Biochemical Pharmacology, Vol. 29, pp. 2769–2776.

0006-2952/80/1015-2769 $02.00/0

DRUG INTERACTIONS WITH MISONIDAZOLE: EFFECTS OF DEXAMETHASONE AND ITS DERIVATIVES ON THE PHARMACOKINETICS AND TOXICITY OF MISONIDAZOLE IN MICE

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(*Received* 17 *March* 1980; *accepted* 31 *May* 1980)

Abstract—Misonidazole [1-(2-nitroimidazol-1-yl)-3-methoxypropan-2-ol; Ro 07-0582] selectively sensitizes hypoxic cells to radiation and is undergoing clinical trial in the radiation treatment of solid tumours. It has been suggested that the glucocorticoid hormone dexamethasone may reduce the incidence of neurotoxicity, the dose-limiting side effect of misonidazole in man. Here it is shown that the absorption and elimination of misonidazole (1 g/kg i.p.) in C3H mice are unaffected by pretreatment (i.p. for 5 days) with dexamethasone (10 mg/kg/day), dexamethasone acetate (10 mg/kg/day) and dexamethasone phosphate (0.5, 10, 25 and 100 mg/kg/day). The apparent half-life of misonidazole in blood and area under the curve (AUC) of misonidazole concentration × time were unaltered. Likewise *O*-demethylation was unaffected. In contrast, phenobarbitone pretreatment (80 mg/kg/day) increased misonidazole clearance through induction of demethylation. Dexamethasone phosphate pretreatment increased pentobarbitone sleeping-time and slightly decreased liver weight, whereas phenobarbitone did the opposite. Dexamethasone phosphate (25 mg/kg) given as an i.v. bolus injection immediately before misonidazole also had no effect on the systemic pharmacokinetics of misonidazole. Broadly, pretreatment with dexamethasone derivatives had little effect on brain misonidazole and desmethylmisonidazole. But after 100 mg/kg/day dexamethasone phosphate the 6 hr misonidazole concentration was reduced 36 per cent. Simultaneous dexamethasone phosphate (25 mg/kg) reduced the concentration at 1 hr by 15 per cent and the brain $AUC_{(0-6\,hr)}$ by 14 per cent. Dexamethasone phosphate pretreatment reduced the acute LD_{50} for misonidazole by up to 19 per cent whereas phenobarbitone increased it by 16 per cent. Simultaneous dexamethasone phosphate had no effect. The drug had little effect on misonidazole-induced hypothermia. The significance of these findings for the putative role of dexamethasone in the protection of misonidazole neurotoxicity is discussed.

It is considered that a major factor contributing to failure of local control by conventional radiotherapy is the presence in human solid tumours of radioresistant hypoxic cells [1]. Such cells may also be resistant to chemotherapy [2]. Misonidazole [1-(2-nitroimidazol-1-yl)-3-methoxypropan-2-ol; Ro 07-0582] selectively sensitizes hypoxic cells to conventional radiation treatment [1] and is preferentially cytotoxic toward them [3]. It also selectively sensitizes hypoxic cells to hyperthermia and some cytotoxic drugs [4, 5].

Misonidazole is undergoing extensive clinical trial as a radiosensitizer. The dose-limiting side effect is neurotoxicity, including peripheral neuropathy and in some cases ototoxicity and encephalopathy [6–9]. The incidence of neurotoxicity is reduced by restricting the total dose to 12 g/m^2 [6–10]. Unfortunately this means that in conventional multifraction radiotherapy the amount given with each radiation dose results in tumour concentrations suboptimal for radiosensitization.

Considerable effort has been directed towards development of improved radiosensitizers (e.g. see Ref. 11). One recent approach involves analogues excluded from nervous tissues by their reduced lipid/water partition coefficients [12]. An alternative, possible interim, strategy is to prevent or repair the toxic lesion(s) with other drugs.

It is not known whether the neurotoxicity is due to misonidazole itself or a metabolite, such as a nitroreduction product [13], but there is a correlation with prolonged drug exposure, i.e. a large area under the curve (AUC) of plasma misonidazole concentration × time [9]. In mice and dogs the drug's half-life ($t_{\frac{1}{2}}$) and AUC were reduced after pretreatment with phenobarbitone or phenytoin [13, 14]. The mechanism involves increased metabolism to desmethylmisonidazole [1-(2-nitroimidazol-1-yl)-2,3-propandiol; Ro 05-9963] following induction of hepatic microsomal mixed function oxidases. Pretreatment with these agents also increased the acute LD_{50} for misonidazole in mice [13].

