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Research Article

# Protection and Reversal by Memantine and Atropine of Carbofuran-Induced Changes in Biomarkers

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ABSTRACT Male Sprague-Dawley rats injected with a single acute dose of carbofuran (1.5 mg/kg, sc) developed chewing movements and fine tremors within 5–7 min. The signs of maximal severity with hypercholinergic preponderance including muscle fasciculations, convulsions, tracheobronchial secretions, and diarrhea were evident within 15–30 min and lasted for about 2 h. Toxic signs were of central, as well as peripheral, origins. Rats were free from toxic signs by 3.5 h. Various antidotal drugs, alone or in combination with atropine sulfate (ATS), were administered as pretreatment or as therapeutic measures to alleviate carbofuran-induced cholinergic toxicity. In fact, pyridine-2-aldoxime methylchloride (2-PAM) or diazepam alone or in combination with ATS did not provide any beneficial antidotal effects. Combined pretreatment with memantine (MEM, 18 mg/kg, sc) and ATS (16 mg/kg, sc) provided complete protection against carbofuran toxicity and reversal of clinical evidence when given therapeutically. Carbofuran intoxication caused significant alterations in the activities of biomarker enzymes such as creatine kinase (CK) and lactic dehydrogenase (LDH) and their isoenzymes patterns in serum as a result of their leakage from the target organs (brain, muscles, and heart). Significant increases in the levels of transaminases (GOT and GPT) and glucose were also noted. MEM in combination with ATS provided significant protection and reversal of the induced changes in the aforementioned parameters, in addition to similar protective effects reported on target enzyme acetylcholinesterase (AChE). These results, along with those reported previously, indicate that MEM antagonizes carbamates toxicity by maintaining cell membrane permeability and integrity through multiple mechanisms, in addition to muscarinic receptor blocking effect of ATS. 

Key Words: carbamate toxicity, biomarker enzymes, antidotal drugs, creatine kinase

## INTRODUCTION

The carbamate compounds, especially those with insecticidal effect, are known to elicit poisoning in man and animals by virtue of acetylcholinesterase (AChE) inhibition at the synapses and neuromuscular junctions resulting in excessive acetylcholine (ACh) accumulation [Casida, 1963; O'Brien, 1967]. Considerable morphological and biochemical evidence also suggest that brain, skeletal muscles, and heart are the target organs for most AChE inhibitors [Petras, 1981; Lemercier et al., 1983; McLeod, 1985; Gupta and Kadel, 1989a, 1990]. Interestingly, these organs are also reported to have a greater abundance of biomarker enzymes, such as creatine kinase (CK) and lactic dehydrogenase (LDH), whose isoenzymes are relatively tissue specific [Bais and Edwards, 1982; Gupta et al., 1991a,b]. Thus, the application of CK and LDH and their characteristic isoenzymes in serum analyses, representing tissue specific damage, has become more common [Codd et al., 1977; Asztalos and Nemcsok, 1985; Sket et al., 1989; Linz et al., 1990; Bhargava et al., 1990; Shina and Condrea, 1990].

In our recent studies, we demonstrated that a commonly used carbamate insecticide carbofuran not only inhibits AChE and carboxylesterases (CarbE) but also perturbs the activities of CK and LDH and their isoenzymes patterns along with transaminases (GOT

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TABLE 1. Protocol for Antidotes Against Carbofuran Intoxication*

| Group | Treatment | Toxicant/antidotes | Schedule of antidote administration | Time of sacrifice |
|---|---|---|---|---|
| I | Control | Dimethyl sulfoxide (DMSO) | None | 1 h after DMSO |
| II | Toxicant | Carbofuran | None | 1 h after carbofuran |
| III | Antidotes | MEM + ATS | ATS 45 min after MEM | 1 h after MEM |
| IV | Prophylactic treatment | MEM + ATS + carbofuran | MEM 1 h and ATS 15 min before carbofuran | 1 h after carbofuran |
| V | Therapeutic treatment | Carbofuran + MEM + ATS | MEM and ATS 5–7 min after carbofuran | 1 h after carbofuran |

*Drug dosages: Carbofuran = 1.5 mg/kg; memantine HCl (MEM) = 18 mg/kg; and atropine sulfate (ATS) = 16 mg/kg. All the drugs were administered via sc route.

and GPT) in target tissues [Gupta et al., 1991a,b]. Alterations in the activities of enzymes and their isoenzymes were reflected in serum as a consequence of their leakage from tissues due to substantial depletion of adenosine triphosphate (ATP), which is essential for retaining intracellular enzymes by maintaining the cell membrane permeability and integrity [Woodman, 1981; Gupta et al., 1991a, 1992]. We have consistently found increased glucose to be another very sensitive biochemical parameter of carbofuran toxicity [Gupta et al., 1991a] and has also been considered as a biomarker in this study.

