Evaluation of hydroxypropyl-β-cyclodextrin in the treatment of aldicarb poisoning in rats

R S Verster[a*] and C J Botha[a]

ABSTRACT

Cyclodextrins are ring-shaped oligosaccharides with a hydrophilic exterior and a hydrophobic interior. The interior cavity is capable of complexing fat-soluble molecules small enough to fit inside. Sprague-Dawley rats were used to evaluate the efficacy of hydroxypropyl-β-cyclodextrin as treatment of aldicarb poisoning in rats. Survival times in the majority of rats dosed with aldicarb and receiving intravenous cyclodextrin were longer compared with the control rats only dosed with aldicarb *per os*.

Key words: aldicarb, cyclodextrin, poisoning, rats.

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INTRODUCTION

Organophosphor and carbamate compounds are some of the most widely used pesticides, making it inevitable that accidental or intentional exposure of animals and humans will occur. Worldwide, several incidences of aldicarb poisoning have been reported[6,10,13,14] and malicious poisoning of dogs with aldicarb occurs frequently in South Africa. Data of confirmed cases of aldicarb mortalities obtained from the Toxicology Division, Onderstepoort Veterinary Institute, indicate the following: 72 cases were confirmed during 1998, 67 cases in 1999 and 72 cases were recorded in 2000; this increased to 115 in 2001 and 114 in 2002 and decreased somewhat to 97 in 2003 (J P J Joubert, ARC-Onderstepoort Veterinary Institute, unpubl. data 2002). The majority of poisonings occurred in dogs and cats, but sporadic occurrences in cattle, birds, monkeys and antelope were also reported. However, the official records reflect only a small percentage of the actual cases, as many poisonings are not reported nor samples submitted to confirm a diagnosis, as the presence of the typical round, black granules in the gastrointestinal tract or vomitus practically confirms a tentative diagnosis.

Aldicarb, an oxime carbamate insecticide, nematocide and acaricide (molecular weight = 190.3 daltons) is registered in terms of the Fertilisers, Farm Feeds, Agricultural and Stock Remedies Act (Act 36 of 1947) for the control of agricultural pests in South Africa. It is one of the most toxic carbamates, with an oral LD_{50} of less than 1 mg/kg in the rat and a dermal LD_{50} of 20 mg/kg[21]. Its mechanism of action is via inhibition of the enzyme acetylcholinesterase, responsible for catabolism of the neurotransmitter acetylcholine, resulting in the accumulation of the latter at postsynaptic cholinergic receptor sites. Muscarinic effects include hypersalivation, lacrimation, urination, diarrhoea, bradycardia, bronchoconstriction with excess bronchial secretions and miosis. Nicotinic effects manifest as tremors, muscle stiffness, weakness and paralysis[8,9]. The muscular hypertonia, tremors and convulsions can lead to exertional rhabdomyolysis[17]. Mortalities are commonly attributed to respiratory failure[9].

In companion animals it would appear that mild intoxication can be successfully treated, but more severe cases usually die, despite intensive treatment. The most important treatment is repeated parenteral administration of atropine, which acts as a competitive antagonist of acetylcholine at the muscarinic receptors. Atropine has no effect on nicotinic receptors and will not counteract muscle tremors, weakness or paralysis[3,9].

Decontamination procedures such as induction of emesis and/or gastric lavage, in conjunction with adsorbents such as activated charcoal can be effective to limit absorption of the toxin from the gastrointestinal tract. Additional supportive treatment must be given until the animal has eliminated the poison. Most cases arrive at veterinary practices only when signs of intoxication become evident. If an additional agent, which will bind or complex aldicarb in the circulation can be administered as soon as clinical signs become evident, mortalities will most probably be curtailed.

Cyclodextrins are capable of binding toxins of low molecular weight and are toroidal ('doughnut-shaped') oligosaccharides produced from starch by the bacterium *Bacillus macerans*[5,7] and contain 6, 7 or 8 α-D-glucopyranosyl residues referred to as α, β and γ-cyclodextrins, respectively[5]. They have a hydrophilic exterior, but a hydrophobic interior cavity and owing to this cyclic structure, cyclodextrins have the ability to form inclusion complexes with many lipophilic molecules[15]. Cyclodextrins may host a great variety of molecules, having the size of 1 or 2 benzene rings, or even larger ones, which have a side chain of comparable size to form crystalline inclusion complexes[12]. The complex renders lipid-soluble molecules relatively more water-soluble and aids renal excretion. Molecules that are hydrophobic, or have a fatty acid side chain, will partition into the cavity of the cyclodextrins, forming a molecular complex, changing the physical and biochemical properties of the 'guest' molecule[22].

