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THE PREVENTION BY SULPHYDRYL COMPOUNDS OF THE TOXICITY IN THE CAT OF 2,6-DIMETHOXYPHENOL AND ITS MORPHOLINOPROPIONYL ESTER

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1 Intravenous (−)-2,6-dimethoxyphenyl-2-morpholinopropionate hydrochloride (M&B 16,573) produced anaesthesia of short duration in the mouse, rat, rabbit, cat, dog and monkey. In the cat but not in other species, a severe and usually fatal toxic reaction was seen 1-2 h after administration.

2 This toxic reaction but not the anaesthetic properties of M&B 16,573 was prevented by the intravenous administration of cysteine or N-acetylcysteine. Cysteamine or dimercaprol were ineffective.

3 Intravenous administration of 2,6-dimethoxyphenol or 2,6-dimethoxyquinol in the cat produced a response similar to the delayed toxic effects of M&B 16,573 but not preceded by anaesthesia. The toxic effects of these compounds were prevented by cysteine.

4 Intravenous 4-allyl-2,6-dimethoxyphenyl-2-morpholinopropionate hydrochloride produced anaesthesia in the cat without the delayed toxic effects seen after M&B 16,573.

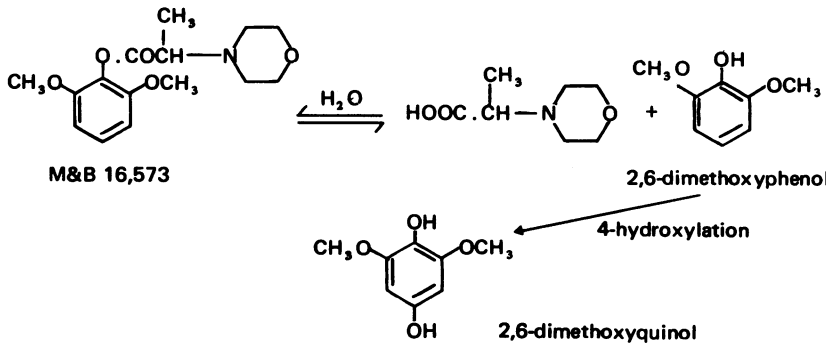
5 The acute toxicity of 2,6-dimethoxyquinol in mice was reduced by the administration of cysteine or N-acetylcysteine.

6 It is postulated that the delayed effects produced by M&B 16,573 in the cat are due to the formation of 2,6-dimethoxyquinol and 2,6-dimethoxybenzoquinone in this species, the toxicity of the latter being reduced by sulphydryl compounds.

Introduction

While evaluating the intravenous anaesthetic properties of a series of basic phenolic esters, and particularly (−)-2,6-dimethoxyphenyl-2-morpholinopropionate hydrochloride (M&B 16,573), it was observed that in the cat but not in the mouse, rat, rabbit, dog or monkey, a delayed toxic and usually fatal reaction was seen. This occurred only after the slow intravenous infusion of approximately twice the anaesthetic dose. The cats appeared to recover normally from the anaesthetic but 0.5-2 h later were seen to be suffering from excessive salivation, facial oedema, swelling of the tongue and respiratory difficulty. The majority of these cats died 2-4 h after administration of the drug.

It seemed likely that M&B 16,573 might be hydrolysed *in vivo* to 2,6-dimethoxyphenol and morpholinopropionic acid and we hypothesized that the phenol might subsequently undergo



4-hydroxylation to form a quinol and the corresponding 2,6-dimethoxybenzoquinone, and that the latter might be a toxic metabolite.

Phillips & Cater (1956) showed that sulphydryl-containing compounds protected against the acute toxicity of menadiol diphosphate (2-methyl-1 : 4-naphthohydroquinone diphosphate) in rats. Hence, if the above hypothesis of the cause for the delayed toxicity of M&B 16,573 were correct, sulphydryl compounds might afford protection against this. Furthermore, this hypothesis suggested that compounds related to M&B 16,573 but suitably substituted in the 4-position of the phenolic ring so that the quinol cannot be formed *in vivo*, should not produce the delayed toxicity seen with M&B 16,573.