Until recently, antidotal treatment against carbamate poisoning consisted of only atropine sulfate (ATS), since the use of oximes or diazepam was either of no therapeutic value or in some cases accentuated the toxicity [Hayes, 1982; Murphy, 1985; Gupta and Kadel, 1989b]. In our recent reports, we demonstrated that MEM plus ATS completely prevented carbofuran and aldicarb toxicity by significant protection and/or reversal of inhibited AChE [Gupta and Kadel, 1989b, 1991a,b]. In this investigation we report that MEM in combination with ATS also reversed alterations in CK, LDH, and other related biomarkers caused by carbofuran acute poisoning. These findings further validate the therapeutic potential of these antidotes in carbamates poisoning.

## MATERIALS AND METHODS

### Animals

Male Sprague-Dawley rats weighing between 160–180 g were purchased from Sasco, Inc. (Omaha, NE). They were housed five per cage in our controlled environmental conditions: 21 ± 1°C room temperature, 50 ± 10% humidity, and 12 h light/12 h dark cycle. They had free access to pellet food (Rodent Laboratory Chow, Purina Mills, Inc., St. Louis, MO) and water. The animals were acclimatized to these conditions for at least 7 to 10 days before being used.

### Chemicals

Carbofuran (2, 3-dihydro-2, 2-dimethyl-7-benzofuranyl N-methylcarbamate), technical grade (98.1%), in a crystalline form from FMC Corporation (Agricultural Chemical Group, Princeton, NJ), and memantine HCl (1, 3-dimethyl-5-aminoadamantane hydrochloride, MEM), technical grade (90.18%), in a powder form from Merz and Company, GMBH, Frankfurt, West Germany, were received as generous gifts. The reagents and materials for the assay of transaminases (GOT and GPT), creatine kinase (CK), and lactic dehydrogenase (LDH) and their isoenzymes were obtained from the following sources: 1) Trace Scientific Pty. Ltd., Baulkham Hills, N.S.W., 2) Ciba-Corning Diagnostic Corp., Palo Alto, CA, and 3) Sigma Diagnostics, St. Louis, MO. All other chemicals used were of highest purity and purchased from Sigma Chemical Co., St. Louis, MO, and Fisher Scientific, Fair Lawn, NJ.

### Experimental Design

The experimental design including protocol for carbofuran and antidotes (MEM and ATS) administration was similar to that described previously [Gupta and Kadel, 1989b, 1991a,b] and is presented in Table 1. Briefly, this investigation was conducted on five groups of rats (five rats in each group), which were given the treatment(s) as follows. Group I, receiving dimethylsulfoxide (DMSO) in an equal volume to the tested drugs (100 μl/100 g body weight), was considered as vehicle control group. Group II rats were administered with a signs-producing sublethal acute dose of carbofuran (1.5 mg/kg). Group III rats received a combination of antidotes: MEM (18 mg/kg) + ATS (16 mg/kg). Group IV rats were treated with MEM followed by ATS, 60 min and 15 min, respectively, prior to carbofuran administration. Group V rats received these antidotes (MEM + ATS) as a therapeutic measure shortly after appearance of carbo-

furan-induced toxic signs (5–7 min after carbofuran). All injections were made subcutaneously (sc) in the back of the neck, and the volume of injection was kept constant (100 μl/100 g body weight).

The rationale for selecting the doses of carbofuran, MEM, and ATS is well described in our recent publications [Gupta and Kadel, 1989a,b, 1990, 1991a,b]. Briefly, the administration of carbofuran (1.5 mg/kg, sc) produced the onset of anticholinesterase signs within 5–7 min. With increasing propensity, the signs of maximal severity including muscle fasciculations and convulsions were attained within 15 min to 30 min. The rats were sacrificed 1 h after carbofuran injection because maximal inhibition of acetylcholinesterase (AChE) and peak toxicity signs were noted at this time [Gupta and Kadel, 1989a,b]. Further, the time to peak memantine concentration in tissues/blood was also attained within 0.5–1 h [Bleidner et al., 1965; Uchiyama and Shibuya, 1969; Osborne et al., 1982]. Since neither MEM nor ATS alone provided complete protection against carbofuran acute toxicity, administration of these two antidotes in combination was considered more appropriate. In pilot studies, prophylaxis or therapy with pyridine-2-aldoxime methylchloride (2-PAM) or diazepam singly or in combination with ATS did not provide any beneficial effects. Therefore, neither 2-PAM nor diazepam was included in the final investigation.

At predetermined times rats were decapitated by guillotine and blood samples were collected. Serum samples were harvested at room temperature by centrifugation (3,000 rpm for 30 min) of the clotted blood.

