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Effect of Sodium Bicarbonate in Rats Acutely Poisoned with Dichlorvos

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Abstract: The development of effective antidotes against organophosphates such as dichlorvos has been a persistent challenge over the past decades. Therapy of organophosphate poisoning is based on the administration of atropine and oxime as standard antidotes. The present study was undertaken to evaluate the ability of sodium bicarbonate to improve protective effects of standard antidotes in rats poisoned with dichlorvos. The aim of this experiment was to establish the correlation between protective effects and biochemical parameters relevant for acid-base status. In order to examine the protective effect of both standard antidotes and their combinations, groups of experimental animals were poisoned subcutaneously with increasing doses of dichlorvos. Immediately thereafter, rats were treated with atropine 10 mg/kg intramuscularly, oximes 10 mg/kg intramuscularly and sodium bicarbonate 3 mmol/kg intraperitoneally. These antidotes were administered either as single doses or in combinations. In the biochemical part of the experiments, rats were poisoned with dichlorvos 1.3 LD_{50} (10.64 mg/kg) subcutaneously and immediately thereafter treated with atropine 10 mg/kg intramuscularly, oximes (trimedoxime or obidoxime) 10 mg/kg intramuscularly and sodium bicarbonate 3 mmol/kg intraperitoneally either as single doses or in combinations. Parameters relevant for acid-base status were measured 10 minutes after the administration of antidotes. The results of our study indicate that addition of sodium bicarbonate to standard antidotes significantly improves protective effects of atropine, obidoxime and trimedoxime. Correlation between protection and biochemical outcome is clearly evident when sodium bicarbonate is being added to atropine.

Development of effective antidotes against organophosphates such as dichlorvos has been a persistent challenge during the past decades. Characteristic signs and symptoms of acute organophosphate intoxication are generally thought to arise from the inhibition of acetylcholinesterase (AChE, EC 3.1.1.7) and the ensuing acute cholinergic crisis. Within the spectrum of pathophysiological changes resulting from acute organophosphate intoxication, ventilatory insufficiency clearly represents the most life-threatening element of their toxicity. Organophosphate-induced ventilatory insufficiency is manifested as: (a) an increased airway resistance and obstruction caused by combined bronchoconstriction (the so-called pulmonary muscarinic syndrome), and excessive mucus secretion, (b) depolarisation neuromuscular blockade of respiratory-related musculature, and (c) failure of central respiratory drive (Adler *et al.* 1992; Karalliedde & Henry 2001).

Therapy of organophosphate poisoning is based on the administration of atropine and oximes as standard antidotes (Willems & Belpaire 1992; Karalliedde & Szinicz 2001). Atropine effectively antagonises the muscarinic actions of acetylcholine excess (increased tracheobronchial secretions, bradycardia, salivation, bronchoconstriction). However, it is well known that atropine does not have effect on nicotinic receptor sites.

Oximes are well recognised as reactivators of inhibited AChE (Bismuth 1992; Worek *et al.* 1996, 1997 & 2004; Thiermann *et al.* 1999). Until today, oxime which possesses properties of "universal" AChE reactivator has not been synthesised. Therefore, oximes are not equally effective in antagonising the toxicity of structurally different organophosphates. It has been shown that among the currently used oximes trimedoxime (1,3-bis-(4-hydroxyiminomethylpyridinium)-1-propane dichloride) and obidoxime (bis-(4-hydroxyiminomethyl-pyridinium)-1-methylether dichloride) are very efficient antidotes in poisonings caused by the majority of OP insecticides (Jokanovic & Maksimovic 1995; Worek *et al.* 1996; Antonijevic *et al.* 2005). Despite the fact that effects of organophosphates were extensively investigated and well documented, there is still a wide range of problems concerning therapeutic approaches to the organophosphate poisoning.

During the last few years further improvement of the therapy was also made with the introduction of sodium bicarbonate ($NaHCO_3$). It has been reported that administration of $NaHCO_3$ potentates therapeutic activity of atropine in acute organophosphate poisonings. According to the

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clinical findings, Balali-Mood *et al.* (2000 & 2002) concluded that $NaHCO_3$ could be useful as a part of therapeutic regimen in human organophosphate poisoning.

The present study was undertaken to evaluate the ability of $NaHCO_3$ to improve protective effects of standard antidotes in rats poisoned with dichlorvos. The aim of this trial was also to investigate the possible correlation between the protective effects and the biochemical parameters relevant for acid-base status.

Materials and Methods

Chemicals. Dichlorvos (2,2-dichlorovinyldimethylphosphate) was purchased from CIBA, Basel, Switzerland. Trimedoxime and obidoxime were obtained from the Military Technical Institute, Belgrade, Serbia and Montenegro. The purity of the oximes was analysed by HPLC technique and it was greater than 99%. Atropine was purchased from Sigma Chemical Company, St. Louis, MO, USA. All the other chemicals of analytical grade were purchased from commercial sources. Stock solution of dichlorvos was prepared in isopropanol. Oximes were dissolved in distilled water and diluted to the required concentration immediately before use.

Animal experiments. Males Wistar rats (180–240 g) were obtained from the Military Medical Academy, Belgrade, Serbia and Montenegro. The animals were acclimatised for at least one week prior to use and received food and tap water *ad libitum.*

The study protocol was based on the Guidelines for Animal Study no. 282–12/2002 (Ethics Committee of the Military Medical Academy, Belgrade, Serbia and Montenegro).