In a recent controlled clinical study we observed similar induction of misonidazole metabolism by phenytoin [15], and several groups have noted unusually short half-lives in patients on phenytoin or phenobarbitone [10, 16]. Patients with brain tumours frequently receive these drugs for sedative and anti-convulsant therapy, and it is of interest that they have a low incidence of neurotoxicity [10, 16]. Hepatic enzyme induction may be involved, but a causal relationship should not be assumed. One complicating factor is that brain tumour patients are frequently maintained on the glucocorticoid dexamethasone to control cerebral oedema [17, 18], and

a recent small study suggested that dexamethasone itself may be protective [16]. More data are required and preliminary clinical trials with dexamethasone, phenobarbitone and phenytoin as protectors are under way.

Dexamethasone and other glucocorticoids affect membrane permeability [17, 19, 20] and the activities of many enzymes including those involved in drug metabolism [21, 22]. This paper concerns the effects of dexamethasone on the metabolism, brain penetration and acute toxicity of misonidazole in mice.

MATERIALS AND METHODS

Animals. Adult male C3H mice were obtained from Olac (Southern) Ltd. or from our own breeding colony. Except for urine collection mice were housed in plastic cages on sawdust bedding from soft white woods (Usher Ltd.). They were fed PRD nuts (Labsure) and allowed water *ad lib.* Care was taken to avoid contact with known microsomal enzyme inducers. Mice were used at 25–35 g body wt.

Drugs. Misonidazole and desmethylmisonidazole were gifts from Roche Laboratories. Phenytoin (5,5-diphenylhydantoin, sodium salt), dexamethasone (9α-fluoro-16α-methylprednisolone) and dexamethasone acetate (9α-fluoro-16α-methylprednisolone-21-acetate) were obtained from Sigma Chem. Co. Dexamethasone phosphate (9α-fluoro-16α-methylprednisolone-21-phosphate, disodium salt) was a gift from Merck, Sharp & Dohme Research Laboratories. Phenobarbitone (sodium salt) was obtained from BDH Laboratories and sodium pentobarbitone from May & Baker as a 60 mg/ml solution for injection (Sagatal).

Drug pretreatment regimes. Pretreatment regimes were similar to those described previously [13]. Drugs were usually prepared in saline (0.85% w/v) and injected i.p. in a volume of 10 ml/kg, once daily for five days (Days 1–5). In experiments to compare dexamethasone, dexamethasone acetate and dexamethasone phosphate the drugs were injected in 10% v/v ethanol in saline (10 ml/kg). Controls received vehicle alone.

Body weights were monitored daily to the end of the experiment (Day 7). In some experiments liver weights and pentobarbitone sleeping times were determined on Day 7. Sodium pentobarbitone was diluted to 6 mg/ml in saline and injected in 10 ml/kg i.p. to give a dose of 60 mg/kg. Sleeping time was the time to regain the righting reflex [13].

Pharmacokinetics of misonidazole. Misonidazole was prepared as a 25 mg/ml solution in Hank's balanced salt solution (HBSS). It was injected i.p. at a dose of 1g/kg (5 mmoles/kg) in 40 ml/kg. In pretreatment experiments misonidazole was given on Day 7, 48 hr after the last injection. In others the misonidazole was given immediately after i.v. bolus dexamethasone phosphate (25 mg/kg in 10 ml saline/kg via the tail vein). Blood samples were obtained from the tail or by cardiac puncture [13]. Whole brain was removed and immediately frozen. In urinary excretion studies, 5–6 mice were contained in a Urimax metabolism cage and urine collected for 24 hr. All samples were stored at −20°.