### Biochemical Procedures

#### Creatine kinase (CK) and CK isoenzymes (EC. 2.7.3.2)

The activity of total CK was determined according to the method of Serono-Baker Diagnostics, Inc. based on the method of Oliver [1955] as modified by Rosalki [1967] and Szasz et al. [1976], using the Encore Chemistry System (Baker Instruments Corp.). The isoenzymes of CK [CK-BB (CK-1), CK-MB (CK-2), and CK-MM (CK-3)] were separated by electrophoresis (Ciba-Corning, Palo Alto, CA), and the activity of each isoenzyme was quantified according to the procedure of Sigma Diagnostics using IL densitometer (Instrumentation Laboratory Inc.).

#### Lactic dehydrogenase (LDH) and LDH isoenzymes (EC. 1.1.1.27)

The activity of total LDH was determined according to the method of Serono-Baker Diagnostics, Inc. based on the method of Gay et al. [1968], using Encore Chemistry System. The isoenzymes of LDH [HHHH (LDH-1), HHHM (LDH-2), HHMM (LDH-3), HMMM (LDH-4), and MMMM (LDH-5)] were separated by electrophoresis, and the activity of each isoenzyme was quantified according to the procedure of Ciba-Corning Diagnostics Corp., using IL densitometer.

#### Transaminases (aspartate aminotransferase, GOT, EC. 2.6.1.1., and alanine aminotransferase, GPT, EC. 2.6.1.2.)

The activities of GOT and GPT were determined according to the methods of Henry et al. [1960] and Amador et al. [1967] modified from the original methods of Karmen [1955] and Wroblewski and LaDue [1956]. The activities of CK, LDH, GOT, and GPT were determined as IU/liter and also expressed in terms of percent activity of controls.

#### Glucose

The determination of glucose was done according to the method of Serono-Baker Diagnostics, Inc. using the Encore Chemistry System.

### Statistical Analyses

Data were analyzed using analysis of variance (ANOVA) coupled with Duncan's new multiple range test [Steel and Torrie, 1980].

## RESULTS

Carbofuran produced the toxic signs of onset such as salivation, chewing movements, and fine tremors within 5–7 min following its administration via sc route. Within 15–30 min animals exhibited signs of peak severity with hypercholinergic activity including muscle fasciculations and convulsions that lasted for about 2 h. Prophylaxis with MEM and ATS afforded complete protection against carbofuran acute toxicity as the animals did not show any toxic signs. Therapeutic administration of these antidotes at the time of onset signs, i.e., within 5–7 min after carbofuran injection, attenuated all the toxic signs. At no time was there evidence of recurrence of any toxic signs. MEM and ATS administration in carbofuran-untreated and treated rats did not produce any untoward effects.

Results presented in Table 2 show that carbofuran significantly enhanced the activity of total CK (163%). Detailed analyses of normal rat serum for CK-isoenzymes revealed very high activity of CK-MM compared to that of CK-BB. Carbofuran caused elevation of CK-BB by 247% and CK-MM by 131%. The isoenzyme CK-MB, which was not detectable in normal serum, showed much higher activity in carbo-

TABLE 2. Effects of MEM and ATS on Total CK and LDH Activities in Serum of Rats Intoxicated With Single Dose of Carbofuran (1.5 mg/kg, sc)†

| Group | Treatment | Enzyme activity (IU/L) | |
|---|---|---|---|
| | | Total CK | Total LDH |
| I | DMSO (control) | 4,644 ± 535 (100) | 675 ± 61 (100) |
| II | Carbofuran | 7,558 ± 283* (163) | 1,081 ± 35* (160) |
| III | MEM + ATS | 4,415 ± 655 (95) | 731 ± 110 (108) |
| IV | MEM + ATS + carbofuran | 4,811 ± 261** (104) | 681 ± 85** (101) |
| V | Carbofuran + MEM + ATS | 5,768 ± 302** (124) | 1001 ± 81 (148) |

†Values are means ± s.e.m. Numbers in parentheses indicate percentage changes compared to controls (100%). For the details of drugs and dosages, see Table 1.

*Significant difference between controls (Group I) and carbofuran-treated (Group II) rats ($P < 0.01$).

**Significant difference between carbofuran treated (Group II) and carbofuran + antidotes (Group IV or Group V)-treated rats ($P < 0.01$).

furan-treated (740 ± 93 IU/liter) rats (Fig. 1). MEM and ATS administration in carbofuran-untreated rats did not alter the activity of CK or its isoenzymes. Prophylaxis or therapy with MEM and ATS provided significant protection and reversal of carbofuran induced changes in total CK and CK isoenzymes activity (Table 2 and Fig. 1).

