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CYCLOPHOSPHAMIDE CYSTITIS

STUDIES AIMED AT ITS MINIMIZATION

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Abstract—Two types of approach were used in an effort to alleviate cyclophosphamide-induced cystitis, which is due to acrolein liberated within the urinary tract. The more successful was the concurrent administration of 2,3-dimercaptosuccinic acid, an orally effective protecting agent. Replacement of cyclophosphamide with 6-methylcyclophosphamide, an effective anti-tumour drug which liberates the less toxic crotonaldehyde in place of acrolein, resulted in less bladder toxicity but it was not sufficiently advantageous to merit further study. 5-d_2-Cyclophosphamide, which on metabolism releases acrolein at less than 20 per cent of the rate of release from cyclophosphamide, was found to be much less toxic than the undeuterated form.

The cause of haemorrhagic cystitis as a side effect of the anti-tumour drugs cyclophosphamide (CP)* and ifosfamide (IP) has now been conclusively demonstrated to be the acrolein released within the bladder from their primary hydroxylated metabolites [1, 2]. Various techniques have been used to alleviate this symptom, including maintenance of a high urine flow [3], protection with *N*-acetyl-L-cysteine [1 and refs. cited therein] and protection with i.v. 2-mercaptoethane sulphonate [4, 5]. Two other approaches seem possible; firstly, the use of an orally effective protecting agent and secondly, the use of an analogue of cyclophosphamide releasing less of, or a less toxic, unsaturated aldehyde.

Recently a new agent for the treatment of lead and arsenic poisoning was reported [6, 7]. 2,3-Dimercaptosuccinic acid (DMS) in aqueous solution by mouth was found to be more effective than EDTA, dimercaptopropanol and D-penicillamine in inducing lead excretion, the major route being through the kidneys. Thus, DMS presented the required properties: absorption from the gastrointestinal tract, water solubility, excretion through the kidneys and two sulphydryl groups.

6-Methylcyclophosphamide (6-MeCP) is almost as good an anti-tumour agent as CP [8] or equally effective [9]. In place of acrolein it releases crotonaldehyde which is acutely less toxic to rats than is acrolein [10–12]. Thus 6-MeCP may be an alternative drug with milder urotoxic side-effects.

On the other hand, 5-d_2-cyclophosphamide (5-d_2-CP) liberates acrolein-2-d from its 4-hydroxylated metabolite at about 18 per cent of the rate of formation of acrolein from 4-hydroxycyclophosphamide [13]. Thus, the induction of cystitis by 5-d_2-CP may require a higher dose than of CP.

MATERIALS AND METHODS

Cyclophosphamide monohydrate was purchased from Koch-Light Laboratories Ltd., Colnbrook, Bucks, England. 6-Methylcyclophosphamide was a gift from Professor N. Brock, Asta-Werke, Bielefeld, West Germany. 5-d_2-CP was synthesised [13] by Dr G. Taylor of this Institute. 2,3-Dimercaptosuccinic acid (DMS), acrolein and crotonaldehyde were purchased from Aldrich Chemical Co. Ltd., Gillingham, Dorset. The aldehydes were redistilled before use.

Male Wistar rats, bred in this Institute, were used. Drugs were administered intraperitoneally (i.p.) in aqueous solution, except for 6-methylcyclophosphamide (i.p. in 10% ethanol) and DMS (orally in 5% $NaHCO_3$). Damage to the bladder was assessed and analysed as previously described [1], values of P less than 0.02 obtained from Student's *t* test being considered significant.

Walker 256 carcinosarcoma ascites cells were maintained and treated in culture as described by Phillips [14]. Cytotoxicity was expressed as the concentration required to cause a 50 per cent inhibition of growth over 72 hr.

Kinetic constants for the microsomal metabolism of CP and 6-MeCP were measured by a method [15] based on the reaction of 3-methyl-2-benzothiazolone hydrazone with aldehydes [16].

RESULTS

Table 1 shows that DMS, when given orally at 0.165 mmoles/kg (30 mg/kg), protected male rats against cyclophosphamide-induced cystitis when given at the same time. Administration of DMS

* Abbreviations used: CP—cyclophosphamide, {2-[bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide}; IP—ifosfamide, [3-(2-chloroethyl)-2-(2-chloroethylamino)tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide]; DMS—2,3-dimercaptosuccinic acid; 6MeCP—6-methylcyclophosphamide; 5-d_2-CP—5-d_2-cyclophosphamide.

