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Short communication

Mesna does not reduce cisplatin induced nephrotoxicity in the rat

Barbara C. Millar[1], Zahid H. Siddik[1], John L. Millar[2], and Sally Jinks[1]

[1] Drug Development Section, Institute of Cancer Research, Clifton Avenue, Sutton, Surrey, England
[2] Section of Medicine, Institute of Cancer Research 'F' Block, Clifton Avenue, Sutton, Surrey SM2 5PX, England

Summary. Although mesna afforded protection against the cytotoxicity of cisplatin in Chinese hamster cells, line V-79-753B, in vitro, there was no evidence for protection against nephrotoxicity when this drug combination was examined in the rat. It seems likely that cisplatin-induced nephrotoxicity is mediated by intracellular events in kidney cells which cannot be inhibited by mesna possibly due to its presence within cells in vivo as the stable and unreactive disulphide. On the basis of these data it is unlikely that combinations of mesna and cisplatin will be of therapeutic benefit in man.

Introduction

Mesna (2-mercaptoethane sulphonate) has been shown to provide selective protection against the bladder toxicity induced by oxazophosphorine compounds, such as cyclophosphamide and ifosphamide, in man [12, 15], largely by the formation of nontoxic addition products with acrolein [2, 3]. This specificity is achieved because the parent compound is rapidly oxidized in the bloodstream to the stable nonreactive disulphide [4]. Enzymatic regeneration of the parent thiol occurs in the renal tubular epithelium, from where mesna crosses the brush border and thereby gains access to the bladder [14].

Damage to normal tissue, which is often tissue-specific, restricts the therapeutic benefit of many antitumour agents in man. In the case of cisplatin, an agent widely used for the treatment of ovarian [16] and testicular tumours [8], the critical normal tissue is the kidney. If this nephrotoxicity could be obviated the therapeutic benefit of cisplatin treatment could be greatly enhanced. Several workers have shown that thiol compounds can protect against cisplatin-induced nephrotoxicity [1, 9–11]. Since mesna is unlikely to affect tumour cell killing due to its oxidation in blood [4], experiments have been undertaken to evaluate the potential reduction in cisplatin-induced nephrotoxicity by means of this drug combination. Initial experiments were carried out in vitro to determine the potential dose reduction that might be achieved by using mesna with cisplatin.

Offprint requests to: B. C. Millar

Materials and methods

Cisplatin (cis-$Pt[NH_3]_2Cl_2$) was a gift from the Johnson Matthey Research Centre, Reading, Berks. Mesna was a gift from Boeringher Ingelheim, Hospital Division, UK.

In vitro. Experiments were undertaken in asynchronous exponential cultures of Chinese hamster cells, V-79-753B, grown in monolayer. The routine handling of these cells has been described elsewhere [5].

In experiments to investigate the toxicity of cisplatin, cultures were trypsinized, counted, and plated into 61-mm glass petri dishes in 2 ml Earle's based MEM supplemented with 15% foetal calf serum and 20 m*M* HEPES buffer. When the cells had attached the medium was aspirated from the dishes and replaced with Dulbecco's phosphate-buffered saline containing cisplatin alone or in combination with mesna. The cultures were incubated at 37 °C for the times indicated in the text. At these times the drugs were removed from the cells and cultures were incubated in fresh medium at 37 °C for 6 days, in an atmosphere of 5% CO_2/95% air to allow colony formation. The resultant colonies were fixed with ethanol, stained with methylene blue and counted. The surviving fraction was calculated with untreated controls.

In vivo. Cisplatin and mesna were dissolved in saline immediately before use. Male Wistar rats weighing 350–400 g received a maximally tolerated dose of cisplatin (6.5 mg/kg, 5 ml/kg) IV via the tail. A single dose of mesna (300 mg/kg, 4 ml/kg) was given IV immediately after cisplatin or the same dose IP on three occassions at 0,2, and 4 h after cisplatin administration. Rats receiving cisplatin alone were given saline to compensate for any effects attributable to hydration in the cisplatin and mesna group. Control animals received saline by the appropriate routes. On day 4, rats were anaesthetised with ether, and blood (2–5 ml) was removed via cardiac puncture into heparinised tubes. Plasma was prepared by centrifuging blood at 1000 *g* for 10 min. Urea levels in the plasma were determined colorimetrically by its reaction with diacetyl monoxime [7].

Results

The data in Figs. 1 and 2 show that mesna decreased the toxicity of cisplatin towards Chinese hamster cells in vitro.