In normal rat serum, the activity of total LDH was less than one seventh compared to that of total CK. Normal serum had five electrophoretically distinct isoenzymes with variable activity in the following order: LDH-5> LDH-4> LDH-3> LDH-2> LDH-1. In other words, isoenzyme LDH-5 with M subunits was in abundance, while the isoenzyme LDH-1 with H subunits and no M subunit was in the least quantity (Fig. 2). Carbofuran acute toxicity raised the total LDH activity by 160% (Table 2). Under the influence of carbofuran intoxication, the activities of four out of five LDH isoenzymes (LDH-1 to LDH-4) were significantly elevated, while LDH-5 was lowered (Fig. 2). The biggest increase was noted in LDH-1 (1655%), followed by LDH-2 (768%), LDH-3 (515%), and LDH-4 (265%). Combined pretreatment of antidotes (MEM and ATS), which did not alter the activity of total LDH or any of its isoenzymes in carbofuran-untreated control rats, provided remarkable protection against carbofuran-induced alterations. These antidotes when given therapeutically reversed the induced changes in LDH isoenzymes but were unable to completely reverse the total LDH activity (Table 2, Fig. 2).

Results in Figure 3 show that carbofuran acute toxicity raised the activities of both transaminases (GOT and GPT) markedly higher than normal levels. The increase in GOT activity was significantly greater than GPT activity (300% and 191%, respectively). Activities of both transaminases were found in the normal range when rats were pretreated or treated with antidotes. This combined antidotal treatment also provided complete protection/attenuation of hyperglycemia (2.5-fold) caused by carbofuran (Fig. 4).

## DISCUSSION

The objective of this investigation was to further evaluate the efficacy of MEM and ATS, on the basis of protection and reversal of alterations in the "biomarkers," in carbofuran acute toxicity. It needs to be emphasized that the overall spectrum of carbofuran acute toxicity signs consists of muscarinic as well as nicotinic receptor associated effects [Gupta and Kadel, 1989a]. MEM alone (18 mg/kg, sc) antagonized the induced nicotinic effects but did not afford protection against muscarinic effects. On the other hand, ATS (16 mg/kg, sc) alone protected against the muscarinic effects, but not the nicotinic effects. Prophylaxis with a combination of MEM and ATS, however, completely prevented the anticipated acute toxic effects of carbofuran or attenuated these effects when given therapeutically [Gupta and Kadel, 1989b]. Therefore, in the present investigation the efficacy of MEM and ATS was evaluated only in combination.

It was demonstrated recently that carbofuran, in addition to causing inhibition of target enzyme AChE and nontarget enzyme CarbE [Gupta and Kadel, 1989b], elicited profound changes in biomarker enzymes such as LDH, CK, and transaminases (GOT and GPT), suggesting severe cytotoxicity [Gupta et

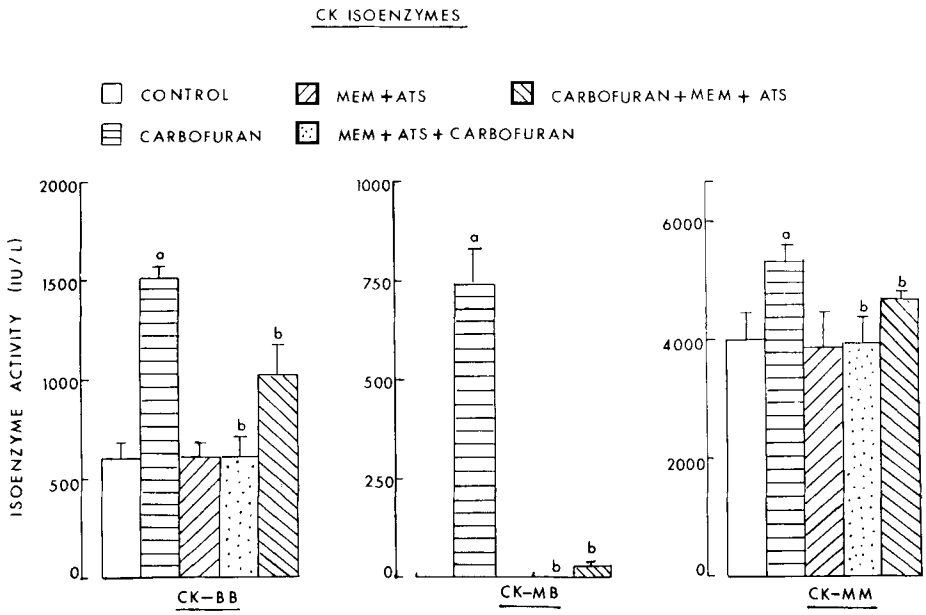


Fig. 1. Effects of MEM and ATS on serum CK isoenzymes in rats acutely intoxicated with carbofuran. The activities of isoenzymes presented as IU/L are means ± s.e.m. (n = 4–5). For the details of drugs and dosages, see Table 1. a, Significant difference between controls (Group I) and carbofuran-treated (Group II) rats ($P < 0.01$). b, Significant difference between carbofuran-treated (Group II) and carbofuran + antidotes (Group IV or Group V)-treated rats ($P < 0.01$).

al., 1991a,b]. Detailed evaluation of serum for isoenzymes of CK and LDH revealed characteristic changes in their patterns which were tissue specific. In fact, determination of isoenzymes in tissues confirmed that the observed changes in serum were reflections of those seen in tissues.

