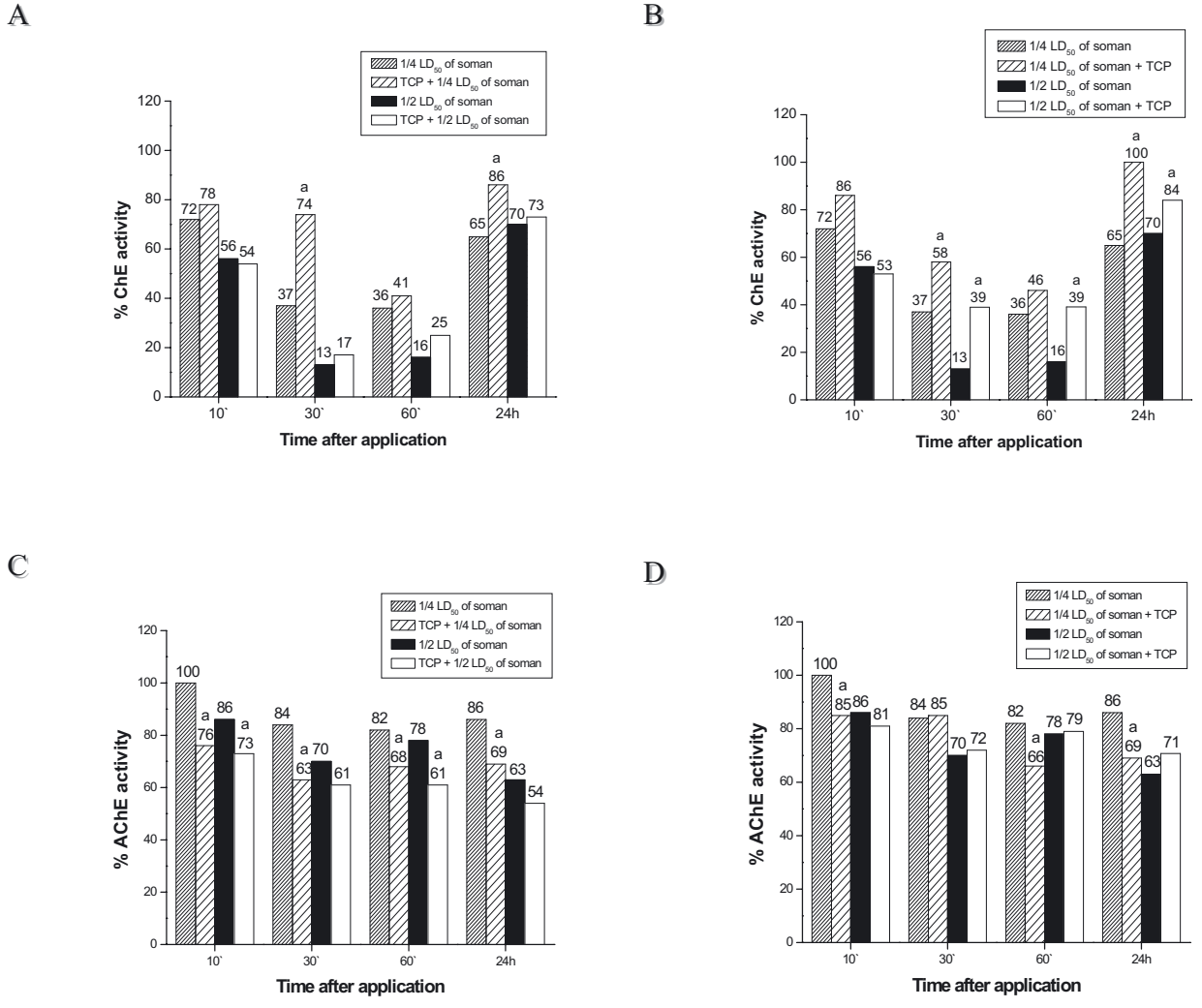

Figure 2. The effects of TCP given as pretreatment or therapy on the catalytic activity of ChE in the plasma (A, B) and AChE in the brain (C, D) of rats poisoned with ¼ LD_{50} or ½ LD_{50} of soman.
[a]Significantly increased enzyme activity as compared to corresponding control.

activity in brain was obtained in all animals administered ¼ or ½ LD_{50} of soman (Fig. 2). Rats poisoned with a single sub-lethal dose of soman s.c. (½ of its LD_{50}) exhibited, within 5–10 min of injection, signs of toxicity, such as convulsions, hypersalivation, muscle fasciculation, seizures and fine tremor progressing. Maximal toxicity was observed within 15–60 min after soman injection. All other animals showed no clinical signs of poisoning.

