Does 2-mercaptoethane sulphonate (mesna) prevent cyclophosphamide and azathioprine induced immunosuppression? *In vitro* studies

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1 The effect of 2-mercaptoethane sulphonate (mesna) on the inhibition of the human MLR by 4-hydroxycyclophosphamide or azathioprine was studied.

2 4-Hydroxycyclophosphamide (34 μM) completely inhibited the MLR and this inhibition was unaffected by 122 μM of 2-mercaptoethane sulphonate or its disulphide.

3 At high concentrations (mM) 2-mercaptoethane sulphonate inhibited the MLR reaching 85% at 24 mM.

4 2-Mercaptoethane sulphonate (15–122 μM) had no effect on azathioprine (36 μM) inhibition.

5 The results of these *in vitro* studies suggest that 2-mercaptoethane sulphonate does not interfere with cyclophosphamide or azathioprine induced suppression of cellular immunity.

Keywords 2-mercaptoethane sulphonate cyclophosphamide azathioprine immunosuppression

Introduction

Regional detoxification with 2-mercaptoethane sulphonate to prevent chemical cystitis in patients treated with high dosage cyclophosphamide and other oxazaphosphorines has been a major advance in therapeutics. The oxazaphosphorine metabolite that causes haemorrhagic cystitis is acrolein (Brock *et al.*, 1979). 2-Mercaptoethane sulphonate prevents cystitis by reacting with acrolein in urine to form a stable non-toxic compound and prevents its release from 4-hydroxycyclophosphamide (Brock *et al.*, 1981; Brock & Pohl, 1983).

As there have been reports of carcinoma of the bladder occurring in patients treated with cyclophosphamide as an immunosuppressive agent (Pearson & Soloway, 1978; Kinlen *et al.*, 1981), the question arises as to whether 2-mercaptoethane sulphonate will prevent it as in the rat experimental model (Habs & Schmahl, 1983). Before giving this prophylactic therapy it is

essential to establish that it does not interfere with the immunosuppressive effect of cyclophosphamide. Here we report the effects of 2-mercaptoethane sulphonate and its disulphide on the suppression of the human mixed lymphocyte reaction by the active metabolite of cyclophosphamide, 4-hydroxycyclophosphamide. As azathioprine is sometimes used in combination with cyclophosphamide (Mukherjee, 1971) and is known to react with thiols (Watanabe *et al.*, 1978; Maddocks, 1979), we have also studied its inhibitory effects on the human MLR in the presence of 2-mercaptoethane sulphonate.

Methods

2-Mercaptoethane sulphonate sodium (mesna) and disodium 2,2'-dithio-bis-ethane sulphonate (dimesna) were kindly provided by Boehringer

Ingelheim Limited, Bracknell, U.K. 2-Mercaptoethane sulphonate is converted to its disulphide in the blood after intravenous administration (Brock *et al.*, 1982) so the effect of 2,2'-dithio-bis-ethane sulphonate (dimesna) was also tested on the human MLR in the presence of 4-hydroxycyclophosphamide.

4-Hydroperoxycyclophosphamide (a gift from Boehringer Ingelheim Limited) was used in these experiments as 4-hydroxycyclophosphamide is very unstable at room temperature. When added to culture medium 4-hydroperoxycyclophosphamide is rapidly and completely converted to 4-hydroxycyclophosphamide (Low *et al.*, 1982; Zincke *et al.*, 1983).

The details of other materials and methods used have been described in detail previously (Al-Safi & Maddocks, 1983, 1984). Briefly, lymphocytes were isolated from blood samples of two healthy blood donors and cultured together for 5 days. Lymphocytes of either individual were pre-treated with mitomycin-C to block their responding capacity of DNA synthesis but retain their stimulating ability. The response was measured by the incorporation of tritiated thymidine ($[^3\text{H}]\text{-TdR}$) into DNA for the last 8 h.

The stability of mesna and dimesna in culture medium was determined by incubating each compound (10 mM) in RPMI 1640 medium containing 20% human serum for 2 h at 37°C. After ultrafiltration through a membrane cone (cut off molecular weight 25 000; Amicon Ltd, Glos., England) the filtrate was applied to cellulose thin layer chromatography plates which were developed in methanol. Mesna was detected by Ellman's reagent (Glaser *et al.*, 1970) and also by monobromobimane (Thiolite, Calbiochem., Cambridge Bioscience, England). Dimesna was detected by applying these thiol reagents after reduction with borohydride (Glaser *et al.*, 1970).

Statistical significance was assessed by analysis of the variance (ANOVA) and the Student's *t*-test for paired samples.

Results

2-Mercaptoethane sulphonate had no effect on the MLR at 4–122 μM while it caused significant inhibition at and above 180 μM (Table 1). Its disulphide derivative had no effect when used at 122 μM .

Mixed lymphocyte cultures incubated with 2-mercaptoethane sulphonate (122 μM) and 4-hydroperoxycyclophosphamide (17 and 34 μM) were suppressed to the same extent as those cultured with 4-hydroperoxycyclophosphamide only (Figure 1). Moreover, 2,2'-dithio-bis-ethane

sulphonate (122 μM) did not interfere with 4-hydroperoxycyclophosphamide (17 and 34 μM) inhibition of the MLR (Figure 1).