Protection. In order to examine the protective effect of antidote(s), five groups of experimental animals (n=8 per group) were poisoned subcutaneously with increasing doses of dichlorvos. Immediately thereafter rats were treated with atropine 10 mg/kg intramuscularly, oximes 10 mg/kg intramuscularly and $NaHCO_3$ 3 mmol/kg intraperitoneally. These antidotes were administered either as single doses or in combinations. After the 24 hr survival registration, median lethal doses (LD_{50}) were calculated according to the method of Litchfield & Wilcoxon (1949), with 95% confidence limits. Protective indices were calculated according to the following equation:

$$\text{Protective indices (antidote)} = \frac{LD_{50}\,(\text{dichlorvos}+\text{antidote})}{\text{LD}_{50}\,(\text{dichlorvos})}$$

Biochemistry. The biochemical experiments were divided into two parts. In the first part of the experiments, aimed to find out the optimal time interval relevant for the assessment of biochemical parameters, arterial blood samples were collected 10, 20 and 30 min. after the administration of $NaHCO_3$ 3 mmol/kg intraperitoneally.

Within the second part of the biochemical experiments, when the time period between the application of tested substances and arterial blood sampling was fixed at 10 min., rats were poisoned with dichlorvos 1.3 LD_{50} (10.64 mg/kg) subcutaneously and immediately thereafter treated with atropine 10 mg/kg intramuscularly, oximes (trimedoxime, obidoxime) 10 mg/kg intramuscularly and $NaHCO_3$ 3 mmol/kg intraperitoneally either as single doses or in combinations. In the biochemical sets of experiments partial pressure of oxygen, partial pressure of carbon dioxide, total CO_2 content, base excess, standard bicarbonate, base excess in extracellular fluids, oxygen saturation calculated at a normal p50 (p50 is the oxygen tension at half saturation of blood), pH and concentration of bicarbonate anion (HCO_3^-) in arterial blood were measured by the Blood Gas Analyser – BGM (model IL 1312), Instrumentation Laboratory, Lexington, USA. Arterial blood samples were drawn by intracardial puncture using heparinised syringe and kept on ice until analysing.

Data analysis. Statistical significance was determined by means of Student's t-test and Mann-Whitney U-test, and differences were considered significant when $P<0.05$, $P<0.01$ and $P<0.001$.

Results

Protection. Co-administration of $NaHCO_3$ significantly increased the protective effects of standard antidotes given alone in rats poisoned with dichlorvos (fig. 1). Addition of bicarbonate increased protective indices of atropine, trime-

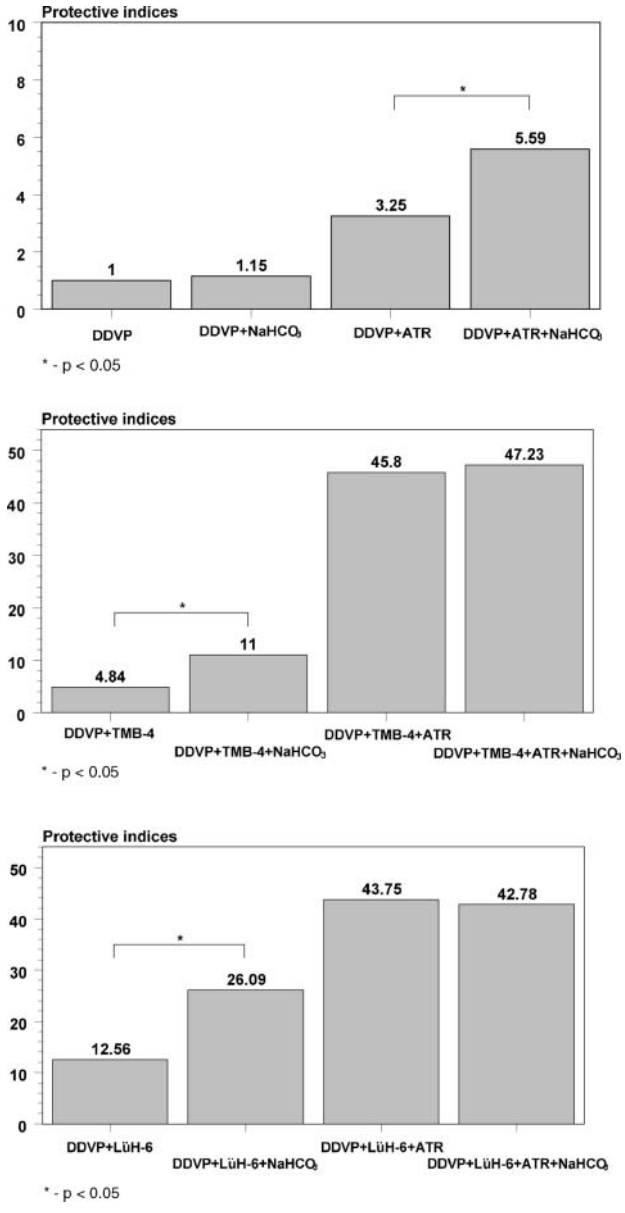

Fig. 1. Protective indices of antidotes and their combinations in rats poisoned by dichlorvos (DDVP). $NaHCO_3$ (3 mmol/kg intraperitoneally), atropine (ATR) (10 mg/kg intramuscularly), trimedoxime (TMB-4) (10 mg/kg intramuscularly) and obidoxime (LüH) (10 mg/kg intramuscularly) were given immediately after the increasing doses of DDVP (subcutaneously).

Table 1.

pO_2* and pCO_2* in the arterial blood samples of non-poisoned rats treated by $NaHCO_3$ (3 mmol/kg intraperitoneally), during 30 min. after administration (mean±S.D.).

Time after administration (min)	pH	HCO_3^- (mmol/l)	pO_2 (kPa)	pCO_2 (kPa)
0	7.43±0.03	22.40±2.12	15.10±4.66	4.46±0.59
10	7.47±0.08	29.80±2.96[b]	9.42±2.71[a]	5.40±0.82
20	7.44±0.04	26.87±3.71[a]	9.81±3.03[a]	5.22±1.02
30	7.44±0.03	25.03±2.83	11.68±0.78	4.82±0.30

[a]$P<0.05$ versus 0 min.
[b]$P<0.001$ versus 0 min.
*Abbreviations: HCO_3^-: concentration of bicarbonate anion, pO_2: partial pressure of oxygen, pCO_2: partial pressure of carbon dioxide.

doxime and obidoxime by 1.72, 2.27 and 2.07 times, respectively. Obidoxime given alone provided better protection of experimental animals than trimedoxime, i.e. protective indice value of obidoxime was 2.59 times greater than that of trimedoxime.