TCP given alone as pretreatment, 5 min before soman (¼ or ½ LD_{50} of soman), seems to be effective in the protection of ChE catalytic activity in the plasma, especially after administration of the lower dose of soman. Activities of the enzyme after a joint treatment with TCP and soman were significantly increased at 30 min ($P < 0.001$) and 24 h ($P = 0.0043$) compared to soman alone (Fig. 2 A). TCP as pretreatment seems to diminish the AChE activity in the brain of soman-intoxicated rats. The catalytic activities of AChE after a joint treatment with TCP and ¼ LD_{50} of soman were significantly lower at time points consecutively studied throughout the experiment ($P < 0.001$, $P = 0.0014$, $P = 0.0337$ and $P = 0.0067$; Fig. 2 C), while those after TCP and ½ LD_{50} of soman were decreased at 10 ($P = 0.016$) and 60 min ($P = 0.014$) compared to respective controls treated with soman alone (Fig. 2 C).

Since animals treated with TCP alone as therapy after soman injection showed severe signs of intoxication and none survived, these investigations included the determination of the enzyme catalytic activities in plasma and brain of rats which received TCP as therapy in combination with atropine given (i.p.) 1 min after soman poisoning (s.c.). Judging by the plasma ChE activity, the pharmacological effect of TCP and atropine was more effective than the effect of TCP given alone as a pretreatment. The above therapy significantly increased activities of the enzyme at 30 min ($P = 0.046$) and 24 h ($P < 0.001$) compared to controls treated with ¼ LD_{50} of soman alone (Fig. 2 B). TCP significantly reversed the decreases in ChE activity induced by the higher dose of soman at time points of 30′, 60′ and 24 h ($P < 0.001$, $P = 0.049$, $P = 0.029$; Fig. 2 B). This combination showed no therapeutic effects on the brain AChE activity (Fig. 2 D).

The alkaline comet assay

Distribution of the individual values measured for each comet parameter in blood samples, along with the median, minimum and maximum, is shown in Fig. 3, while the corresponding values measured in the brain tissue are displayed in Fig. 4.