This feature has been used to treat mice suffering from experimental hypervitaminosis A. Vitamin A and other retinoids are known to form complexes with cyclodextrins[19]. β-Cyclodextrin administered at 470 mg/kg over 24 hours intravenously, had been used to treat a child suffering from hypervitaminosis A, with empirical success, although no conclusion could be made from only 1 case[4]. Hydroxypropyl-β-cyclodextrin has been shown to protect sheep against tunicaminyluracil (corynetoxin) toxicity (annual ryegrass toxicity), when administered intraperitoneally at 100 mg/kg twice daily[22].

In an *in vitro* study to evaluate the inactivation of the organophosphorous nerve

[a]Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa.

*Author for correspondence.
E-mail: christo.botha@up.ac.za

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agents it was observed that cyclodextrins catalysed the inactivation of the compounds sarin and soman, but did not inactivate tabun and VX. Furthermore, sarin and soman showed greater affinity for β-cyclodextrins than for the α- or γ-cyclodextrins[7].

β-Cyclodextrin has been used to complex the E-isomer of mevinphos, an organophosphor insecticide[2] and also enhanced biological detoxification of industrial waste-waters containing numerous pesticides[18]. α-Dicyclopropylmethane was complexed with γ-cyclodextrin to decrease the acute oral toxicity of the parent compound in rats[16].

Several acute toxicity studies of cyclodextrins have been conducted in mice, rats and dogs[1,12,24]. The parenteral LD_{50} of γ-cyclodextrin in mice exceeds 4000 mg/kg and in rats more than 2400 mg/kg[12]. In mice single intraperitoneal injection of 10 000 mg/kg and intravenous administration of 2000 mg/kg hydroxypropyl-β-cyclodextrin did not induce mortalities[12].

β-Cyclodextrin and hydroxypropyl-β-cyclodextrin distribute rapidly after intravenous administration to rats[11]. Both are eliminated by glomerular filtration and over 90 % of the dose is recovered unchanged in the urine within 24 h of administration[11].

The objective of the current experiments was to determine if hydroxypropyl-β-cyclodextrin (30 times more water-soluble than β-cyclodextrin)[20] is effective in the treatment of rats poisoned with aldicarb.

MATERIALS AND METHODS

Chemicals

Aldicarb (99.9 % pure, Riedel-de Haën) was dissolved in sterile water (0.1 % m/v) before dosing and hydroxypropyl-β-cyclodextrin (97+ %, Acros Organics) was dissolved in distilled water (10 % m/v) before intravenous administration.

First experiment

Male Sprague-Dawley rats (n = 12), weighing 280–305 g, were randomly divided into 3 groups. Four rats in the positive control group each received an estimated lethal dose of aldicarb (1.5 mg/kg) by oral gavage. Six rats received the same aldicarb dose orally of which 3 were injected with hydroxypropyl-β-cyclodextrin (200 mg/kg) in the caudal vein immediately before aldicarb administration and the other 3 rats were dosed with aldicarb just prior to intravenous cyclodextrin administration. Two rats, serving as negative controls, only received 200 mg/kg cyclodextrin intravenously.