Effects of sodium bicarbonate. In the first part of biochemical experiments, maximal changes were obtained 10 min. after the administration of $NaHCO_3$ (tables 1 & 2). During the biochemical experiment, blood gas partial pressure of oxygen value decreased significantly from the saline group and caused the fall in oxygen saturation value. Expectedly, the partial pressure of carbon dioxide value increased, as well as total CO_2 content. Compared to the control groups, the values of the base excess and the base excess in extracellular fluids were significantly higher during the 20 min. period after the administration of $NaHCO_3$. Elevation in standard bicarbonate value was also present and significantly different from saline group in the first two time-points observed.

Effects of dichlorvos. Since there was a statistically significant difference in blood partial pressure of oxygen and partial pressure of CO_2 values between dichlorvos-treated rats

Table 2.

Acid-base status parameters in the arterial blood samples of non-poisoned rats treated by $NaHCO_3$ (3 mmol/kg intraperitoneally) during 30 min. time period after administration (mean±S.D.).

Time after administration (min.)	ct CO_2* (mmol/l)	BEb* (mmol/l)	SBC* (mmol/l)	BEecf* (mmol/l)	sO_2c* (%)
0	23.45±2.24	−0.56±1.74	24.46±1.36	−2.06±2.07	97.83±1.48
10	31.06±2.93[a2]	6.24±3.78[a1]	29.64±3.01[a1]	6.00±3.89[a1]	92.50±5.93
20	28.10±3.89[a]	3.25±2.82[a]	27.28±2.17[a]	2.55±3.59[a]	91.28±10.90
30	26.13±2.89	1.76±2.91	26.26±2.28	0.71±3.29	96.96±0.49

[a, a1, a2]$P<0.05$, 0.01, 0.001 versus 0 min.
* Abbreviations: ct CO_2: total CO_2 content, BEb: base excess, SBC: standard bicarbonate, BEecf: base excess in extra-cellular fluids, sO_2c: oxygen saturation calculated at a normal p50 (p50 is the oxygen tension at half saturation of blood) in arterial blood.

Table 3.

Values (mean±S.D.) of pH, bicarbonate (HCO_3^-), partial pressure of oxygen (pO_2) and carbon dioxide (pCO_2) in the arterial blood of rats 10 min after poisoning with 1.3 LD_{50} of dichlorvos (DDVP) (10.64 mg/kg subcutaneously) and treatment with atropine (10 mg/kg intramuscularly), trimedoxime (TMB-4)/obidoxime (LüH-6) (10 mg/kg intramuscularly) and $NaHCO_3$ (3 mmol/kg intraperitoneally).

Treatment	pH	HCO_3^- (mmol/l)	pO_2 (kPa)	pCO_2 (kPa)
Saline	7.43±0.03	22.40±2.12	15.02±4.34	4.52±0.61
$NaHCO_3$	7.47±0.08	29.80±2.96[a2,b1]	9.42±2.71[a]	5.40±0.82
DDVP	7.11±0.02[a]	23.15±2.12	2.73±2.87[a1]	10.28±4.24[a2]
DDVP+atropine	7.14±0.18	22.48±4.11	3.33±2.37[a1]	9.08±3.35[a2]
DDVP+atropine+$NaHCO_3$	7.39±0.07[b1,c]	26.92±2.75[a1,b,c]	6.20±3.88[a]	5.98±1.21[a1,b]
DDVP+TMB-4	7.35±0.07[a,b]	18.55±4.12[b]	10.26±4.42[b2]	4.47±1.21[b2]
DDVP+TMB-4+$NaHCO_3$	7.38±0.13[b]	20.28±4.38	11.16±3.15[b2]	5.36±1.39[b]
DDVP+LüH-6	7.33±0.09[a,b]	15.88±1.54[a2,b2]	10.82±4.62[b1]	4.06±0.95[b]
DDVP+LüH-6+$NaHCO_3$	7.37±0.15[b]	19.75±3.55[d]	12.81±2.32[b2]	4.50±1.08[b1]

[a,a1,a2]$P<0.05$, 0.01, 0.001 significantly different from the saline group.
[b,b1,b2]$P<0.05$, 0.01, 0.001 significantly different from DDVP group.
[c]$P<0.05$ significantly different from DDVP+atropine group.
[d]$P<0.05$ significantly different from DDVP+LüH-6 group.

Table 4.

Acid-base status parameters (mean±S.D.) in the arterial blood of rats 10 min. after poisoning with 1.3 LD_{50} dichlorvos (DDVP) (10.64 mg/kg subcutaneously) and treatment with atropine (10 mg/kg intramuscularly) and $NaHCO_3$ (3 mmol/kg intraperitoneally).

Treatment	ct CO_2* (mmol/l)	BEb* (mmol/l)	SBC* (mmol/l)	BEecf* (mmol/l)	sO_2c* (%)
Saline	23.08±2.19	−0.52±1.86	24.98±1.30	−2.16±2.01	98.65±1.62
$NaHCO_3$	31.06±2.93[a2]	6.24±3.78[a1]	29.64±3.01[a1]	6.00±3.89[a1]	92.50±5.93
DDVP	25.51±2.01	−7.25±5.50[a]	17.31±5.11[a1]	−6.53±4.57	31.00±37.34[a1]
DDVP+atropine	24.60±3.99	−7.13±6.80[a]	17.63±5.79[a]	−6.71±6.44	38.05±29.59[a2]
DDVP+atropine+$NaHCO_3$	28.30±2.93[a1]	2.05±2.4[b1,c]	25.71±1.81[b1,c1]	1.71±2.78[a,b1,c]	69.88±14.49[a2,b,c]

[a,a1,a2]P<0.05, 0.01, 0.001 significantly different from the saline group.
[b,b1]P<0.05, 0.01 significantly different from DDVP group.
[c,c1]P<0.05, 0.01 significantly different from DDVP+atropine group.
* Abbreviations: ct CO_2: total CO_2 content, BEb: base excess, SBC: standard bicarbonate, BEecf: base excess in extra-cellular fluids, sO_2c: oxygen saturation calculated at a normal p50 (p50 is the oxygen tension at half saturation of blood) in arterial blood.

and saline group, a shift in oxygen calculated value from 98.65% to 31.00% was expected (table 3). Depletion of both the base excess and the base excess in extra-cellular fluids was also present (table 4).