2-Mercaptoethane sulphonate (15–122 μM) did not have any significant effect on azathioprine (36 μM) suppression of the MLR (Table 2). Further, addition of 2-mercaptoethane sulphonate (122 μM) to mixed lymphocyte cultures 2 h prior to azathioprine (18 and 36 μM) did not affect the inhibition of azathioprine.

Thin layer chromatography studies showed that 2-mercaptoethane sulphonate and 2,2'-dithio-bis-ethane sulphonate were stable in the lymphocyte culture medium. In particular, there was no evidence of oxidation of 2-mercaptoethane sulphonate to 2,2'-dithio-bis-ethane sulphonate in the presence of 20% human serum.

Discussion

We have shown that the cyclophosphamide metabolite 4-hydroxycyclophosphamide (34 μM) completely inhibited the human MLR. This inhibition was unaffected by 2-mercaptoethane sulphonate or its disulphide, a metabolite formed in plasma, at therapeutic concentrations [100–1000 μM] (Pohl, 1980). At these concentrations neither 2-mercaptoethane sulphonate nor its disulphide had any inhibitory effect on the human MLR.

With relatively high concentrations (mM) 2-mercaptoethane sulphonate inhibited the MLR and caused 85% inhibition at 24 mM. The mechanism of this inhibitory effect is unknown. 2-Mercaptoethane sulphonate is highly ionised so that cellular entry would be expected to be small. Possible sites of action would therefore include thiol and/or disulphide groups on the lymphocyte membrane or components of the culture medium.

Although it is well established that 2-mercaptoethane sulphonate does not affect the antitumour activity of cyclophosphamide and other oxazaphosphorines (Burkert, 1983) its effect on the immunosuppressive activity of these drugs has been little studied. Brock *et al.* (1982) showed that 2-mercaptoethane sulphonate had no effect on cyclophosphamide suppression of humoral antibody responses in mice and rats challenged with sheep red blood cells and Brucella, respectively. In contrast, Hows *et al.* (1983) reported that five out of eight bone marrow transplant recipients rejected their grafts whilst being treated with cyclophosphamide and 2-mercaptoethane sulphonate. In another series of eight bone marrow graft recipients treated

Table 1 Effect (mean $\pm$ s.e. mean) of 2-mercaptoethane sulphonate (2-MES) on the MLR

2-MES $\times 10^{-5}M$	n	% Inhibition	Significance P	2-MES $\times 10^{-3}M$	n	% Inhibition	Significance P
0.40	10	2 $\pm$ 12	NS	0.18	12	19 $\pm$ 4	< 0.01
0.80	10	3 $\pm$ 9	NS	0.30	12	25 $\pm$ 5	< 0.02
1.50	10	7 $\pm$ 12	NS	0.48	12	35 $\pm$ 3	< 0.005
3.00	10	7 $\pm$ 7	NS	6.08	12	49 $\pm$ 6	< 0.001
6.10	10	9 $\pm$ 8	NS	12.16	12	66 $\pm$ 4	< 0.001
12.20	10	-23 $\pm$ 32	NS	24.33	12	85 $\pm$ 3	< 0.001

Figure 1 Effect (mean $\pm$ s.e. mean) of 2-mercaptoethane sulphonate and 2,2'-dithio-bis-ethane sulphonate on 4-hydroperoxycyclophosphamide inhibition of the MLR. MLR measured by [3H]-Tdr (thymidine) incorporation into DNA. n = number of pairs of test and control cultures. A = 4-hydroperoxycyclophosphamide (17 μM). B = 4-hydroperoxycyclophosphamide (34 μM). C = 2-Mercaptoethane sulphonate (122 μM). D = disodium 2,2'-dithio-bis-ethane sulphonate (122 μM). * = statistically non-significant.**Table 2** Effect (mean $\pm$ s.e. mean) of 2-mercaptoethane sulphonate (2-MES) on azathioprine (AZ) inhibition of the MLR ($n = 12$)

AZ (μM)	2-MES (μM)	% Inhibition	Significance P	% reversal of AZ inhibition by 2-MES	Significance P
36	Nil	84 $\pm$ 5	< 0.005		
36	15	88 $\pm$ 3	< 0.005	-7 $\pm$ 4	NS
36	30	90 $\pm$ 3	< 0.005	-10 $\pm$ 5	NS
36	61	90 $\pm$ 3	< 0.005	-10 $\pm$ 6	NS
36	122	89 $\pm$ 3	< 0.005	-9 $\pm$ 5	NS

with high dose cyclophosphamide and forced diuresis there was no graft rejection. These authors suggested that 2-mercaptoethane sulphonate might have interfered with the immunosuppressive activity of cyclophosphamide. Clearly a controlled trial is needed urgently to test this hypothesis.

The lack of effect of 2-mercaptoethane sulphonate on azathioprine inhibition of the MLR was surprising as it is readily split by other thiols such as glutathione and cysteine to 6-mercaptopurine. However, it is possible that other thiol groups such as those on the lymphocyte membrane are more reactive than 2-mercaptoethane sulphonate.

In conclusion, there is no evidence from these studies using the human MLR to suggest that 2-mercaptoethane sulphonate (mesna) therapy interferes with cyclophosphamide and azathioprine induced depression of cellular immunity.

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