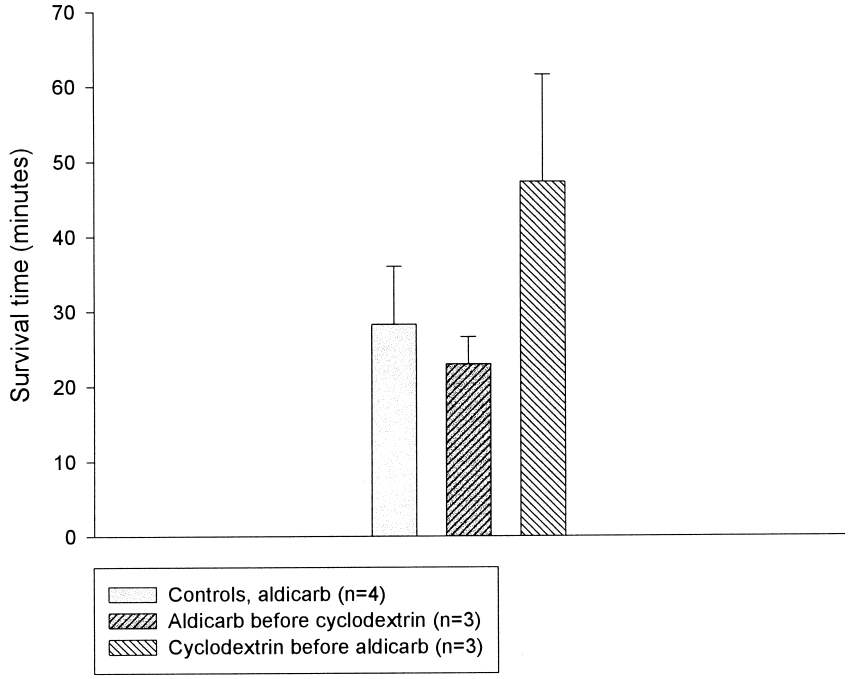

Fig. 1: **Survival times of rats dosed with aldicarb** (1.5 mg/kg) **with and without hydroxypropyl-β-cyclodextrin** (200 mg/kg) **administered intravenously.**

Second experiment

Male Sprague-Dawley rats (n = 12), weighing 170–215 g, were randomly divided into 2 equal groups. Group 1 rats were first dosed with aldicarb, at 1.5 mg/kg, orally and immediately thereafter were injected with 250 mg/kg cyclodextrin intravenously. Group 2 rats received 250 mg/kg cyclodextrin intravenously immediately prior to being dosed with aldicarb (1.5 mg/kg) per os.

Third experiment

Male Sprague-Dawley rats (n = 14), weighing 245–300 g, were randomly divided into 2 groups. Control rats (n = 7) received a slightly lower dose of 1.3 mg/kg aldicarb orally and the rats in the experimental group (n = 7) received the same dose of aldicarb, by gavage, immediately after the intravenous administration of cyclodextrin at 250 mg/kg. The lower, but still estimated fatal, dose of 1.3 mg/kg aldicarb was decided upon to allow slightly more time for the hypothesised cyclodextrin-aldicarb complex formation.

Data analysis

The data obtained from the experimental groups were captured and analysed with the t-test and the Mann-Whitney rank sum test for statistical differences (Sigma Stat, Jandel Scientific, San Rafael, CA).

RESULTS

First experiment

In the control rats, only dosed with aldicarb, the mean survival time was 28.25 ± 7.52 minutes and in the experimental group (aldicarb plus cyclodextrin) mean survival time was 35.16 ± 16.26 minutes. No statistical significant differences occurred between groups. Although the survival times of 4 rats in the experimental group were similar to the control group, 2 of the 3 rats, which first received cyclodextrin and then aldicarb, noticeably survived longer (Fig. 1). The 2 rats only receiving cyclodextrin, survived without exhibiting any adverse effect.

Second experiment

Four rats that were first injected with cyclodextrin and then dosed with aldicarb survived longer than any of the rats receiving aldicarb before intravenous administration of cyclodextrin. The mean survival time of the group of rats that were first dosed with aldicarb followed by cyclodextrin was 34 ± 7.77 minutes and for the group that received cyclodextrin before aldicarb it was 57.5 ± 24.52 minutes (Fig. 2); these differences were statistically significant ($P < 0.05$).

Third experiment

Statistical significant differences (P = 0.002) were determined in mean survival time between the control group (35.28 ± 11.78 minutes; range 20–52) and the experimental group (1896.42 ± 2267.17 minutes; range 45–4320) (Fig. 3). Six of the 7 rats in the experimental group survived longer than any of the rats in the control group. Three rats that had survived for 72 hours (4320 minutes) were euthanased (overdose of pentobarbitone administered intraperitoneally). Although they did not exhibit any clinical signs reminis-