From various laboratories, including ours, it is well established that MEM exerts directly/indirectly multiple biochemical and pharmacological effects. For example, its effects are studied on i) transmitter processes [Osborne et al., 1982; Sontag et al., 1982], ii) synaptic and nonsynaptic actions [Wand et al., 1977; Wesemann and Ekenna, 1982; Wesemann et al., 1983], iii) prevention of neural hyperexcitability [McLean, 1987], iv) reversible neuromuscular transmission blockade by interaction with ACh receptor-ion channel complex [Masuo et al., 1986], and v) central muscle relaxation [Grossman and Jurna, 1977]. The principal mechanism in antagonizing carbofuran and other anti-AChE poisoning by MEM could be its potential for protection and/or reactivation of AChE [Gupta and Kadel, 1989b, 1990, 1991a,b]. Another important mechanism investigated was protection of CarbE by MEM providing blockage of nonspecific binding of carbofuran allowing its rapid elimination [Gupta and Kadel, 1989b]. The mechanism of action of ATS in partial protection and reversal of anticholinesterase agents poisoning is well established [Hulme et al., 1978; Taylor, 1985]. In brief, ATS competitively blocks muscarinic receptors and thereby attenuates the hypersecretory activities of accumulated ACh.

The leakage of cytoplasmic enzymes (CK, LDH, GOT, and GPT) into serum from tissues could be attributed to marked depletion of high-energy phosphate ATP and possibly phosphocreatine (PCr), since ATP is required to maintain the cell membrane permeability and membrane characteristics, including electrophysiology and stability [Gupta and Dettbarn, 1987; Bricknell and Opie, 1978; Gupta et al., 1991a,b, 1992], and thereby retains the vital intracellular contents. It was evident from these studies that down-regulation of high-energy phosphates (40–60%) was a result of their greater demand to cope with energy required for high-frequency muscle fasciculations, the most characteristic feature of carbofuran or other carbamate toxicity [Gupta and Kadel, 1989a; Gupta et al., 1991a]. The characteristic changes in isoenzymes patterns of CK and LDH are suggestive of cytotoxic insult at least in target organs. Data presented in Table 2 and Figures 1–3 demonstrate that combined treatment with MEM and ATS provided significant protection/reversal of carbofuran-induced changes in the cytoplasmic biomarker enzymes (CK, LDH, and transaminases). It can be hypothesized that MEM, in addition to the aforementioned multiple actions,

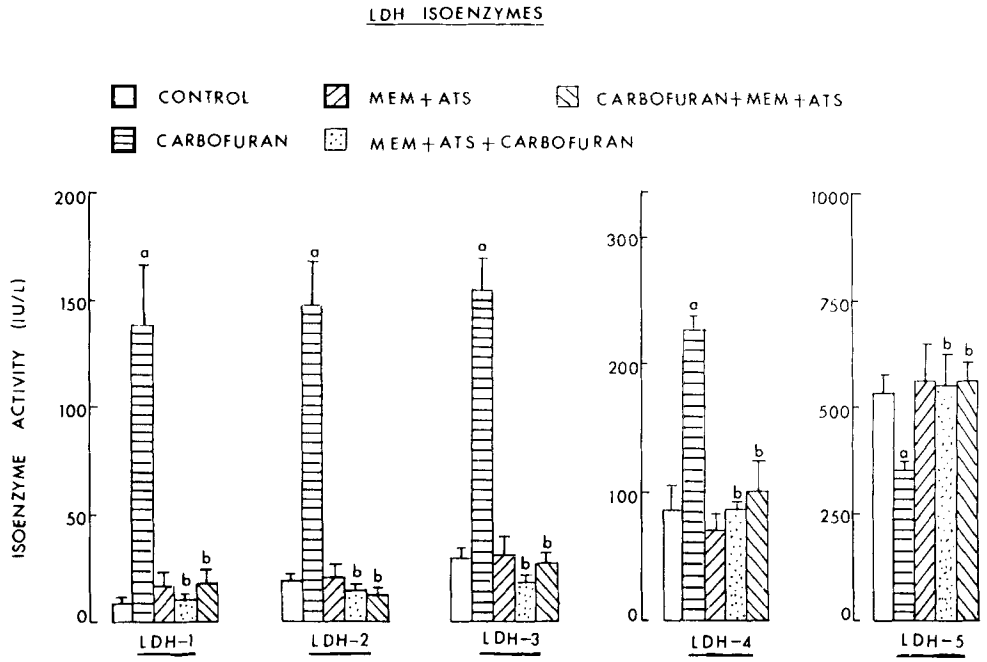


Fig. 2. Effects of MEM and ATS on serum LDH isoenzymes in rats acutely intoxicated with carbofuran. The activities of isoenzymes presented as IU/L are means ± s.e.m. (n = 4–5). For the details of drugs and dosages, see Table 1. a, Significant difference between controls (Group I) and carbofuran-treated (Group II) rats ($P < 0.01$). b, Significant difference between carbofuran-treated (Group II) and carbofuran + antidotes (Group IV or Group V)-treated rats ($P < 0.01$).

might facilitate in maintaining normal cell membrane permeability probably by preserving normal ATP and PCr levels, since MEM is demonstrated to alleviate the hyperactivity of muscles, brain, and heart in carbofuran intoxicated rats [Gupta and Kadel, 1989b].