Estimation of misonidazole and desmethylmisonidazole. Concentrations of misonidazole and desmethylmisonidazole in blood, urine and tissue homogenates were determined by reversed-phase high-performance liquid chromatography (h.p.l.c.) [23] with the following minor modifications. The octadecylsilane h.p.l.c. columns used (10 μm particle size) were obtained from Hichrom (25 cm × 4.9 mm I.D. packed with Spherisorb S10 ODS) and from Waters Associates (25 × 3.9 mm I.D. packed with μBondapack C_{18}). The mobile phases for these columns were 35 and 25 per cent methanol/water, respectively. The internal standard was either 1-(2-nitroimidazol-1-yl)-3-chloropropan-2-ol (Ro 07-0269, Roche) or 1-(2-nitroimidazol-1-yl)-3-fluoropropan-2-ol (Ro 07-0741, Roche).

Concentrations of misonidazole and desmethylmisonidazole glucuronides in urine were determined after hydrolysis with β-glucuronidase. Samples were diluted × 10 in water and incubated in the dark at 37° for 24 hr with an equal volume of Glucurase (bovine liver β-glucuronidase solution, pH 5; Sigma).

Determination of misonidazole acute LD_{50}. This was carried out as before [13] except that the doses ranged from 1.1 to 2.3 g/kg injected i.p. in 80 ml HBSS/kg. Mice were observed for 7 days but deaths occurred within 3–4 days of treatment.

Measurement of body temperature. Rectal temperatures were measured with a thermistor probe connected to an externally calibrated electric thermometer (Light Laboratories Ltd) [13].

Estimation of pharmacokinetic parameters and statistical analysis. AUC values were estimated by Simpson's Rule. The apparent half-life ($t_{\frac{1}{2}}$) for drug elimination was calculated from the equation $t_{\frac{1}{2}} = \ln 2/K_{el}$, where K_{el} is the apparent elimination rate constant given by the slope of ln concentration × time. Lines of best fit, with standard errors, were calculated by least squares regression analysis. The LD_{50} values and confidence limits were calculated by probit analysis using a computer installation and programme at the Department of Radiology, Stanford University School of Medicine, CA, U.S.A. Confidence limits and significance levels were calculated using Student's t distribution.

RESULTS

Effect of dexamethasone phosphate pretreatment on pentobarbitone sleeping time and liver and body weight. At doses of 0.5, 25 and 100 mg/kg/day dexamethasone phosphate for 5 days some slight weight loss was observed, but this did not exceed 7–8 per cent. Occasional deaths were seen at 100 mg/kg/day and this was treated as the maximum tolerated. The acute LD_{50} for a *single* i.p. dose was between 500 and 1000 mg/kg.

The effects of dexamethasone phosphate on pentobarbitone sleeping time and liver weight are shown in Table 1, with phenytoin (40 mg/kg/day) and phenobarbitone (80 mg/kg/day) data for comparison. Phenobarbitone and phenytoin reduced the pentobarbitone sleeping time and increased liver weight. In contrast, with all three doses of dexamethasone phosphate the median pentobarbitone sleeping time was almost doubled, and the range increased with

Table 1. Effects of dexamethasone phosphate, phenobarbitone and phenytoin pretreatment on pentobarbitone sleeping time and liver weight in C3H male mice

	Sleeping time			Liver weight	
	N per group	Median (si range)‡ (min)	% Control	Mean (± S.E.) (g)	% Control
Control	8	48 (5)	100	1.39 ± 0.02	100
Phenobarbitone 80 mg/kg/day	8	16 (1)	33	1.56 ± 0.04*	112
Phenytoin 40 mg/kg/day	8	31 (5)	65	1.58 ± 0.03†	114
Dexamethasone 0.5 mg/kg/day	7	92 (20)	192	1.20 ± 0.03†	86
Dexamethasone 25 mg/kg/day	6	88 (44)	183	1.29 ± 0.01†	93
Dexamethasone 100 mg/kg/day	13	86 (>150)	179	1.35 ± 0.02	97

* $0.01 > P > 0.001$, significantly different from control.
† $P < 0.001$, significantly different from control.
‡ Semi-interquartile range.