Table 1. Protection of the male rat bladder from cyclophosphamide-induced cystitis by orally administered 2,3-dimercaptosuccinic acid

	Time of dosing of DMS relative to CP (min)	Number of animals	Dry weight (mg/100 g body weight)	Water content (mg/100 g body weight)
Control	—	9	6.7 ± 0.3	14.6 ± 0.5
CP	—	10	11.1 ± 0.5*	53.9 ± 4.0*
CP + DMS	0	15	7.0 ± 0.4†	21.6 ± 3.6†
CP + DMS	−30	5	9.4 ± 0.4*	41.0 ± 4.6*
CP + DMS	+30	10	7.9 ± 0.6†	28.1 ± 5.9†

Cyclophosphamide (CP) was injected ip at 0.365 mmoles/kg. Dimercaptosuccinic acid (DMS) was dosed orally (in 5% $NaHCO_3$) at 0.165 mmoles/kg.

* $P < 0.02$ compared to controls.

† $P < 0.02$ compared to CP.

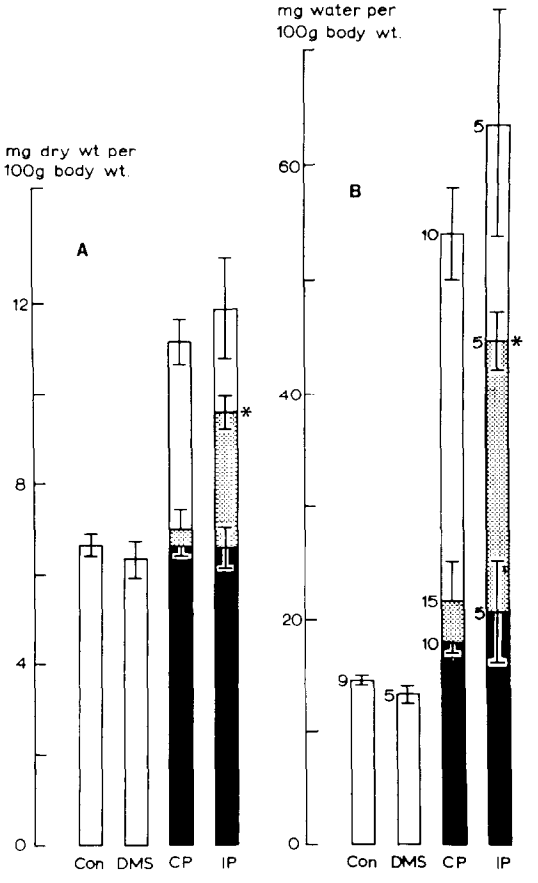

Fig. 1. A. Bladder dry weights (expressed as mg/100 g body weight) and B. Bladder water content (expressed as mg/100 g body weight), in male rats treated i.p. with CP and IP at 0.365 mmoles/kg. Dotted bars indicate combination of test compound with DMS at 0.165 mmoles/kg and black bars combination with DMS at 0.55 mmoles/kg. Error bars show the standard error. Figures show the number of animals in each group. CP and IP caused highly significant changes. Those bars marked* did not differ significantly from IP.

30 min prior to CP was not protective. Thus the relative timing was important, as previously shown with *N*-acetylcysteine [1]. Increasing the dose of DMS to 0.55 mmoles/kg (100 mg/kg) did not affect the degree of protection against cyclophosphamide cystitis (Fig. 1). However, ifosfamide toxicity was only eliminated by the higher dose of DMS; 0.165 mmoles/kg failed to cause a significant difference in either dry weight or water content.

Table 2 shows the comparative toxicities of acrolein and crotonaldehyde. The toxicity of acrolein-2-d would not differ from that of the non-deuterated form, as no precedent exists of deuterium influencing the reactivity of an adjacent double bond. Figure 2 shows dose–response curves for CP, 6-MeCP and 5-d_2-CP where response is expressed as percentage increase in bladder water content/100 g compared to controls.

DISCUSSION

The water soluble compound DMS was shown to be an orally effective protecting agent (Table 1 and Fig. 1) against cyclophosphamide-induced cystitis. The oral dose required was less than 1 per cent of the intraperitoneal LD_{50} [7], making DMS comparable to 2-mercaptoethane sulphonate [4] in terms of a benefit-risk ratio. If prophylactic protection against sub-clinical cystitis is to be practised [17], it is arguable that an orally effective agent is desirable as it would permit simpler out-patient and home treatments. DMS appears to fulfil that role but would require clinical evaluation.