This paper describes experiments carried out to test this hypothesis.

Methods

Cats

Cats of either sex weighing 1-2 kg were used. All drugs were administered into a cephalic vein. The cats were kept for 48 h after injection and observed for oedema of face and tongue, respiratory difficulty and colour of urine. The number which died was noted. Cats receiving 0.9% w/v NaCl solution (saline) or other vehicle were used as controls and were studied simultaneously with the cats treated with active drugs.

Mice

Male albino mice (M&B strain) weighing 18-25 g were used. To determine the protective activity of the sulphydryl compounds, mice were injected intravenously with the compound, followed immediately by an intravenous injection of either 2,6-dimethoxyphenol or menadiol sodium diphosphate. The mice dying within 24 h were counted. Control mice receiving only the toxic compound were used in all experiments. The doses of 2,6-dimethoxyphenol or of menadiol sodium diphosphate used produced approximately 80% mortality in control animals.

Experiments in other species

An anaesthetic dose of M&B 16,573 was injected intravenously in the mouse, rat, rabbit, dog and Rhesus monkey and this initial injection was followed by an intravenous infusion to maintain anaesthesia. Animals were observed for 48 h after recovery.

Drugs

The following drugs were used: (−)-2,6-dimethoxyphenyl-2-morpholinopropionate hydrochloride (M&B 16,573); 4-allyl-2,6-dimethoxyphenyl-2-morpholinopropionate hydrochloride (M&B 15,920); 2,6-dimethoxyphenol (Aldrich); 2,6-dimethoxyquinol; Dimercaprol (BAL) injection (Boots); cysteine hydrochloride; cysteamine (2-mercapto-ethylamine hydrochloride); N-acetylcysteine (Airbron, BDH); menadiol sodium diphosphate (Synkavit, Roche); atropine sulphate; promethazine hydrochloride; methysergide hydrogen maleate; dimethothiazine mesylate.

Doses throughout the text refer to the compounds as listed above.

Results

Cat

The rapid intravenous administration of M&B 16,573 100 mg/kg to the cat was followed by general anaesthesia (loss of righting reflex, withdrawal reflexes and corneal reflex). This anaesthesia lasted 2-5 min and was followed by complete and apparently uneventful recovery. No delayed toxic effects were observed at this dose in any of the 12 cats studied. When the initial intravenous injection of 100 mg/kg was followed by an infusion of a further 100 mg/kg over a period of 2-5 min, anaesthesia was maintained throughout the period of drug infusion and for some 5 min afterwards and was followed by apparently complete recovery from anaesthesia. However, after a delay of 0.5-2 h, a characteristic syndrome developed in all these animals. This consisted of facial oedema, swelling of the tongue, respiratory difficulty and, eventually, prostration and death. Urine collected from animals which had received 200 mg/kg M&B 16,573 was dark brown or black in colour. M&B 16,573 (200 mg/kg) was administered in this way to a total of 50 cats. All developed these toxic effects and 37 died within 2-4 h after drug administration.

The precise cause of death was somewhat obscure and, although it involved respiratory difficulty, it was not prevented by intubation and artificial respiration. Post-mortem examination revealed no gross pathological change, other than oedema of the face and tongue. There was no observable oedema of the lung.

Attempts were made to prevent the development of these toxic effects. Atropine (2 mg/kg, 3 cats), promethazine (10 mg/kg, 3 cats), methysergide (1 mg/kg, 2 cats) or dimethothiazine (4 mg/kg, 4 cats) given immedi-

ately before M&B 16,573 (200 mg/kg) did not modify the development of the fatal toxic effect.

However, the intravenous administration of cysteine, 200 mg/kg (6 cats) or 600 mg/kg (8 cats) immediately after the infusion of M&B 16,573 (200 mg/kg) completely protected all the animals treated (Table 1). The duration of the anaesthetic activity of M&B 16,573 was unaltered.