Effects of sodium bicarbonate and atropine in dichlorvos-poisoned rats. Introduction of atropine had no influence on tested biochemical parameters in rats poisoned with DDVP (tables 3 & 4). Addition of $NaHCO_3$ to atropine induced statistically significant correction in partial pressure of CO_2 value. Much better results were developed for four parameters: oxygen saturation, base excess, standard bicarbonate and base excess in extracellular fluid.

Effects of sodium bicarbonate and oximes in dichlorvos-poisoned rats. Introduction of trimedoxime and obidoxime as single doses or with sodium bicarbonate significantly increased values of arterial blood (tables 3 & 5). Co-administration of $NaHCO_3$ to obidoxime produced statistically significant improvement in total CO_2 content. However, no statistically significant improvement could be seen when the following three parameters were evaluated: base excess, standard bicarbonate, base excess in extracellular fluids, although addition of $NaHCO_3$ improved base excess and base excess in extracellular fluids values obtained when obidoxime was given alone (table 5).

Discussion

Since the $NaHCO_3$-induced improvement in protective effects of of atropine and oximes in rats poisoned with dichlorvos could not be attributed to the increase in the AChE activity neither in brain, nor in the respiratory muscles (Antonijevic *et al.* 2002), our assumption was that this benefitial effect could be ascribed to mere correction of the respiratory acidosis, caused by combined central and peripheral effects of dichlorvos.

In the present paper co-administration of sodium bicarbonate improved significantly protective effects of atropine, trimedoxime and obidoxime used in rats poisoned with dichlorvos and this result confirms the similar findings reported by the other authors.

So far, it has been reported that administration of $NaHCO_3$ potentiates therapeutic activity of atropine in acute organophosphate poisonings (Balali-Mood *et al.* 2000 & 2002; Bajgar & Portmann 2001; Karalliedde & Szinicz 2001). Animal studies on this topic have suggested that $NaHCO_3$ therapy decreased the rate of mortality in organo-

Table 5.

Acid-base status parameters (mean±S.D.) in the arterial blood of rats 10 min. after poisoning with 1.3 LD_{50} dichlorvos (DDVP) (10.64 mg/kg subcutaneously) and treatment with trimedoxime (TMB-4) or obidoxime (LüH-6) (10 mg/kg intramuscularly) and $NaHCO_3$ (3 mmol/kg intraperitoneally).

Treatment	ct CO_2* (mmol/l)	BEb* (mmol/l)	SBC* (mmol/l)	BEecf* (mmol/l)	sO_2c* (%)
Saline	23.08±2.19	−0.52±1.86	24.98±1.30	−2.16±2.01	98.65±1.62
$NaHCO_3$	31.06±2.93[a2]	6.24±3.78[a1]	29.64±3.01[a1]	6.00±3.89[a1]	92.50±5.93
DDVP	25.51±2.01	−7.25±5.50[a]	17.31±5.11[a1]	−6.53±4.57	31.00±37.34[a1]
DDVP+TMB-4	19.58±4.39[b]	−5.46±3.73[a]	20.35±2.77[a1]	−7.23±4.34[a]	87.48±14.28[b1]
DDVP+TMB-4+$NaHCO_3$	21.50±4.48	−4.98±5.37	20.75±4.10	−6.16±5.55	87.88±6.38[a1,b1]
DDVP+LüH-6	16.82±1.59[a2,b2]	−8.12±2.54[a2]	18.32±2.18[a2]	−10.28±2.31[a2]	88.42±13.61[b1]
DDVP+LüH-6+$NaHCO_3$	20.80±3.38[b,c]	−3.98±5.96	21.76±4.72	−5.66±5.75	96.25±3.88[b1]

[a,a1,a2]P<0.05, 0.01, 0.001 significantly different from the saline group.
[b,b1,b2]P<0.05, 0.01, 0.001 significantly different from DDVP group.
[c]P<0.05 significantly different from DDVP+LüH-6 group.
* Abbreviations: ct CO_2: total CO_2 content, BEb: base excess, SBC: standard bicarbonate, BEecf: base excess in extra-cellular fluids, sO_2c: oxygen saturation calculated at a normal p50 (p50 is the oxygen tension at half saturation of blood) in arterial blood.

phosphate poisonings (Cordoba *et al.* 1983; Wong *et al.* 1998; Bajgar & Portmann 2001). Bajgar & Portmann (2001) examined the potential beneficial role of $NaHCO_3$ (3 mmol/kg intraperitoneally) in rats intoxicated with 2 LD_{50} subcutaneously of sarin, dichlorvos or pyridostigmine and reported that administration of $NaHCO_3$ had a therapeutic effect in organophosphate and pyridostigmine intoxication, even more so when combined with atropine. In the study published by Balali-Mood *et al.* (2000), patients with diagnosed organophosphate poisonings (mainly suicidal) were treated intravenously with atropine plus $NaHCO_3$ (3 meq./kg over one hour, followed by 3 meq./kg in 1 l of dextrose per day until recovery/death). According to the clinical findings, these authors concluded that $NaHCO_3$ could be useful as a part of therapeutic regimen in human organophosphate poisoning. Another study of the same group of authors showed that infusion of high doses of $NaHCO_3$ (5 meq./kg during 30 min., followed by 5–6 meq./kg/day to obtain a pH in the arterial blood of around 7.5) was effective and significantly decreased the total atropine dose used in the treatment of patients with organophosphate pesticide poisoning (Balali-Mood *et al.* 2002). However, in the papers cited, there was no evidence/explanation about the underlying mechanism(s) of beneficial action of $NaHCO_3$.