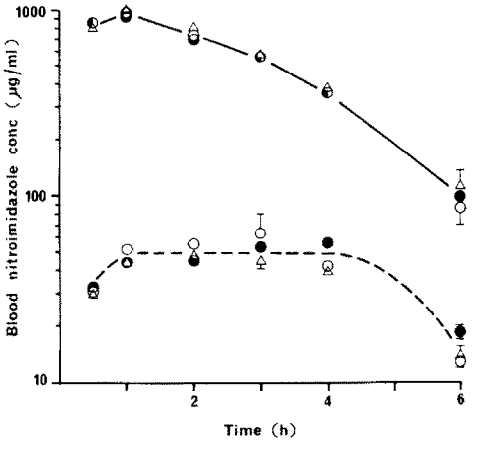

Fig. 1. Effect of dexamethasone phosphate pretreatment (i.p.) on the concentrations of misonidazole (upper solid line) and desmethylmisonidazole (lower broken line) in blood. Mice were pretreated with saline (●) or dexamethasone phosphate at 25 mg/kg/day (△) or 100 mg/kg/day (○) for 5 days. Misonidazole (1g/kg, i.p.) was given on Day 7. Error bars at 3 and 6 hr indicate one standard error (N = 8).

dose. Mean liver weight was reduced at 0.5 and 25 but not 100 mg/kg/day.

Effect of dexamethasone phosphate pretreatment on misonidazole pharmacokinetics. Pretreatment with 25 and 100 mg/kg/day dexamethasone phosphate had no effect either on the absorption of misonidazole or the subsequent concentrations of misonidazole and its metabolite desmethylmisonidazole in blood (Fig. 1). The apparent $t_{\frac{1}{2}}$ values for misonidazole (with 95 per cent confidence limits) were 1.76 (1.59–1.97) hr for the controls, 1.79 (1.58–2.06) hr after 25 mg/kg/day and 1.62 (1.44–1.85) hr after 100 mg/kg/day ($P > 0.1$). Values for the $AUC_{(0-6\,hr)}$ represented 109 and 102 per cent of the control for 25 and 100 mg/kg/day respectively. There was a similar lack of effect after 0.5 mg/kg/day (not shown). In contrast, phenobarbitone pretreatment (80 mg/kg/day) shortened the misonidazole $t_{\frac{1}{2}}$ to 0.81 (0.73–0.91) hr ($P < 0.001$), and reduced the AUC by 44 per cent. The peak desmethylmisonidazole concentration was doubled after phenobarbitone.

Effects of dexamethasone phosphate pretreatment on brain concentrations. Table 2 summarizes the results of experiments to assess the effects of 25 and 100 mg/kg/day dexamethasone phosphate pretreatment on the concentrations of misonidazole and

Table 2. Effects of pretreatment with dexamethasone phosphate (i.p. for 5 days) on misonidazole and desmethylmisonidazole concentrations in whole mouse brain. Misonidazole (1 g/kg i.p.) was given on Day 7

Group	N per group	Time	Misonidazole (μg/g ± S.E.)	Desmethylmisonidazole (μg/g ± S.E.)
Control	15		661 ± 27	9.9 ± 0.45
Dexamethasone phosphate (25 mg/kg/day)	15	1 hr	728 ± 23	11.3 ± 0.59
Dexamethasone phosphate (100 mg/kg/day)	16		696 ± 26	12.2 ± 0.69*
Control	15		98.5 ± 7.7	12.1 ± 0.36
Dexamethasone phosphate (25 mg/kg/day)	15	6 hr	93.4 ± 8.8	12.0 ± 0.68
Dexamethasone phosphate (100 mg/kg/day)	14		63.4 ± 7.6†	11.6 ± 1.06

* $P = 0.01$, significantly different from control.
† $0.01 > P > 0.001$, significantly different from control.

Table 3. Effects of dexamethasone, dexamethasone phosphate and dexamethasone acetate pretreatment (10 mg/kg/day i.p. for 5 days) on the pharmacokinetics of misonidazole (1g/kg i.p.)