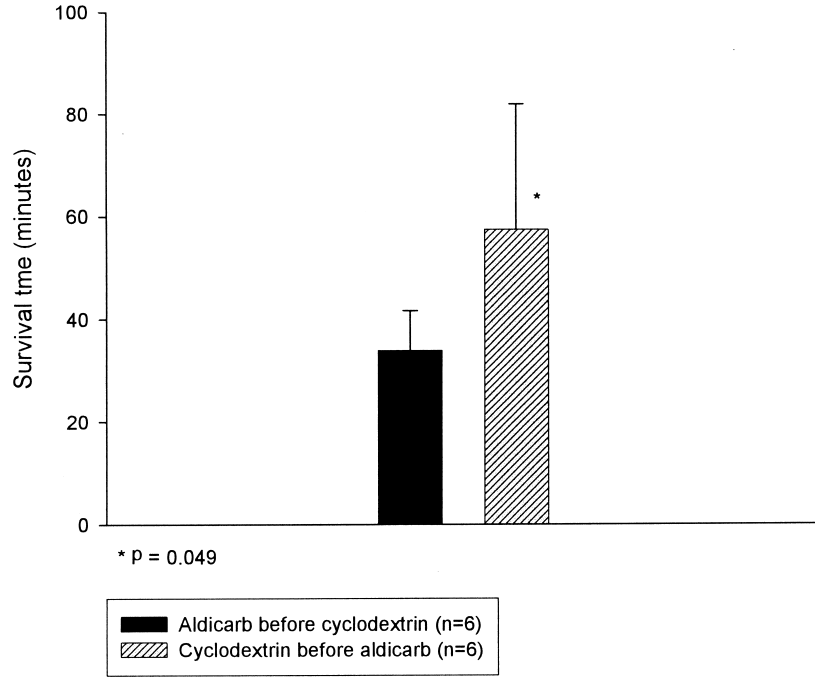

Fig. 2: **Survival times of rats dosed with aldicarb** (1.5 mg/kg) **before and after intravenous hydroxypropyl-β-cyclodextrin** (250 mg/kg)**.**

cent of aldicarb poisoning at the time of euthanasia they lost between 7 and 10 % body mass and it was decided to terminate the experiment.

DISCUSSION

This study indicates that intravenous administration of hydroxypropyl-β-cyclodextrin to rats before oral dosing of aldicarb prolongs survival and it would appear that hydroxypropyl-β-cyclodextrin complexes aldicarb to some extent. However, this will limit the therapeutic use of cyclodextrins because pets will ingest aldicarb and treatment will only commence once clinical signs become apparent. Possible explanations for the unsatisfactory results of hydroxypropyl-β-cyclodextrin in these experiments are the exceptionally high doses of aldicarb administered to the rats and the fact that aldicarb is rapidly metabolised *in vivo* to aldicarb sulphoxide and sulphone, more water-soluble compounds[23], which arguably bind poorly to cyclodextrin. Larger doses of hydroxypropyl-β-cyclodextrin, being a relatively safe compound, could also be evaluated as the increase in dose improved survival times in rats. The intravenous doses of hydroxypropyl-β-cyclodextrin, administered in this study, were up to 250 mg/kg and deaths were attributed to aldicarb and not cyclodextrin.

Administration of atropine remains the most important treatment for carbamate and organophosphor poisoning; however, hydroxypropyl-β-cyclodextrin may be included as a supportive treatment. Considering the safety of cyclodextrins and the ability to complex organophosphors[2,7] it might contribute to the survival of organophosphor-poisoned pets.

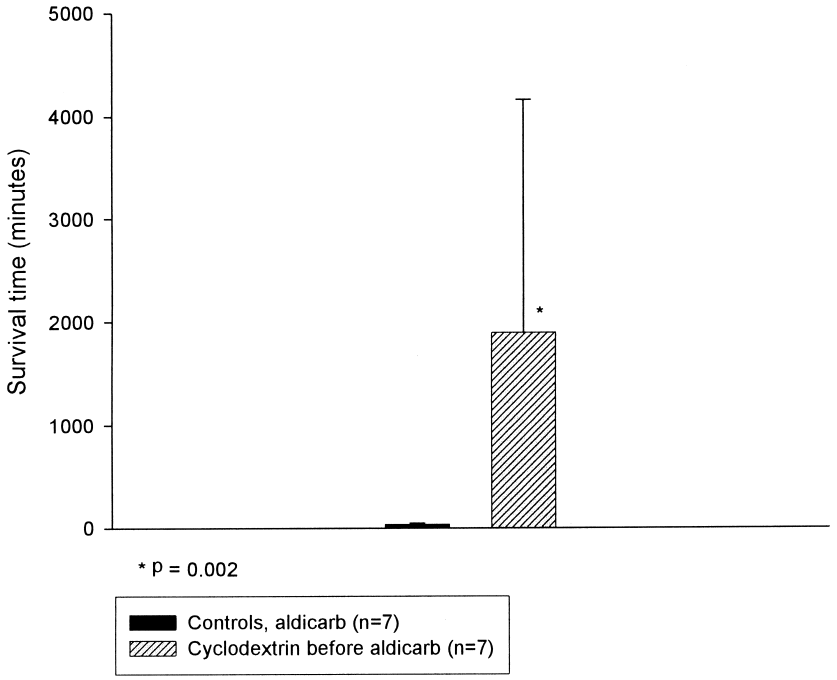

Fig. 3: **Survival times of rats dosed with aldicarb** (1.3 mg/kg) **and the same dose of aldicarb following intravenous administration of hydroxypropyl-β-cyclodextrin** (250 mg/kg)**.**

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