In our comprehensive studies on carbofuran intoxication, one of the most consistently observed biochemical changes, was a 2- to 3-fold increase in glucose (Fig. 4). This marked increase in glucose was probably due to enhanced levels of catecholamines (epinephrine and norepinephrine) [Gupta et al., 1984] and elevation in the activities of key quartet gluconeogenic enzymes (pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1, 6-biphosphatase, and glucose 6-phosphatase), i.e., the reversal of glycolysis [Kacew and Singhal, 1973]. MEM and ATS treatment provided complete protection/reversal against hyperglycemia caused by carbofuran (Fig. 4). Whether MEM diminishes the hyperglycemia by preventing the release of catecholamines or by normalizing the activity of muscles and brain thereby lowering the need of energy is yet to be studied.

It is also important to note that combined administration of MEM and ATS did not affect the activity of any target or non-target biomarkers in normal (carbofuran-untreated) rats [Table 1 and Figs. 1–4; and Gupta and Kadel, 1989b, 1991a,b,]. In addition,

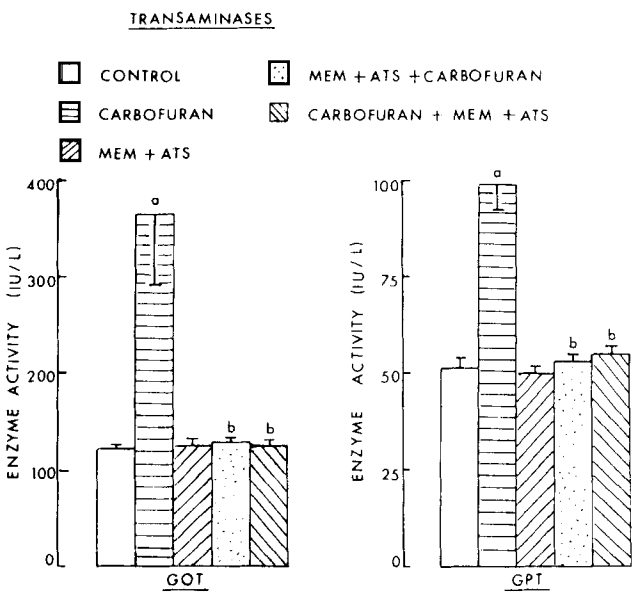


Fig. 3. Effects of MEM and ATS on serum transaminases (GOT and GPT) in rats acutely intoxicated with carbofuran. The activities of enzymes presented as IU/L are means ± s.e.m. (n = 4–5). For the details of drugs and dosages, see Table 1. a, Significant difference between controls (Group I) and carbofuran-treated (Group II) rats ($P < 0.01$). b, Significant difference between carbofuran-treated (Group II) and carbofuran + antidotes (Group IV or Group V)-treated rats ($P < 0.01$).

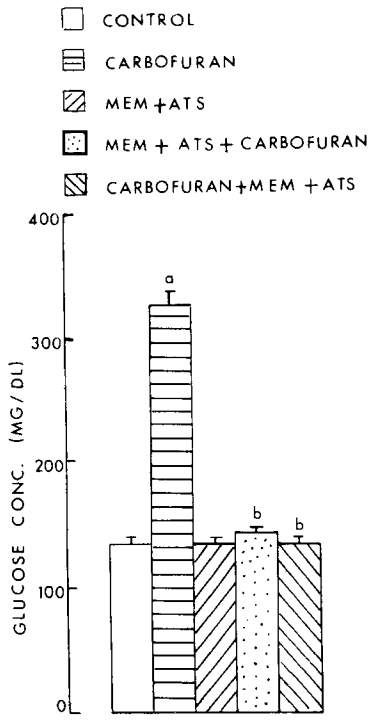


Fig. 4. Effects of MEM and ATS on serum glucose in rats acutely intoxicated with carbofuran. The concentrations of glucose presented as mg/dl are means ± s.e.m. (n = 4–5). For the details of drugs and dosages, see Table 1. a, Significant difference between controls (Group I) and carbofuran-treated (Group II) rats ($P < 0.01$). b, Significant difference between carbofuran-treated (Group II) and carbofuran + antidotes (Group IV or Group V)-treated rats ($P < 0.01$).

MEM does not produce any obvious side effects, thus imparting an obvious advantage over other antidotes [Miltner, 1982; Gupta and Kadel, 1989b].