		Blood misonidazole			Blood desmethylmisonidazole		Brain misonidazole	Brain desmethylmisonidazole
			$AUC_{(0-6\ hr)}$		$AUC_{(0-6\ hr)}$		Concentration at 6 hr (± S.E.) (μg/ml)	
	N per group	$t_{\frac{1}{2}}$ (95% confidence limits) (hr)	(μg.hr.m^{-1})	% saline/ ethanol control	(μg.hr.ml^{-1})	% saline/ ethanol control		
Saline	10	1.67 (1.47–1.95)	2847	104	217	95	78.0 ± 14.2	9.7 ± 0.97
10% ethanol/saline	10	1.53 (1.34–1.78)	2750	100	228	100	53.9 ± 12.1	8.7 ± 0.51
Dexamethasone	12	1.74 (1.56–1.98)	2853	104	240	105	75.8 ± 11.8	10.1 ± 0.71
Dexamethasone phosphate	10	1.68 (1.44–2.01)	3053	111	223	98	87.0 ± 18.2	8.2 ± 0.70
Dexamethasone acetate	10	1.56 (1.37–1.82)	2770	101	210	92	62.4 ± 12.2	7.2 ± 0.44*

* Significantly different from saline/ethanol control ($0.05 > P > 0.02$).

desmethylmisonidazole in whole brain, 1 and 6 hr after misonidazole (1 g/kg i.p.). Concentrations at 1 hr were slightly higher than the controls, but apart from the desmethylmisonidazole after 100 mg/kg/day ($P = 0.01$) this was not significant ($P > 0.05$). In general, the concentrations at 6 hr were unaltered ($P > 0.1$) but the misonidazole was reduced by 36 per cent after 100 mg/kg/day ($0.01 > P > 0.001$). There was no effect after 0.5 mg/kg/day (not shown).

Comparison of dexamethasone, dexamethasone acetate and dexamethasone phosphate pretreatment. These experiments were done to compare directly the effects of pretreatment with dexamethasone, dexamethasone acetate and dexamethasone phosphate on the pharmacokinetics of misonidazole. The dexamethasone derivatives were injected i.p. at a dose of 10 mg/kg/day for 5 days in 10% ethanol/saline as vehicle. None of the dexamethasone derivatives had any significant effect on misonidazole or desmethylmisonidazole in blood and brain ($P > 0.1$); the one exception was the brain desmethylmisonidazole concentration which was slightly lower in the dexamethasone acetate group ($0.05 > P > 0.02$) (Table 3).

Effect of dexamethasone phosphate pretreatment on misonidazole urinary excretion. Table 4 shows the effect of pretreatment with dexamethasone phosphate (0.5 mg/kg/day i.p. for 5 days) on the 24 hr urinary excretion profile of misonidazole and its metabolites, with phenobarbitone (80 mg/kg/day i.p. for 5 days) data for comparison. Neither agent significantly altered the ratio of misonidazole glucuronide/misonidazole or desmethylmisonidazole glucuronide/misonidazole ($P > 0.1$), though there was a tendency for the latter to be increased after phenobarbitone. However, phenobarbitone doubled the desmethylmisonidazole/misonidazole ratio ($0.02 > P > 0.01$) whereas dexamethasone phosphate had no effect ($P > 0.1$).

Effect of simultaneous dexamethasone phosphate on misonidazole pharmacokinetics. Given as a single bolus i.v. injection immediately before misonidazole, dexamethasone phosphate (25 mg/kg) altered neither the absorption of misonidazole nor the subsequent concentrations of misonidazole and desmethylmisonidazole in blood. The apparent $t_{\frac{1}{2}}$ values (with 95 per cent confidence limits) were 1.19 (1.08–1.32) hr for the controls and 1.16 (1.02–1.33) hr with dexamethasone phosphate ($P > 0.1$). The corresponding $AUC_{(0-6\ hr)}$ values for misonidazole were 2361 μg.hr.ml^{-1} for controls and 2367 μg.hr.ml^{-1} with dexamethasone phosphate.

Effect of simultaneous dexamethasone phosphate on brain concentrations. Table 5 summarizes the results of experiments to assess the effects of 25 mg/kg dexamethasone phosphate i.v. given immediately before misonidazole on the concentrations of misonidazole and desmethylmisonidazole in blood and whole brain. As above, there was no effect on the blood concentrations ($P > 0.05$). Though there were no differences at 3 and 6 hr, the brain misonidazole concentration at 1 hr was reduced by 15 per cent and the $AUC_{(0-6\ hr)}$ by 14 per cent. Also the concentration of desmethylmisonidazole in the brain was significantly lower than the control at 3 hr ($0.02 > P > 0.01$) but not at other times ($P > 0.1$).