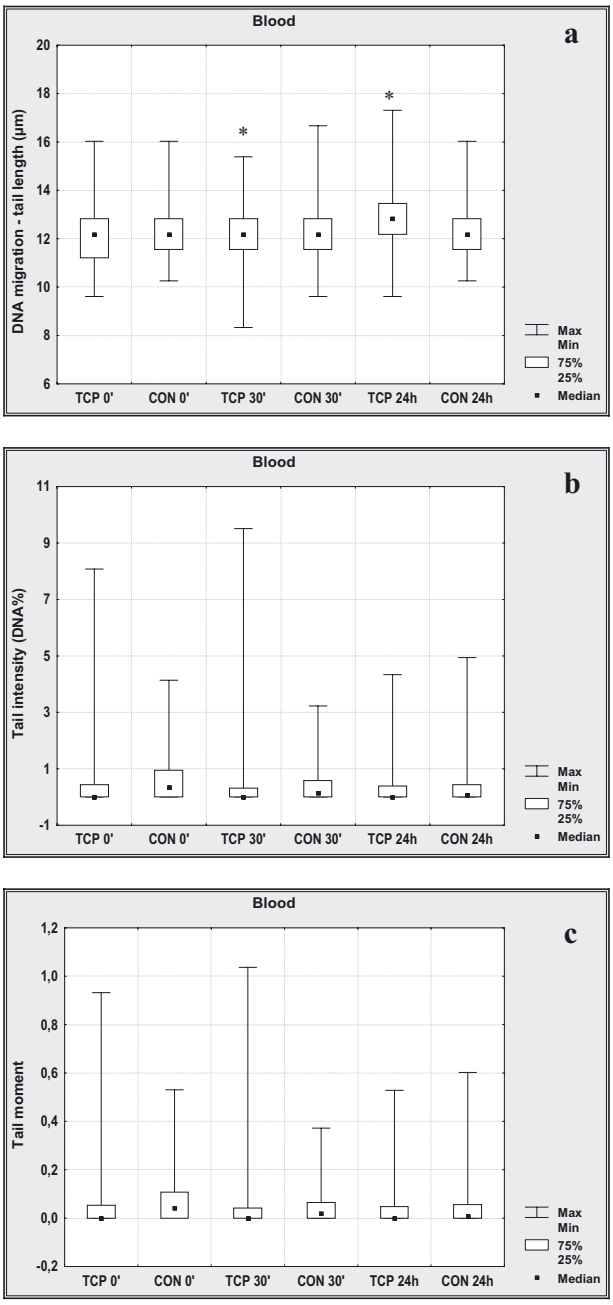

Figure 3. Distribution of comet parameters measured in white blood cells of rats pre-treated with TCP and in corresponding negative controls (CON).
Blood samplings were made before application of TCP (time point 0′), as well as following treatments that lasted for 30′ or 24 h. *Significantly increased values with regard to negative control ($P < 0.01$; ANOVA, post-hoc Scheffé test).

Blood

In white blood cells of rats administered physiological saline only (negative controls), slight differences in three comet parameters were observed. Mean tail lengths were: 12.14 ± 0.08 at time point 0′, 12.00 ± 0.08 at time point 30′, and 12.27 ± 0.08 at

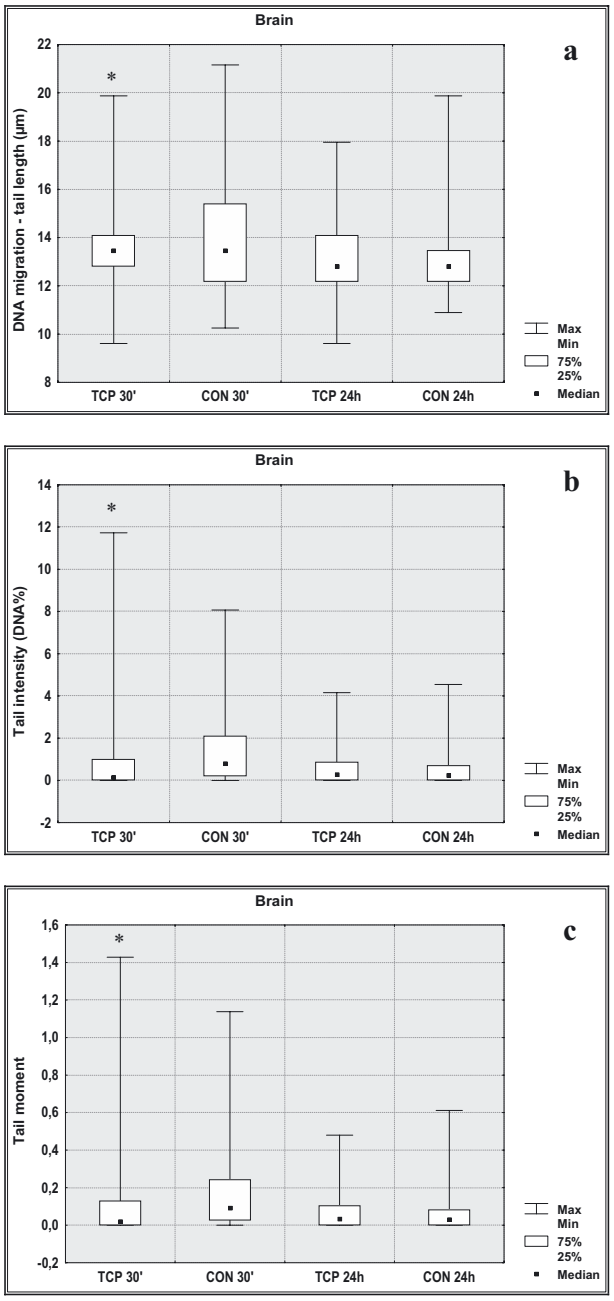

Figure 4. Distribution of comet parameters measured in brain cells of rats pre-treated with TCP for 30′ or 24 h and in corresponding negative controls (CON).
*Significantly lowered values with regard to negative control ($P < 0.05$; ANOVA, post-hoc Scheffé test).

time point 24 h. Mean tail intensities recorded in the same samples were: 0.70 ± 0.06 at time point 0′, 0.40 ± 0.04 at time point 30′, and 0.33 ± 0.05 at time point 24 h. The corresponding mean tail moments were: 0.08 ± 0.01 at time point 0′, 0.05 ± 0.005 at time point 30′, and 0.04 ± 0.01 at time point 24 h.

Before application of TCP, the mean tail length of comets measured in white blood cells was 12.14 ± 0.09, with the corresponding tail intensity of 0.33 ± 0.05 and tail moment 0.04 ± 0.01. Pretreatment with TCP induced a slight time-dependent increase in the comet tail length (mean: 12.34 ± 0.08 at 30′ and 12.68 ± 0.09 at 24 h). However, statistically significant was only the difference between samples taken before application and 24 h following treatment with TCP.