The alternative approach, of using a cyclophosphamide analogue which liberates crotonaldehyde, an aldehyde of lower toxicity than acrolein, proved less successful. Table 2 shows a three- to five-fold difference in acute whole-body toxicity, and a five-fold difference in the toxicities to ascites tumour cells grown in the presence of the aldehydes. Treatment of the tumour cells for only 15 min widened the gap in toxicities to 21.5-fold. In addition, whilst K_m values for the microsomal metabolism of CP and 6Me-CP

Table 2. Toxicities of acrolein and crotonaldehyde

	Acrolein	Crotonaldehyde
LD_{50} p.o. (mg/kg)*	46	300
LD_{50} s.c. (mg/kg)†	50	140
ID_{50} values (μg/ml) for Walker ascites tumour cells in suspension culture		
15 min treatment	0.7	15.0
Continuous (72 hr) treatment	0.2	1.0
Dose reduction factor (15 min/72 hr)	3.5	15

* Data from Smyth *et al.* [10, 11].
† Data from Skog [12].

were similar (0.60 and 0.52 mM respectively), the maximal rate of metabolism was 3-fold faster for CP than for 6MeCP (24.4 and 8.2 nmoles aldehyde formed/min/mg protein, respectively). However, when the bladder toxicity was examined (Fig. 2) 6-MeCP was found to be only slightly less urotoxic than CP. 5-d_2-CP, which following metabolism releases acrolein quite slowly [13], was significantly less toxic than CP indicating that the rate of release and the extent of release of acrolein into the bladder is of considerable importance to urotoxicity. However, 5-d_2-CP is thought to be an inferior anti-tumour agent [13].

Thus it would seem that the principal means available for reducing or eliminating the bladder toxicity of CP and IP is not the substitution of an analogue for the drug, but is the protection of the patient with an acrolein-reactor. DMS seems particularly suited to the purpose.

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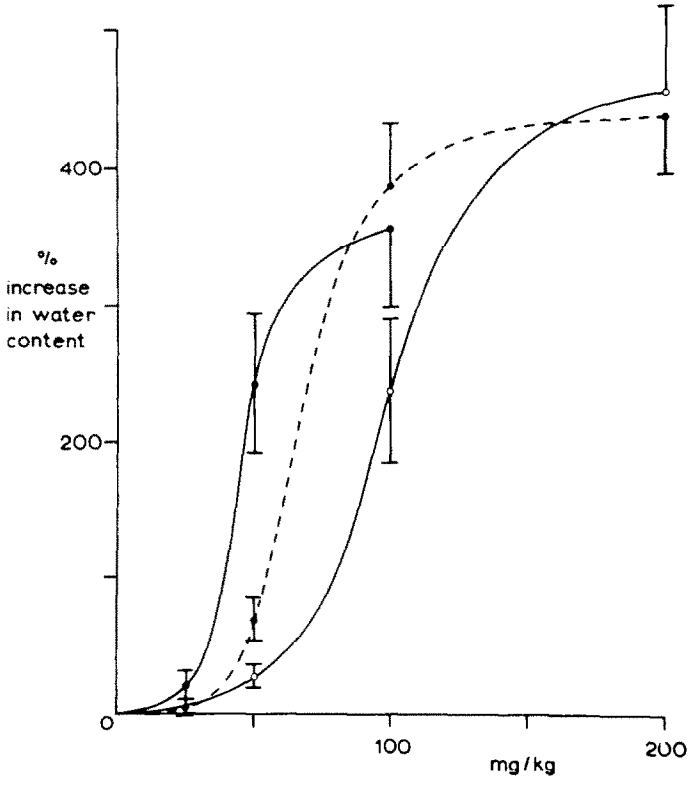

Fig. 2. Dose–response curves for CP (——●——) 6-MeCP (– – ● – –) and 5-d_2-CP (——○——) injected i.p. into male rats, where response is measured as the percentage increase in mg water content of the bladder per 100 g body weight when compared to control animals. Maxima and minima of the error bars were obtained by comparing the mean + 1 S.E. of the test value with the mean − 1 S.E. of the control value, and vice versa.

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