Table 4. Effect of pretreatment with dexamethasone phosphate (0.5 mg/kg/day for 5 days) and phenobarbitone (80 mg/kg/day for 5 days) on the 24 hr urinary excretion of misonidazole and its metabolites. Misonidazole (1g/kg i.p.) was given on Day 7*

	Desmethylmisonidazole	Misonidazole-gluc	Desmethylmisonidazole-gluc
	Misonidazole	Misonidazole	Misonidazole
Control	0.71 ± 0.099	0.39 ± 0.026	0.032 ± 0.024
Dexamethasone phosphate	0.69 ± 0.070	0.43 ± 0.0067	0.014 ± 0.0099
Phenobarbitone	1.42 ± 0.12†	0.53 ± 0.16	0.112 ± 0.047

* Results are expressed as metabolite/misonidazole ratios (means ± S.E. of three independent determinations).

† Significantly different from control, $0.02 > P > 0.01$.

Table 5. Effect of a single i.v. bolus dose of 25 mg/kg dexamethasone phosphate on the concentrations of misonidazole in blood and whole brain. Misonidazole (1g/kg i.p.) was given immediately after dexamethasone or saline*

		Misonidazole		Ro 05-9963	
Group	Time (hr)	Blood (μg/ml)	Brain (μg/g)	Blood (μg/ml)	Brain (μg/g)
Control	1	869 ± 21	606 ± 30	51.2 ± 3.6	11.1 ± 0.62
Dexamethasone Phosphate	1	806 ± 23	516 ± 21‡	45.3 ± 1.6	10.6 ± 0.41
Control	3	413 ± 43	235 ± 28	49.6 ± 3.3	17.3 ± 1.4
Dexamethasone phosphate	3	400 ± 27	195 ± 15	45.0 ± 1.9	13.8 ± 0.76†
Control	6	52.4 ± 6.5	45.0 ± 4.8	14.7 ± 1.6	13.0 ± 0.74
Dexamethasone phosphate	6	65.0 ± 12.7	52.0 ± 9.6	12.7 ± 1.2	11.6 ± 0.62

* N = 15 for 1 hr and 6 hr and 10 for 3 hr groups.

† $0.05 > P > 0.02$, significantly different from control.

‡ $0.02 > P > 0.01$, significantly different from control.

Table 6. Effects of dexamethasone phosphate on the acute LD_{50} for misonidazole*

	Misonidazole LD_{50} (95 per cent confidence limits) (g/kg)
(A) Pretreatment (5 days)	
Saline	1.84 (1.76–1.93)
Phenobarbitone (80 mg/kg/day)	2.14 (2.02–2.27)‡
Dexamethasone phosphate (0.5 mg/kg/day)	1.70 (1.60–1.79)†
Dexamethasone phosphate (25 mg/kg/day)	1.49 (1.36–1.62)‡
Dexamethasone phosphate (100 mg/kg/day)	1.51 (1.41–1.61)‡
(B) Simultaneous treatment	
Simultaneous saline	1.61 (1.44–1.80)
Simultaneous dexamethasone phosphate (25 mg/kg)	1.72 (1.62–1.82)§

* In panel A, 6–9 dose levels of misonidazole were used for each pretreatment, and 2–9 mice per dose level of misonidazole. In panel B, 5 dose levels of misonidazole were used for each treatment, and 6–16 mice per dose level of misonidazole.

† $P = 0.02$.

‡ $P < 0.01$.

§ $P > 0.1$.

Effect of dexamethasone phosphate on the acute LD_{50} *for misonidazole.* The results of experiments to assess the effects of dexamethasone phosphate pretreatment (0.5, 25 and 100 mg/kg/day i.p. for 5 days) or a 25 mg/kg single i.v. bolus injection immediately before misonidazole are summarized in Table 6, with phenobarbitone pretreatment data (80 mg/kg/day i.p. for 5 days) for comparison.

In the pretreatment experiments (Table 6A) phenobarbitone increased the acute LD_{50} dose by 16 per cent ($P < 0.001$). However, dexamethasone phosphate decreased the LD_{50} by 8 per cent after 0.5 mg/kg/day ($P = 0.02$), 19 per cent after 25 mg/kg/day ($P < 0.001$) and 18 per cent after 100 mg/kg/day ($P < 0.001$). On the other hand, simultaneous dexamethasone phosphate (25 mg/kg i.v.) increased the LD_{50} by 7 per cent but this was not significant ($P > 0.1$) (Table 6B).