From the results presented in this study and elsewhere, it can be concluded that MEM in combination with ATS protect target tissues from cytotoxic insult elicited by carbofuran. It remains to be seen whether this antidotal treatment would provide such antagonizing effects on biomarkers against other anti-AChE compounds, as demonstrated on AChE [Gupta and Kadel, 1989a, 1990, 1991a,b].

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## REFERENCES

Amador E, Massod MF, Franey RJ (1967): Reliability of glutamic-oxalacetic transaminase methods. Am J Clin Pathol 47:419–428.

Asztalos B, Nemcsok J (1985): Effect of pesticides on the LDH activity and isoenzyme pattern of carp (*Cyprinus carpio L.*) sera. Comp Biochem Physiol 82C:217–219.

Bais R, Edwards JB (1982): Creatine kinase. CRC Crit Rev Clin Lab Sci 16:291–335.

Bhargava AS, Preus M, Khater AR, Grünzel, P (1990): Effect of iloprost on serum creatine kinase and lactate dehydrogenase isoenzymes after isoprenaline-induced cardiac damage in rats. Arzneimittelforschung 40:248–252.

Bleidner WE, Harmon JB, Hewes WE, Lynes TE, Hermann EC (1965): Absorption, distribution and excretion of amantadine hydrochloride. J Pharmacol Exp Ther 150:484–490.

Bricknell OL, Opie LH (1978): Glycolytic ATP and its production during ischemia in isolated Langendorff-perfused rat heart. In Kobayashi T, Sano T, Dhalla NS (eds): Recent Advances in Studies on Cardiac Structure and Metabolism, Heart Function and Metabolism, Vol. II. Baltimore: University Park, pp 509–519.

Casida JE (1963): Mode of action of carbamates. Annu Rev Entomol 8:39–58.

Codd JE, Sullivan RG, Weins RD, Barner HB, Kaiser GC, Willman VL (1977): Myocardial injury following myocardial revascularization: Detection by isoenzyme analysis. Circulation 56: 49–53.

Gay RJ, McComb RB, Bowers GN Jr (1968): Optimum reaction conditions for human lactate dehydrogenase isoenzymes as they affect total lactate dehydrogenase activity. Clin Chem 14:740–753.

Grossman W, Jurna I (1977): Die Wirkung von memantin auf die membranen sensibler nervenfaserbundel. Arzneimittelforschung 27:1483–1487.

Gupta RC, Dettbarn W-D (1992): Role of carboxylesterases in the prevention and potentiation of N-methylcarbamate toxicity. J Biochem Toxicol, in press.

Gupta RC, Kadel WL (1989a): Concerted role of carboxylesterases in the potentiation of carbofuran toxicity by iso-OMPA pretreatment. J Toxicol Environ Health 26:447–457.

Gupta RC, Kadel WL (1989b): Prevention and antagonism of acute carbofuran intoxication by memantine and atropine. J Toxicol Environ Health 28:111–122.

Gupta RC, Kadel WL (1990): Methyl parathion acute toxicity: Prophylaxis and therapy with memantine and atropine. Arch Int Pharmacodyn Ther 305:208–221.

Gupta RC, Kadel WL (1991a): Novel effects of memantine in antagonizing acute aldicarb toxicity: Mechanistic and applied considerations. Drug Dev Res 24:329–341.

Gupta RC, Kadel WL (1991b): Subacute toxicity of aldicarb: Prevention and treatment with memantine and atropine. Drug Dev Res 24:343–353.

Gupta M, Bagchi GK, Gupta SD, Samal D, Chatterjee T, Dey SN (1984): Changes of acetylcholine catecholamines and amino acids in mice brain following treatment with nuvacron and furadan. Toxicology 30:171–175.

Gupta RC, Goad JT, Kadel WL (1991a): Carbofuran-induced alterations (in vivo) in high-energy phosphates, creatine kinase (CK) and CK isoenzymes. Arch Toxicol 65:304–310.

Gupta RC, Goad JT, Kadel WL (1991b): In vivo alterations in lactate dehydrogenase (LDH) and LDH isoenzymes patterns by acute carbofuran intoxication. Arch Environ Contam Toxicol 21: 263–269.

Gupta RC, Goad JT, Kadel WL (1992): Partial depletion of high-energy nucleotides and phosphagens and changes in the related enzymes and isoenzymes in diaphragm muscle by acute methomyl toxicity. Neurotoxicology, in press.

Hayes WJ Jr (1982): Pesticide Studies in Man, 2nd ED. Baltimore: The Williams and Wilkins Co., pp 284–435, 436–467.

Henry RJ, Chiamori N, Golub OJ, Berkman S (1960): Revised spectrophotometric methods for the determination of glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase and lactic dehydrogenase. Am J Clin Pathol 34:381–390.

Hulme EC, Birdsall NJM, Burgen ASV, Mehta P (1978): The binding of antagonists to brain muscarinic receptors. Mol Pharmacol 14:737–750.