Cysteine (600 mg/kg) was also effective when given 1 h (4 cats) or 2.5 h (4 cats) after M&B 16,573, except in one animal in which the signs were already severe when the cysteine was administered 2.5 h after the anaesthetic (Table 1). Other doses of cysteine were not studied.

N-acetylcysteine (200 mg/kg) administered immediately after M&B 16,573 prevented the lethal effects of M&B 16,573 in five of six treated cats (Table 2), whilst cysteamine (300 mg/kg) and dimercaprol (40 mg/kg) were ineffective at the doses used. Cats injected with morpholinopropionic acid in amounts (200 mg/kg) greater than would be formed from M&B 16,573 showed no toxic reaction.

If the toxic effects of M&B 16,573 were due to the formation of 2,6-dimethoxyquinol and 2,6-dimethoxybenzoquinone, similar toxic effects might follow administration of either 2,6-dimethoxyphenol or 2,6-dimethoxyquinol. The intravenous administration of 2,6-dimethoxyphenol (100 mg/kg) to four cats (Table 3) produced no significant anaesthesia but all developed the toxic effects described above and died 1-2 h after the injection. Cysteine (600 mg/kg) given intravenously immediately after 2,6-dimethoxyphenol protected three of the four treated animals (Table 3).

2,6-Dimethoxyquinol (30 mg/kg, 4 cats) produced less severe toxic effects than either M&B 16,573 (200 mg/kg) or dimethoxyphenol (100 mg/kg). However, all the treated animals died and the delay between dosing and death was 20-30 min, as compared with the 30-120 min after M&B 16,573 (Table 3). The toxicity of 2,6-dimethoxyquinol was reduced by cysteine (600 mg/kg). The effects of 2,6-dimethoxybenzoquinone were not studied in the cat because of its poor water solubility.

The intravenous administration of 4-allyl-2,6-dimethoxy-2-morpholinopropionate (M&B 15,920, 200 mg/kg) to three cats produced some

Table 1 Effect of cysteine on the delayed toxicity of M&B 16,573 (200 mg/kg) in the cat

Cysteine		*Number dying/number dosed*		
Dose (mg/kg)	*Time after M&B 16,573 (h)*	*M&B 16,573 + cysteine*	*M&B 16,573 only (control)*	P*
200	0	0/6	4/6	<0.02
600	0	0/8	8/8	<0.001
600	1	0/4	2/3	0.05
600	2.5	1/4	4/4	<0.05

All cats received M&B 16,573 (200 mg/kg intravenously). Control cats received intravenous saline. Cysteine-treated cats received the stated dose of cysteine at the stated time after M&B 16,573.
* Determined by χ^2 test.

Table 2 Effect of sulphydryl compounds on the delayed toxicity of M&B 16,573 (200 mg/kg) in the cat

		Number dying/number dosed		
Sulphydryl compound	*Dose (mg/kg, i.v.)*	*M&B 16,573 + sulphydryl compound*	*M&B 16,573 only (control)*	P*
Cysteine	200	0/6	4/6	<0.02
N-acetylcysteine	200	1/6	4/4	0.01
Cysteamine	300	4/6	5/6	0.5
Dimercaprol	40	6/6	6/8	<0.2

The sulphydryl compounds were administered immediately after M&B 16,573. Data on cysteine are from Table 1 and are included for comparison.
* χ^2 test.

anaesthesia of short duration but none of the animals developed the delayed effects seen after M&B 16,573. The quality of the anaesthesia produced by M&B 15,920 was not so good as that produced by M&B 16,573.

Mouse

Experiments with mice were carried out to obtain more information on the protective action of various sulphydryl compounds.

Intravenous injection of M&B 16,573 (120 mg/kg) was followed by anaesthesia of 1-2 min duration, followed by rapid and uneventful recovery. When the initial injection of 120 mg/kg was followed by a slow intravenous infusion of up to 1000 mg/kg, the duration of anaesthesia was prolonged but the animals recovered with no delayed toxic effects.