Effect of dexamethasone phosphate on misonidazole-induced hypothermia. Misonidazole (1g/kg) causes a drop in mouse body temperature [13, 24]. Mice injected i.p. with HBSS (misonidazole vehicle) showed little variation in rectal temperature (Fig. 2). Those pretreated with saline, 0.5 and 25 mg/kg/day dexamethasone phosphate exhibited similar temperature reductions after misonidazole, but with 100 mg/kg/day dexamethasone phosphate the temperature loss was less marked (Fig. 2A). Simultaneous dexamethasone phosphate (25 mg/kg) had no effect (Fig. 2B).

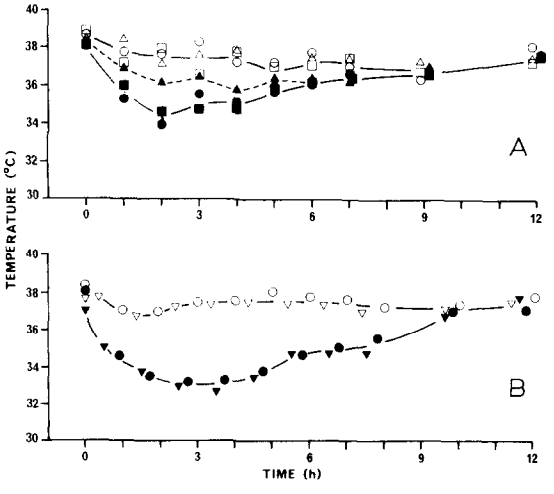

Fig. 2. Effects of dexamethasone phosphate on misonidazole-induced decrease in rectal temperature. Panel A: Effect of pretreatment. Mice were pretreated (i.p. for 5 days) with saline (circles), 0.5 mg/kg/day dexamethasone phosphate (squares) or 100 mg/kg/day (triangles). They then received i.p. misonidazole (1g/kg) (closed symbols) or HBSS (open symbols). Data for 25 mg/kg/day (not shown) were very similar to those for 0.5 mg/kg/day. Ambient temperature was 23.1 ± 0.9 (S.D.)°. Panel B: Effect of simultaneous treatment. Mice were injected i.v. with saline (circles) or 25 mg/kg dexamethasone phosphate (triangles) immediately before i.p. misonidazole (1g/kg) (closed symbols) or HBSS (open symbols). Ambient temperature was 23.5 ± 0.5 (S.D.)°. Data shown are median values for 10 mice per group in Panel A and 5 mice per group in Panel B.

DISCUSSION

Various dexamethasone derivatives are available for clinical use. For cerebral oedema oral dexamethasone is widely used, and the more water soluble dexamethasone sodium phosphate is given by injection [17, 18]. Here, most experiments were done with dexamethasone phosphate because of its water solubility. In addition, dexamethasone, dexamethasone phosphate and dexamethasone acetate were directly compared. In man, intramuscular dexamethasone phosphate is hydrolysed rapidly to dexamethasone, whereas hydrolysis of the acetate is slower [25].

None of the dexamethasone derivatives had any effect on the absorption of misonidazole from the peritoneal cavity into the blood, or the subsequent blood concentrations of misonidazole in C3H mice. The $t_{\frac{1}{2}}$ and AUC were unaltered. In addition, the *O*-demethylation of misonidazole was unchanged, as measured by desmethylmisonidazole in the blood. In contrast, phenobarbitone (80 mg/kg/day) reduced the $t_{\frac{1}{2}}$ and AUC considerably by inducing demethylation. Similar results were observed with phenobarbitone and phenytoin using BALB/c and B10 mice [13]. In that study it was reported that these agents had no effect on urinary excretion of misonidazole and its metabolites. However, when the data are expressed as the ratio of desmethyl-misonidazole/misonidazole in the 24 hr urine we find that phenobarbitone increased this ratio from 0.81 to 1.51 in BALB/c mice. Here phenobarbitone gave a similar increase from 0.71 to 1.42 in C3H mice but dexamethasone phosphate pretreatment (0.5 mg/kg/day) had no effect. Gangji *et al.* [26] independently observed no effect on misonidazole urinary excretion in rats pretreated with 0.23 mg/kg/day dexamethasone i.p. for 7–10 days. The apparent lack of induction of hepatic mixed-function oxidases by dexamethasone phosphate pretreatment is consistent with its effects on liver weight and pentobarbitone sleeping time. Whereas, in agreement with previous results [13], phenobarbitone, and phenytoin significantly increased the liver weight and reduced the sleeping time, dexamethasone phosphate did not.