Early findings on this topic (Jeevarathinam *et al.* 1988) demonstrated that $NaHCO_3$ pretreatment in rats poisoned with diisopropyl fluorophosphate significantly enhanced the therapeutic efficacy of pralidoxime chloride, with an increase of protective indices from 7.63 to 11.7. In order to explain the mode of action of $NaHCO_3$, the same authors also investigated pharmacokinetic properties of pralidoxime, showing that bicarbonate application led to a significant increase in oxime distribution into the tissue compartment.

In our previously published study (Antonijevic *et al.* 2002), where standard antidotes were given along with $NaHCO_3$ in rats poisoned with 0.75 LD_{50} of dichlorvos, brain, diaphragmal and erythrocyte AChE activities were evaluated. Concerning the results of Jeevarathinam *et al.* (1988), it was expected to obtain better reactivation of the inhibited AChE in target tissues. However, introduction of $NaHCO_3$ increased neither the trimedoxime- nor obidoxime reactivating effects. Therefore, in the present study, we made an attempt to further explain the improvement of the protective effects of all three antidotes obtained by their coadministration with sodium bicarbonate, through analysis of the biochemical parameters that are relevant for the assessment of the acid-base status.

If we briefly summarise our current results, there are several important findings worthy of being pointed out and discussed in view of the other available publications.

First, administration of bicarbonates to non-poisoned rats does not result in change of the pH of arterial blood and partial pressure of CO_2 levels but, especially 10 min. after bicarbonate administration, significantly increases the bicarbonate content of the blood, total CO_2 content, standard bicarbonate and base excess in blood and extracellular fluid and decreases the partial pressure of O_2 values, a finding that matches the description of metabolic alkalosis.

Experiments in bigger animals, like cats, in whom a larger quantity of $NaHCO_3$ can be administered (7 mmol/kg), such a metabolic alkalosis led also to the decrease in the partial O_2 pressure and increase in the partial pressure of CO_2, but these changes were accompanied by an increase in the arterial blood pH, from 7.383, to 7.650. The proposed mechanism was that the increased arterial pH caused a decrease in chemoreceptor activity, thus rendering this feed-back mechanism less susceptible to hypercapnia (Pokorski & Lahiri 1982). This mechanism can explain the increase in partial pressure of CO_2 in non-poisoned rats injected with $NaHCO_3$.

Second, intoxication with dichlorvos leads to a severe respiratory acidosis, characterised by pH of 7.11, almost a 6 times decrease in partial pressure of O_2, a drop 3 times in oxygen saturation, doubling of partial pressure of CO_2 and a big base and bicarbonate deficit. These findings are consistent with respiratory failure, shown by (Takahashi *et al.* 1991).

Experiments in goats with intravenous injection of a sublethal dose of dichlorvos have shown that, in the absence of hypersecretion of saliva and nasal discharge, the respiratory distress developed, characterised by an increase in the total pulmonary resistance and skeletal muscle faciculations could be explained by the overstimulation of bronchial smooth muscle muscarinic and myoneural nicotinic receptors, respectively. In three out of five animals even a post-inspiratory pause was noted, suggesting the impairment of the central respiratory drive. Due to the low dose of dichlorvos used, these changes did not result in significant changes of minute ventilation and arterial oxygen and carbon dioxide partial pressures (Bakima *et al.* 1989).

An additional proof to the fact that a dose of dichlorvos sufficiently high to induce the detrimental respiratory effects is the experimental study performed in German shepherd dogs, where dichlorvos 60 mg/kg orally failed to cause any cholinergic symptoms, although the erythrocyte acetylcholinesterase activity was inhibited by 43.7% (Dellinger *et al.* 1987).

Respiratory failure, following acute exposure to organophosphate anticholinesterase agents has been well studied. Rickett *et al.* (1986) examined the effects of the nerve agents, soman, sarin, tabun and VX on diaphragm contraction, diaphragm electromyogram, phrenic nerve activity, medullary respiratory-related unit activity and airflow in the cat. They concluded that the loss of central respiratory drive was the predominant cause of the nerve agent-induced respiratory failure. Soman-induced respiratory failure in awake, non-anaethetised guinea pigs has been investigated by Chang *et al.* (1990). Respiratory response to soman typically begins with hyperpnoea, which is followed by dyspnoea, hypopnoea and finally respiratory failure. Another study has been conducted by using guinea-pigs in sarin poisoning (Taysse *et al.* 2003). The results have shown that respiratory distress was revealed by a significant decrease in

minute ventilation, tidal volume and frequency rate due to an apnoeic state. Worek *et al.* (1994a & 1995) reported that VX and sarin poisoning caused a rapid respiratory arrest in a guinea-pig model.

Using domestic pigs percutaneously exposed to VX, at the time of apnoea, Chilcott *et al.* (2003) demonstrated statistically significant elevation in K^+, inorganic phosphates, partial pressure of CO_2 and partial pressure of CO_2, as well as the decrease in arterial blood pH. In the same study value of HCO_3^- remained unchanged. In the study published by Peng *et al.* (2004), aimed to assess the efficacy of haemoperfusion in the treatment of the patients with acute severe dichlorvos poisoning, respiratory failure was diagnosed as respiratory distress, hypoventilation (partial pressure of O_2 of <60 mmHg, partial pressure of $CO_2>45$ mmHg) accompanied by acidaemia ($pH<7.30$).