Inter-group comparisons (TCP *vs*. negative control) made by ANOVA with post-hoc Scheffé test revealed that the application of TCP significantly affected only the tail length in white blood cells, at both time points following the treatment. The F values obtained were: 9.58 ($P = 0.002$) for time point 30′ and 12.21 ($P = 0.0005$) for time point 24 h. The differences between the tail intensities and tail moments measured in blood cells of TCP-treated rats and negative controls were not statistically significant.

Brain tissue

In samples of single brain cells of negative controls, significant differences in three comet parameters were also observed. The mean tail lengths were: 14.03 ± 0.17 at time point 30′, and 12.97 ± 0.10 at time point 24 h. The mean tail intensities recorded in the same samples were: 1.46 ± 0.12 at time point 30′, and 0.50 ± 0.05 at time point 24 h. The corresponding mean tail moments were: 0.19 ± 0.02 at time point 30′, and 0.06 ± 0.01 at time point 24 h.

In samples of single brain cells of rats pretreated with TCP for 30 min, the mean tail length of comets was 13.61 ± 0.11, with corresponding tail intensity of 0.94 ± 0.13 and tail moment 0.12 ± 0.02. Prolonged treatment with TCP (24 h) induced a time-dependent decrease in all three comet parameters evaluated. The mean tail length of comets measured in brain cells of rats was 13.03 ± 0.10, with corresponding tail intensity of 0.60 ± 0.06 and tail moment 0.07 ± 0.01. Comparisons between two sampling times made by ANOVA with post-hoc Scheffé test revealed significantly lowered tail length ($F = 14.79$, $P = 0.0001$) and tail moment ($F = 5.96$, $P = 0.015$) in brain cells of rats at time point 24 h.

Inter-group comparisons (TCP *vs*. negative control) were also made by ANOVA with post-hoc Scheffé test. They indicate significantly lower values of all comet parameters in the brain cells of rats pretreated for 30 min with TCP as compared to negative control. The F values obtained were: 4.19 for comparisons regarding tail length ($P = 0.04$), 8.67 for comparisons regarding tail intensity ($P = 0.003$) and 9.05 for comparisons regarding tail moment ($P = 0.003$). The comparison of comet parameters measured in the brain tissue collected after prolonged treatment with TCP (24 h) and the corre-

Table 1. Efficacy of adamanyl tenocyclidine derivative Tamorf as ChE protector in soman-poisoned rats*

Time (min)	Enzyme activity (%)					
	¼ LD_{50} of soman plasma/brain	pretreatment/therapy		½ LD_{50} of soman plasma/brain	pretreatment/therapy	
		plasma	brain		plasma	brain
10	72/100	87/74	94/95	56/86	73/56	99/95
30	37/84	49/44	100/87	16/70	31/18	93/70
60	36/82	60/53	93/86	25/78	39/10	83/49
24h	65/86	98/85	95/97	73/63	97/85	85/56

*Reported data are adapted according to our previous study (Lucić Vrdoljak *et al.,* 2006)

sponding negative control revealed no statistically significant differences.

DISCUSSION

AChE mediates hydrolysis of the neurotransmitter acetylcholine (ACh), which is involved in the numerous cholinergic pathways in both central and peripheral nervous system. Consequently, inhibition of AChE with OPc results in severe cholinergic toxic signs caused by increased concentration of ACh at cholinergic nerve-nerve and nerve-muscle synapses (Somani & Romano, 2001). However, experimental data suggest that the neurotoxic properties of nerve agents cannot be explained solely by their direct action on AChE.