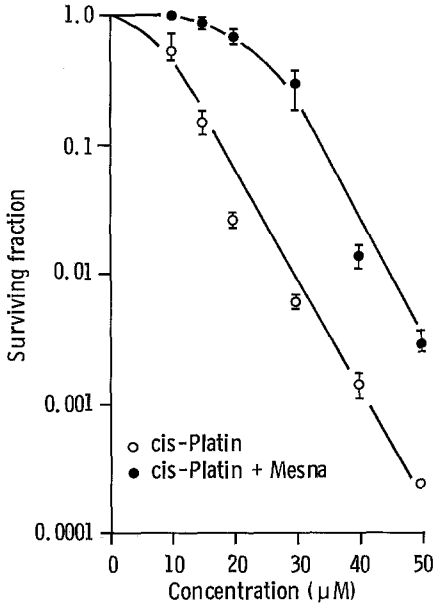

Fig. 1. Effect of 1.0 m*M* mesna on the survival of Chinese hamster cells, V-79-753B, exposed to different concentrations of cisplatin for 1 h at 37 °C. Curves are each plotted from three repeat experiments. *Bars*, ranges of data

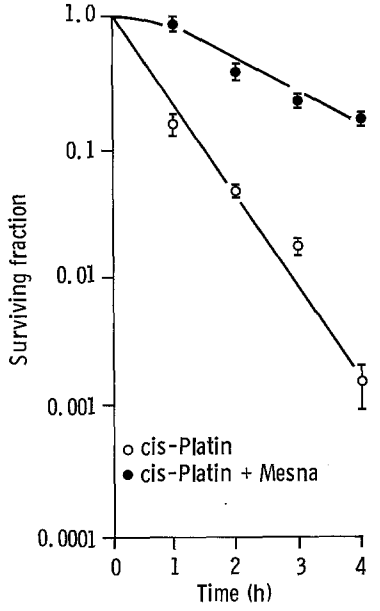

Fig. 2. Effect of 1.0 m*M* mesna on the survival of Chinese hamster cells, V-79-753B, exposed to 15 μ*M* cisplatin at 37 °C. Curves are each plotted from three repeat experiments. *Bars*, ranges of data

The data in Table 1 show that there was a five- to eight-fold increase in blood urea levels, indicating nephrotoxicity in animals receiving cisplatin alone on day 4 after treatment. In animals receiving mesna IV or IP in combination with cisplatin there was no significant decrease in these values. If anything, blood urea levels were higher in animals receiving the drug combination than in animals receiving cisplatin alone, irrespective of the route of administration.

Table 1. The effect of mesna on cisplatin-induced rises in blood urea levels

Treatment	No. of animals	Blood urea nitrogen (mg/100 ml): $\bar{X} \pm$ SD
Saline	4	13.6 ± 2.3
Cisplatin + IV saline	3	76.0 ± 13.2
Cisplatin + IV mesna	3	95.0 ± 7.6
Cisplatin + IP saline	4	108.7 ± 15.0
Cisplatin + IP mesna	4	148.3 ± 33.9

Discussion

The data show that mesna is an effective protector against cisplatin-induced cytotoxicity in vitro. This protection is likely to be mediated by the extracellular reaction of mesna with the drug, since previous work has shown that other thiols, such as cysteine, but not mesna, can protect against radiation-induced cell killing, indicating that mesna is poorly transported across the plasma membrane [13].

Ormstad et al. [14] have suggested that regenerated intracellular mesna is almost exclusively transported across the brush border in the kidneys to the tubular fluid, where it is neither reabsorbed nor oxidized. The inability of mesna to protect against cisplatin-induced nephrotoxicity suggests that this toxicity is mediated by intracellular events and that when the disulphide is reduced to the parent compound in the kidney it is excreted rapidly before reaction with cisplatin or its metabolites can occur. It is unlikely that the concentration of mesna in the kidney tubules is the limiting factor per se, since the molar ratio of mesna to cisplatin was at least 80:1. This contrasts sharply with a dose ratio of less than 1 for the uroprotective activity against cyclophosphamide [2] or ifosphamide [3], where irritation of the bladder wall by acrolein is the principal cause of toxicity [6]. The observation that nephrotoxicity may be increased in animals receiving mesna in combination with cisplatin may indicate that endogenous repair processes in kidney epithelium are less effective, possibly because of a reduction in the availability of glutathione [14].

In conclusion, it seems unlikely that the combination of mesna and cisplatin will provide any therapeutic advantage by obviating nephrotoxicity in man.

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