The aforementioned respiratory distress induced by the organophosphates is a sum of the central and peripheral cholinergic effects, the former consisting of impairment and, finally, loss of the respiratory drive and the latter comprising the so-called "pulmonary muscarinic syndrome" (laryngospasm, bronchospasm and bronchorrhoea) and the depolarisational nicotinic receptor-mediated respiratory skeletal muscle blockade (Rivet & Potgieter 1987; van Helden *et al.* 1996; Chatonnet *et al.* 2003). It seems that the muscarinic receptors responsible for central nervous system effects, including the impairment of the respiratory centre, belong to the M_2 subtype, while the ones, whose hyperstimulation is responsible for the bronchial symptomatology, are identified as M_1 subtype (Maynard & Beswick 1992).

Third, bicarbonates significantly increased the protective indices of atropine, trimedoxime and obidoxime monotherapeutic regimens in dichlorvos intoxication, but failed to add some more protection to atropine + oxime combinations. It is well known that atropine can protect experimental animals exposed to various incapacitating or lethal doses of dichlorvos (Savic *et al.* 2003). Effectiveness of atropinisation was also proven in human beings poisoned with dichlorvos (Singh *et al.* 2001).

It seems that atropine, being both centrally and peripherally acting antimuscarinic drug, is especially important in antagonising the central effects of dichlorvos, since it was shown that only atropine and not only peripherallyacting antimuscarinic drugs glycopyrrolate or ipratropium bromide pre-treatment can protect rats from the death 10 min. following intoxication with dichlorvos (Bird *et al.* 2003; Dickson *et al.* 2003).

It is, however, evident that the peripheral component of the toxicity of dichlorvos in our experiment was also important, since the bispyridinium oxime obidoxime, known for its limited passage through the blood-brain barrier into the brain, also exerted an efficient protection against dichlorvos-induced lethality in our previous and current experiments (Antonijevic *et al.* 2002; Stefanovic *et al.* 2004). These effects should be ascribed to their reactivating potencies, proven in several species poisoned with dichlorvos (Gyrd-Hansen & Kraul 1984). It was even shown *in vitro* that obidoxime is more effective than 2-PAM, HI-6 and HLö-7 in reactivation of human erythrocyte AChE inhibited with dichlorvos (Worek *et al.* 1996).

Fourth, although offering some protection in survival of rats intoxicated with dichlorvos, atropine treatment failed to correct the dichlorvos-induced respiratory acidosis. This finding contrasts the results of an experimental study, performed in calves, where atropine, promptly and completely, antagonised the decreased pulmonary compliance and arterial oxygen tension and increased total pulmonary resistance (Lekeux *et al.* 1986). Similar results were obtained after treatment of dichlorvos-poisoned goats with atropine, which could not antagonise only the central nervous symptoms and skeletal muscle fasciculations (Bakima *et al.* 1989).

The explanation for these discrepancies is the different dose of dichlorvos used, the one in our experiment being larger than in the others. Part of the explanation could also be the various doses of atropine and different animal used in these experiments. In accordance with these assumptions are the results of the experimental study performed in guinea-pigs, where atropine 10 mg/kg intravenously could antagonise the respiratory failure caused by tabun 60 μg/kg intravenously, but was ineffective against 300 μg/kg intravenously of tabun (Worek *et al.* 1994b).

Fifth, addition of bicarbonate to atropine, but even more oxime monotherapies, corrected the dichlorvos-induced respiratory acidosis. In case of the atropine+$NaHCO_3$ combination, the result mentioned could be explained by the additive muscarinic-blockig action of atropine and the blood acidity-neautralising action of $NaHCO_3$. In addition, it was shown in man that the intravenous injection of $NaHCO_3$ can induce a significant increase in the cerebrospinal fluid partial pressure of O_2 (Ryba & Pokorski 1981).

As mentioned before, the bispyridinium oximes trimedoxime and obidoxime can reactivate practically only the dichlorvos-inhbited AChE outside of the brain, i.e. the peripheral AChE, due to their extremely limited access to the CNS. This finding was confirmed in our previous experiments, where trimedoxime and obidoxime effectively reactivated erythrocyte and diaphragmal AChE, but failed to reactivate brain AChE in rats poisoned with 0.75 LD_{50} of dichlorvos (Antonijevic *et al.* 2002).

Logical explanation of the aforementioned efficacy of the oximes in correcting the dichlorvos-induced arterial blood gas impairment would be that reactivation of the peripheral AChE alleviates the toxic effects of dichlorvos on respiratory muscles and lungs.

Sixth, addition of bicarbonate to atropine plus oxime therapeutic regimen failed to afford extra protection and did not further add to the correction of biochemical respiratory parameters. It is to be assumed that the atropine + trimedoxime or obidoxime combinations were sufficiently effective to antagonise the respiratory-impairments caused by 1.3 LD_{50} of dichlorvos. Poisonings with larger doses of dichlorvos, however, might require the use of $NaHCO_3$, as well.

Possibly new insight into the problem of the mechanism of toxic effects of the organophosphates on respiration and of the mechanism of antidotal action of the oximes may have recently been produced by monitoring the respiratory function in wild-type and AChE-knock-out mice, lacking all (homozygotes) or half of the usual AChE activity (heterozygotes). It was concluded that butyrylcholinesterase might also play a significant role in the respiratory toxicity of organophosphates (Boudinot *et al.* 2005).

Based on the results presented in this paper it is evident that administration of $NaHCO_3$ improved antidotal effects of oximes and atropine, and that clear correlation between protection and tested biochemical parameters has been obtained only when sodium bicarbonate was added along with atropine.

The beneficial role of $NaHCO_3$ is a result of complex biochemical reactions focused on compensation of acidosis induced by severe organophosphate poisoning. Additionally, alkalinisation of the blood with sodium bicarbonate might increase the hydrolysis of the esteratic portion of organophosphate molecule, thus decreasing its toxicity (Karalliedde & Szinicz 2001).