In the first part of the experiment we evaluated *in vivo* efficiency of TCP as an antidote in soman poisoning. The obtained results were compared with the findings of our previous study with adamantyl tenocyclidine derivative TAMORF (1-[2-(2-thienyl)-2-adamantyl] morpholine) (Lucić Vrdoljak *et al*., 2006). At this point, we demonstrated that therapy with TCP and atropine given 1 min after soman poisoning afforded especially good protection of the rats from soman-induced seizures and also from toxic effects associated with muscarinic receptors, such as salivation, lacrimation, diarrhea, and other secretory activities. Conversely, atropine alone provided very little or no protection of rats poisoned by soman (unpublished). These results are in accordance with the fact that experimental animals intoxicated with OPc survived only if treated with atropine, which reduces the muscarinic receptor-associated effects, but provided little or no protection against nicotinic receptor effects (Gupta *et al*., 2007). On the other hand, TCP is a compound with low affinity for nicotinic and muscarinic receptors and its antimuscarinic efficacy can be improved with increased bulkiness of the group in the cyclohexyl ring (Gabryelewicz *et al*., 1980). However, TAMORF, newly synthesized and modified compound with the greatest bulkiness of the substituted group, was less effective in the same experimental conditions (Table 1) (Lucić Vrdoljak *et al*., 2006). Data presented in Fig. 2 B show that TCP in combination with atropine as therapy (regardless of the applied dose of soman) afforded significant protection of plasma enzyme activities by preventing a critical level of inhibition, and consequently prevented rats from convulsions and seizures induced by soman toxicity. Although TCP itself does not cause an additional inhibition of the enzyme activity or other side effects, it seems to be less effective as a pretreatment in both plasma and brain (Fig. 2. A, C). Accordingly, our results are very affirmative since conventionally used pyrydostigmine and physostigmine are cholinesterase inhibitors that, applied as a pretreatment, might exhibit additional adverse effects (Petrioanu *et al*., 2005; Lucić Vrdoljak *et al*., 2006). Generally, our findings confirm that not only pretreatment with TAMORF is able to protect animals from the lethal effects of soman, but also TCP as a therapy is able to eliminate most of soman-induced signs of toxicity in rats. The obtained data also suggest the possibility that, applied in combination, TAMORF as a pretreatment and TCP and atropine as a therapy, can adequately block all neuronal hyperactivities and processes associated with soman exposure.

Previous studies revealed that both tested compounds exert various pharmacological effects by multiple mechanisms that are not related to AChE interactions (Škare *et al*., 2002; Ferle-Vidović *et al*., 1993; 1995; Wang *et al*., 2004a; 2004b; Radić *et al*., 2006). Since both TCP and TAMORF lack the oxime group, it is hard to believe that they can reactivate an already inhibited AChE, like oxime does (Radić *et al*., 2007). A more plausible hypothesis concerning the biochemical mechanism of TCP and TAMORF interaction with the enzyme could be allosteric modulation of the active centre of AChE, which in turn could lead to their hindrance and unavailability to soman. For this reason, it seems that the antidotal mechanism of these compounds is related only to the excitatory amino acid glutamate neurotransmitter system and especially to NMDA receptors involved in the processes associated with soman exposure

(McDonough & Shih, 1993; Carpentier, 1994; Lallement *et al*., 1998b; Raveh *et al*., 1999; 2002). In this view, as an NMDA receptor antagonist, TCP might be protective simply by blocking the seizure activity following soman intoxication (Carpentier *et al*., 1994; 2001; De Groot *et al*., 2001).

Given that TCP is often used as a basic structure in the synthesis of many other biologically active compounds applied in the treatment of neurological disorders (Geerts & Grossberg, 2006), it seems reasonable to evaluate its toxicity profile. Since there are no published data on TCP genotoxicity *in vivo*, the second part of this study aimed to elucidate that subject. The results of our earlier *in vitro* study on human white blood cells suggest limited genotoxicity and promissing radioprotective properties of TCP (Radić *et al*., 2006). The present study reports for the first time the results concerning genotoxic and cytoprotective effects of TCP on rat blood and brain cells *in vivo*. The results obtained indicate that TCP shows an acceptable genotoxicity to rats in the concentration tested, similarly as it was observed earlier with TAMORF (Lucić Vrdoljak *et al*., 2006). However, genotoxicity studies indicate a higher biocompatibility of TCP than of TAMORF, since *in vitro* TAMORF acts as a mitotic inhibitor in human peripheral blood lymphocytes (Radić *et al*., 2006). Nevertheless, before the final decision on a wider use of TCP it is necessarry to study other biological aspects, for instance its possible interference with metabolic pathways and modification of its structure, which might occur and affect its activity.

Taken together, our results reveal an acceptable genotoxicity profile of tenocyclidine but indicate its controversial role in brain protection of soman-poisoned rats that should be studied further.

Acknowledgements

We wish to thank Mrs. J. Mileković, Mrs. M. Kramarić and Mrs. M. Matašin for technical assistance. The authors also wish to thank Dr. D. Škare, Institute Ruđer Bošković (Zagreb, Croatia), for preparing tenocyclidine. This study was supported by the Ministry of Science, Education and Sports of the Republic of Croatia (Grants No. 022-0222148-2139 and 022-0222148-2137).

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