Dexamethasone is metabolized to more polar unconjugated forms and glucuronide conjugates [27], and substrate competition between steroids and drugs such as cyclophosphamide has been reported [28]. However, concomitant dexamethasone phosphate had no effect on misonidazole demethylation. The dexamethasone dose (25 mg/kg) was considerably lower than that of misonidazole (1g/kg) but this ratio (1:40) is likely to be even lower in clinical practice.

Pretreatment with dexamethasone derivatives had little effect on brain concentrations of misonidazole and desmethylmisonidazole. A reduction in brain misonidazole was seen only at the maximum tolerated dose of dexamethasone phosphate and at 6 hr but not 1 hr. However, i.v. injection of a single dose of 25 mg/kg immediately before misonidazole reduced brain misonidazole concentrations at 1 hr by 15 per cent and the AUC by 14 per cent.

Dexamethasone inhibits the rate of amino acid

transport in rat hepatoma cells *in vitro* [29] and causes capillary vasoconstriction and reduced capillary permeability [20]. Increased cerebro-vascular permeability in experimental vasogenic oedema can be normalized by steroids [17], and is probably important for their use in brain tumour patients. It may be that the observed reduction in brain misonidazole concentrations by dexamethasone phosphate is due to reduced cerebrovascular permeability and/or blood flow.

In contrast to phenobarbitone and phenytoin pretreatments which increase the LD_{50} of misonidazole, dexamethasone phosphate pretreatment reduces the LD_{50}. But simultaneous i.v. dexamethasone phosphate has no effect. The acute LD_{50} of misonidazole is considerably lower when mice are maintained at normal body temperature [24]. But the dexamethasone phosphate had little or no effect on misonidazole-induced hypothermia. The mechanism of the increased toxicity of misonidazole after dexamethasone phosphate pretreatment is therefore unclear. However, there may be no correlation between neurotoxicity and toxicity measured by acute lethality assay. Small animal models are being developed for the assay of neurotoxicity [30, 31] and may be suitable for protection studies with dexamethasone, phenobarbitone and phenytoin.

Regardless of whether misohidazole neurotoxicity involves the parent compound or a metabolite such as a nitroreduction product, a diminution of the AUC for misonidazole in critical neural structures is likely to reduce neurotoxicity. A decrease in brain AUC through liver microsomal enzyme induction occurs with phenobarbitone and phenytoin but is not seen with dexamethasone. However, there is a small but significant reduction by a different mechanism.

Because of the wide-ranging and complex biochemical and physiological effects of dexamethasone, other mechanisms for the possible protection or repair of misonidazole neurotoxicity may be envisaged. For example, misonidazole causes increased lysosomal enzyme activity in mouse brain and peripheral nerve [31], and dexamethasone is known to stabilize lysosomal membranes [19]. Alternatively, the mechanism may involve reduction of oedema, which has been reported in rat peripheral nerve after misonidazole [32].

Clinical trials will evaluate the possible protective role of dexamethasone, phenobarbitone and phenytoin in the control of misonidazole neurotoxicity. The present studies may facilitate interpretation of the clinical data.

Acknowledgements—I thank Professor N. M. Bleehen for his advice and continued support; Ms. Jane Donaldson and Dr. Nancy Smith for expert technical assistance; Ms. Paula Rayner and Ms. Jill Shaw for help in some of the animal experiments; and Mr. Laurence S. Freedman for statistical advice and helpful discussions. I also thank Dr. C. E. Smithen of Roche Products Ltd. for supplies of nitroimidazoles, Mr. R. G. Benfield of Merck, Sharp & Dohme Research Laboratories for supplies of dexamethasone sodium phosphate, and Dr. J. M. Brown and Mr. E. Parker, Department of Radiology, Stanford School of Medicine, California, for the probit analysis of LD_{50} data.

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