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References

Adler, M., D. H. Moore & M. G. Filbert: Effects of anticholinesterases on airway smooth muscle. In: *Clinical and experimental toxicology of organophosphates and carbamates.* Eds.: B. Ballantyne & T. C. Marrs. Butterworth-Heinemann, Oxford 1992, pp. 149–156.

Antonijevic, B., D. Stefanovic, Z. A. Milovanovic, M. P. Stojiljkovic, D. Bokonjic & M. Đukic: *Standard antidotes along with sodium bicarbonate in organophosphate poisoning.* Proceedings of CB Medical Treatment Symposium IV, April 28–May 3; Spiez, Switzerland 2002, pp. 12–16.

Antonijevic, B., D. Bokonjic, M. P. Stojiljkovic, V. Kilibarda, Z. A. Milovanovic, M. Nedeljkovic & M. Maksimovic: Efficacy of trimedoxime in mice poisoned with dichlorvos, heptenophos or monocrotophos. *Basic & Clinical Pharmacology & Toxicology* 2005, **96**, 111–117.

Bajgar, J. & R. Portmann: *The treatment of intoxication with selected organophosphates and a carbamate: comparison of different therapeutic approaches.* Proceedings of CBMTS-Industry II, World Congress on Chemical and Biological Terrorism. Dubrovnik, Croatia, April 22–27, 2001, pp. 180–84.

Bakima, M., H. M. Baudet, P. Lekeux & F. Lomba: Respiratory and pulmonary haemdynamic changes during experimental organophosphate poisoning in goats. *Vet. Res. Commun.* 1989, **13**, 127–133.

Balali-Mood, M., A. Shahab-Ahmadi, M. Salimifar & M. Shariate: *Effects of sodium bicarbonate in human organophosphate poisoning.* Proceedings of Chemical and Biological Medical Treatment Symposium III, Spiez, Switzerland, May 7–12, 2000, pp. 1–4.

Balali-Mood, M., M. H. Ayati & H. Ali-Akbarian: *Effects of high doses of sodium bicarbonate in acute organophospate pesticide poisoning.* Proceedings of Chemical and Biological Medical Treatment Symposium IV, Spiez, Switzerland, April 28–May 28–3, 2002, pp. 21–25.

Bird, S. B., R. J. Gaspari & E. W. Dickson: Early death due to severe organophosphate poisoning is a centrally mediated process. *Acad. Emerg. Med.* 2003, **10**, 295–298.

Bismuth, C., R. H. Inns & T. C. Marrs: Efficacy, toxicity and clinical use of oximes in anticholinesterase poisoning. In: *Clinical and experimental toxicology of organophosphates and carbamates.* Eds.: B. Ballantyne & T. C. Marrs. Butterworth-Heinemann, Oxford, 1992, pp. 555–577.

Boudinot, E., L. Taysse, S. Daulon, A. Chatonnet, J. Champagnat & A. S. Foutz: Effects of acetylcholinesterase and butyrylcholinesterase inhibition on breathing in mice adapted or not to reduced acetylcholinesterase. *Pharmacol. Biochem. Behav.* 2005, **80**, 53–61.

Chang, F. C. T., R. E. Foster, E. T. Beers, D. L. Rickett & M. G. Filbert: Neurophysiological concomitants of soman-induced respiratory depression in awake, behaving guinea pigs. *Toxicol. Appl. Pharmacol.* 1990, **102**, 233–250.

Chatonnet, F., E. Boudinot, A. Chatonnet, L. Taysse, S. Daulon, J. Champagnat & A. S. Foutz: Respiratory survival mechanisms in acetylcholinesterase knockout mouse. *Eur. J. Neurosci.* 2003, **18**, 1419–1427.

Chilcott, R. P., C. H. Dalton, I. Hill, C. M. Davidson, K. L. Blohm & M. G. Hamilton: Clinical manifestations of VX poisoning following percutaneous exposure in the domestic white pig. *Hum. Exp. Toxicol.* 2003, **22**, 255–261.

Cordoba, D., S. Cadavid, D. Angulo & I. Ramos: Organophosphate poisoning: modifications in acid-base equilibrium and use of sodium bicarbonate as an aid in the treatment of toxicity in dogs. *Vet. Hum. Toxicol.* 1983, **25**, 1–3.

Dellinger, J. A., B. C. McKiernan, G. D. Koritz & B. C. Richardson: Latent dichlorvos neurotoxicity detected by vagal tone monitoring in dogs. *Neurotoxicol. Teratol.* 1987, **9**, 197–201.

Dickson, E. W., S. B. Bird, R. J. Gaspari, E. W. Boyer & C. F. Ferris: Diazepam inhibits organophosphate-induced central respiratory depression. *Acad. Emerg. Med.* 2003, **10**, 1303–1306.

Gyrd-Hansen, N. & I. Kraul: Obidoxime reactivation of organophosphate-inhibited cholinesterase activity in pigs. *Acta vet. scand.* 1984, **25**, 86–95.

Jeevarathinam, K., A. K. Ghosh, A. Srinivasan & S. Das Gupta: Pharmacokinetics of pralidoxime chloride and its correlation to therapeutic efficacy against diisopropyl fluorophosphate intoxication in rats. *Pharmazie* 1988, **43**, 114–15.

Jokanovic, M. & A. Maksimovic: A comparison of trimedoxime, obidoxime, pralidoxime and HI-6 in the treatment of oral organophosphorus insecticide poisoning in the rat. *Arch. Toxicol.* 1995, **70**, 119–23.

Karalliedde, L. & L. Szinicz: Management of organophosphorus compound poisoning. In: *Organophosphates and health.* Eds.: L. Karalliedde, S. Feldman, J. Henry & T. Marrs. Imperial College Press, London, 2001, pp. 257–94.

Karalliedde, L. & J. Henry: The acute cholinergic syndrom. In: *Organophosphates and health.* Eds.: L. Karalliedde, S. Feldman, J. Henry & T. Marrs. Imperial College Press, London, 2001, pp. 257–94.