Kacew S, Singhal RL (1973): The influence of p, p'-DDT, alpha-Chlordane, heptachlor and endrin on hepatic and adrenal carbohydrate metabolism and cyclic AMP-adenyl cyclase system. Life Sciences 13:1363–1371.

Karmen A (1955): A note on the spectrophotometric assay of glutamic-oxalacetic transaminase in human blood serum. J Clin Invest 34:131–133.

Lemercier G, Carpentier P, Sentenac-Roumanou H, Morelis P (1983): Histological and histochemical changes in the central nervous system of rat poisoned by an irreversible anticholinesterase organophosphorus compound. Acta Neuropathol (Berlin) 61:123–129.

Linz W, Martorana PA, Grotsch H, Bie-Yin Q, Scholkens BA (1990): Antagonizing bradykinin (BK) obliterates the cardioprotective effects of bradykinin and angiotensin-converting enzyme (ACE) inhibitors in ischemic hearts. Drug Dev Res 19:393–408.

Masuo K, Enomoto K-I, Maeno T (1986): Effects of memantine on the frog neuromuscular junction. Eur J Pharmacol 130:187–195.

McLean MJ (1987): In vitro electrophysiological evidences predicting anticonvulsant efficacy of memantine and flunarizine. Polish J Pharmacol Pharm 39:81–93.

McLeod CG Jr (1985): Pathology of nerve agents: Perspectives on medical management. Fundam Appl Toxicol 5:S10–S16.

Miltner FO (1982): Wertigkeit der symptomatischen therapie mit memantine beim zerebralen koma. I. Korrelation von Komastadien und EEG-Spectralverlaufen. Arzneimittelforschung 32: 1268–1270.

Murphy SD (1985): Pesticides. In Klaassen CD, Amdur MO, Doull J (eds): The Basic Science of Poisons, 3rd ED. New York: Macmillan Publishing Co., pp 539–543.

O'Brien RD (1967): Insecticides, Action and Metabolism. New York: Academic Press, pp 86–106.

Oliver IT (1955): A Spectrophotometric method for the determination of creatine phosphokinase and myokinase. Biochem J 61: 115–122.

Osborne NN, Beale R, Golombiowska-Nikitin K, Sontag, KH (1982): The effect of memantine on various neurobiological processes. Arzneimittelforschung 32:1246–1255.

Petras JM (1981): Soman Neurotoxicity. Fundam Appl Toxicol 1: 242–250.

Rosalki SB (1967): An improved procedure for serum creatine phosphokinase determination. J Lab Clin Med 69:696–705.

Shina R, Condrea E (1990): Inhibition of lactate dehydrogenase by an EDTA $Ca^{2+}$ complex. Clin Chem Acta 189:231–236.

Sket D, Brzin M, Vreca I (1989): Effect of HI-6 and diazepam on the increase of creatine kinase isoenzymes activity in plasma of atropinized soman-poisoned rats. Acta Pharm Yugosl 39:151–159.

Sontag K-H, Wand P, Schwarz M, Wesemann W, Osborne NN (1982): The effect of memantine on spinal alpha-motoneurones and on content of dopamine, noradrenaline and serotonin of striatum and lumbar spinal cord. Arzneimittelforschung 32: 1236–1240.

Steel RGD, Torrie JH (1980): Principles and Procedures of Statistics, 2nd ED. New York: McGraw-Hill, pp 187–188.

Szasz G, Gruber W, Bernt E (1976): Creatine kinase in serum 1. Determination of optimum reaction conditions. Clin Chem 22: 650–656.

Taylor P: Anticholinesterase Agents. In Gilman AG, Goodman LS, Rall TW, Murad F (eds) (1985): Goodman and Gilman's The Pharmacological Basis of Therapeutics, 7th ED. New York: Macmillan Publishing Co., pp 110–129.

Uchiyama M, Shibuya M (1969): Distribution and excretion of $^{3}H$-amantadine HCl. Chem Pharmaceut Bull 17:841–843.

Wand P, Sontag K-H, Cremer H (1977): Effects of 1, 3-dimethyl-5-aminoadamantane hydrochloride (DMAA) on the stretch-induced reflex tension of flexor muscles and the excitability of the gamma-loop in decerebrate and spinal cats. Arzneimittelforschung 27:1477–1481.

Wesemann W, Ekenna O (1982): Effect of 1-aminoadamantanes on the MAO activity in brain, liver and kidney of the rat. Arzneimittelforschung 32:1241–1243.

Wesemann W, Sontag K-H, Maj J (1983): Zur pharmacodynamic und pharmacokinetik des memantin. Arzneimittelforschung 33: 1122–1134.

Woodman DD (1981): Plasma enzymes in drug toxicity. In Gorrod JW, (ed): Testing for Toxicity. London: Taylor and Francis, pp 145–156.

Wroblewski F, LaDue JS (1956): Serum glutamic-pyruvic transaminase in cardiac and hepatic disease. Proc Soc Exp Biol Med 91:569.