In contrast, the LD_{50} of 2,6-dimethoxyquinol for mice was approximately 35 mg/kg. The protective action of various sulphydryl compounds

Table 3 Effect of cysteine (600 mg/kg) on the delayed toxicity of 2,6-dimethoxyphenol and 2,6-dimethoxyquinol in the cat

Drug	*Dose (mg/kg)*	*Number dead/number dosed*		P*
		Drug + cysteine	*Drug alone*	
M&B 16,573	200	0/8	8/8	<0.001
2,6-dimethoxyphenol	100	1/4	4/4	<0.05
2,6-dimethoxyquinol	30	1/4	4/4	<0.05

Control cats received the phenol or quinol alone. Treated cats received the phenol or quinol + cysteine (600 mg/kg, i.v.) immediately. Data on M&B 16,573 are from Table 1 and are included for comparison.
* χ^2 test.

Table 4 Protective action of sulphydryl compounds in mice treated with 2,6-dimethoxyquinol or menadiol sodium diphosphate

Sulphydryl compound	*Dose (mg/kg)*	*Mortality as % of mortality in controls*	
		2,6-dimethoxyquinol	*Menadiol*
Cysteine	25	75	86
	50	59	72
	100	46	33
	200	0	38
	400	0	26
N-acetylcysteine	31	73	82
	62	60	82
	125	0	65
	250	0	85
	500	0	76
	1000	6	36
Cysteamine	12.5	69	79
	25	72	62
	50	47	24
	100	12.5	0
	200	28	7
Dimercaprol	5	84	100
	10	78	84
	20	125	28
	40	168	14

The sulphydryl compounds were administered immediately before 2,6-dimethoxyquinol (50 mg/kg) or menadiol sodium diphosphate (270 mg/kg). All drugs were administered intravenously. Data obtained from groups of a minimum of 10 mice.

against the acute toxicity of 2,6-dimethoxyquinol was investigated and compared with their ability to reduce the toxicity of menadiol sodium diphosphate. Mice were pre-treated with graded doses of the sulphydryl compound and, immediately afterwards, 2,6-dimethoxyquinol (50 mg/kg) or menadiol sodium diphosphate (270 mg/kg) was administered intravenously. These doses of the quinols killed approximately 80% of un-premedicated mice.

Cysteine and N-acetylcysteine were the most effective of the agents tested in protecting mice from the lethal effects of 2,6-dimethoxyquinol (Table 4). Somewhat surprisingly, however, these compounds did not afford any great degree of protection from the acute toxicity of menadiol sodium diphosphate. In contrast, cysteamine and dimercaprol appeared to be more effective in protecting from the lethal effects of menadiol sodium diphosphate than of 2,6-dimethoxyquinol.

Other species

The anaesthetic properties of M&B 16,573 were also studied in the rat, rabbit, dog and monkey. In these species, anaesthesia of short duration was produced by a suitable intravenous dose of the compound (about 100 mg/kg). This anaesthesia could be prolonged by following the induction dose with a slow intravenous infusion. The total dose infused to each species (number of animals) was: rat (20), 1000 mg/kg; rabbit (4), 400 mg/kg; dog (4), 400 mg/kg; and Rhesus monkey (4), 400 mg/kg. All the animals recovered uneventfully from the anaesthesia and no delayed toxic effects appeared during the following 48 hours.

Discussion

The observation that the lethal reaction described occurred only in cats suggests that, in this species, M&B 16,573 may follow a metabolic pathway which differs from that in the other laboratory animals. M&B 16,573 ((−)-2,6-dimethoxy-2-morpholinopropionate hydrochloride) was designed as a short-acting intravenous anaesthetic whose duration of action was limited by hydrolysis of the ester linkage (Bamford, Biggs, Lee, Owen, Pulsford & Wragg, 1969). The duration of the anaesthetic action of M&B 16,573 in the cat was not different from that observed in the mouse, rabbit, dog or monkey: the hydrolysis of the ester which determines its duration of action is, thus probably common to all the species studied.