Lekeux, P., A. Kyavu, C. Clercx & M. Ansay: Pulmonary function changes induced by experimental dichlorvos toxicosis in calves. *Res. Vet. Sci.* 1986, **40**, 318–323.

Litchfield, J. T. & F. Wilcoxon: A simplified method of evaluating dose-effect experiments. *J. Pharmacol. Exp. Therap.* 1949, **96**, 99–113.

Maynard, R. L. & F. W. Beswick: Organophosphorus compounds as chemical warfare agents. In: *Clinical and experimental toxicology of organophosphates and carbamates.* Eds.: B. Ballantyne & T. C. Marrs. Butterworth-Heinemann, Oxford, 1992, pp. 373–385.

Peng, A., F.-Q. Meng, L.-F. Sun, Z.-S. Ji & Y.-H. Li: Therapeutic efficacy of charcoal hemoperfusion in patients with acute severe dichlorvos poisoning. *Acta pharmacol. sin.* 2004, **25**, 15–21.

Pokorski, M. & S. Lahiri: Inhibition of aorticchemoreceptor responses by metabolic alkalosis in the cat. *J. Appl. Physiol. Resp. Environ. Exer. Physiol.* 1982, **53**, 75–80.

Rickett, D. L., J. F. Glenn & E. T. Beers: Central respiratory effects versus neuromuscular actions of nerve agents. *Neurotoxicolology* 1986, **7**, 225–236.

Rivet, K & P. D. Potgieter: Diaphragmatic paralysis after organophosphate poisoning. *S. Afr. Med. J.* 1987, **72**, 881–882.

Ryba, M. & M. Pokorski: Decrease of oxygen difference between arterial blood and cerebrospinal fluid after intravenous injection of sodium bicarbonate in hyperoxic patients, anaesthetized, paralyzed and artificially ventilated. *Acta Physiol. Polon.* 1981, **32**, 611–612.

Savic, M. M., D. I. Obradovic, N. D. Ugresic & D. R. Bokonjic: The influence of diazepam on atropine reversal of behavioural impairment in dichlorvos-treated rats. *Pharmacology & Toxicology* 2003, **93**, 211–218.

Singh, S., D. Chaudhry, D. Behera, D. Gupta & S. K. Jindal: Aggressive atropinisation and continuous pralidoxime (2-PAM) infusion in patients with severe organophosphate poisoning: experience of a northwest Indian hospital. *Hum. Exp. Toxicol.* 2001, **20**, 15–18.

Stefanovic, D., B. Antonijevic, D. Bokonjic, M. P. Stojiljkovic, Z. A. Milovanovic & M. Nedeljkovic: Influence of sodium bicarbonate and standard antidotes on acid-base status in rats poisoned with dichlorvos. *Jugoslov. Med. Biohem.* 2004, **23**, 165–170.

Takahashi, H., T. Kojima, T. Ikeda, S. Suda & Y. Shirasu: Differences in the mode of lethality produced through inravenous and oral administration of organophosphorus insecticides in rats. *Fund. Appl. Toxicol.* 1991, **16**, 459–468.

Taysse, L., J.-H. Calvet, J. Buee, D. Christin, S. Delamanche & P. Breton: Comparative efficacy of diazepam and avizafone against sarin-induced neuropathology and respiratory failure in guinea pigs: Influence of atropine dose. *Toxicology* 2003, **188**, 197–209.

Thiermann, H., L. Szinicz, F. Eyer, F. Worek, P. Eyer, N. Felgenhauer & T. Zilker: Modern strategies of organophosphate poisoning. *Toxicol. Lett.* 1999, **107,** 233–239.

Van Helden, H. P., R. W. Busker, B. P. Melchers & P. L. Bruijnzeel: Pharmacological effects of oximes: how relevant are they? *Arch. Toxicol.* 1996, **70**, 779–786.

Willems, J. L. & F. M. Belpaire: Anticholinesterase poisoning: an overwiev of pharmacotherapy. In: *Clinical and experimental toxicology of organophosphates and carbamates.* Eds.: B. Ballantyne & T. C. Marrs. Butterworth-Heinemann, Oxford, 1992, pp. 536–542.

Wong, A., M. S. B. Pinheiro, C. Andrusaitis & E. Y. Matida: Comparative efficacy of *i.v.* pralidoxime vs. $NaHCO_3$ in rats lethally poisoned with o-p insecticide. *Toxicol. Lett.* 1998, **95**, 83.

Worek, F., M. Bäcker, H. Thiermann, L. Szinicz, U. Mast, R. Klimmek & P. Eyer: Reappraisal of indications and limitations of oxime therapy in organophosphate poisoning. *Hum. Exp. Toxicol.* 1997, **16**, 466–472.

Worek, F., T. Kirchner, M. Bäcker & L. Szinicz: Reactivation by various oximes of human erythrocyte acetylcholinesterase inhibited by different organophosphorus compounds. *Arch. Toxicol.* 1996, **70**, 497–503.

Worek, F., T. Kirchner & L. Szinicz: Effects of atropine and bispyridinium oximes on respiratory and circulatory function in guinea-pigs poisoned by sarin. *Toxicology* 1995, **95**, 123–133.

Worek, F., T. Kirchner & L. Szinicz: Effects of atropine, HLö 7 and HI 6 on respiratory and circulatory function in guinea-pigs poisoned by O-ethyl S-[2-(diisopropylamino) ethyl] methylphosponotioate (VX). *Pharmacology & Toxicology* 1994a, **75**, 302–309.

Worek, F., T. Kirchner & L. Szinicz: Treatment of tabun poisoned guinea-pigs with atropine, HLo 7 or HI 6: effect on respiratory and circulatory function. *Arch. Toxicol.* 1994b, **68**, 231–239.

Worek, F., H. Thiermann & L. Szinicz: Reactivation and aging kinetics of human acetylcholinesterase inhibited by organophosphonylcholines. *Arch. Toxicol.* 2004, **78**, 212–217.