The equivalent amount of 2,6-dimethoxyphenol given to cats produced toxic effects identical with those due to M&B 16,573. It seems likely, therefore, that it is in the metabolism of this phenol that cats differ from other species. Robinson & Williams (1958) state that the normal metabolic pathway for phenols involves conjugation with glucuronic acid and that cats do not readily form glucuronides. Dutton & Greig (1957) showed that cat liver slices failed to synthesize glucuronides, although this synthesis was observed in liver preparations from guinea-pig, rabbit, mouse and rat.

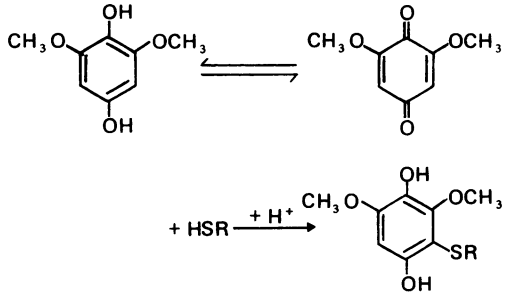
Miller, Powell, Olavesen & Curtis (1973) studied the metabolism of 2,6-dimethoxy [U-^{14}C] phenol in the cat. Following the intravenous administration of 20 mg/kg, 93% of the radioactivity in the urine was identified as 2,6-dimethoxyquinol disulphate, with small amounts of the parent phenol (5%) and a glucuronic acid conjugate (2%). After intravenous administration of 40 mg/kg, similar results were obtained, except that some 3% of the radioactivity in the urine was identified as 2,6-dimethoxyquinol. While sulphate conjugates are the major metabolites from the administration of phenols to cats, this detoxication process may become saturated when higher doses of phenols are administered and the urine might contain more parent phenols, together with their oxidation products (quinones).

In the rat (Miller, Powell, Olavesen & Curtis, 1974), intravenous 2,6-dimethoxy [U-^{14}C] phenol was excreted both as the glucuronide and the sulphate conjugate. The corresponding quinol was not apparently detected.

In the experiments described here, M&B 16,573, 200 mg/kg, given intravenously invariably produced delayed toxic effects. Assuming that most of the dose is hydrolysed, this would be equivalent to an intravenous dose of 94 mg/kg 2,6-dimethoxyphenol. According to the data of Miller *et al.* (1973), some of this 2,6-dimethoxyphenol would be converted to 2,6-dimethoxyquinol. Some of this 2,6-dimethoxyquinol would be detoxified by the formation of sulphate conjugates, but this detoxication process may become saturated, leading to the presence of free 2,6-dimethoxyquinol and possibly the corresponding 2,6-dimethoxybenzoquinone in the circulation.

It is well-known that quinones with unsubstituted positions in the nucleus form addition compounds with substances containing thiol groups (Redfearn, 1965). This reaction accounts for the inhibitory action of certain quinones on the mitochondrial electron transport system (Redfearn & Whittaker, 1962; Smith &

Lester, 1971). Thus, not only would this reaction, e.g.



explain the toxicity of 2,6-dimethoxyquinol in the cat, but it would also account for the protective action of sulphydryl compounds.

That the toxicity of M&B 16,573 in the cat was due to the formation of the 2,6-dimethoxyquinol rather than the toxicity of the free phenol was confirmed by the finding that a related compound in which the 4-position was blocked (4-allyl-2,6-dimethoxyphenyl-2-morpholinopropionate) did not produce the delayed toxic effects. Furthermore, Oettel (1936) described a hydroquinone syndrome after the chronic administration of hydroquinone to cats, which is similar to that following M&B 16,573.

The sulphydryl compounds may react with the 2,6-dimethoxybenzoquinone and prevent its interaction with thiol-containing substances involved in mitochondrial electron transport. The differences between the protective action of the sulphydryl compounds examined against the toxicity of 2,6-dimethoxyquinol and menadiol sodium diphosphate may be related to the ease with which these sulphydryl compounds react with the two quinones in question.

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