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Depletion of total cysteine, glutathione, and homocysteine in plasma by ifosfamide/mesna therapy

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Abstract The sulfhydryl status of cells, particularly the intracellular concentration of glutathione, is a critical determinant of the response of tumor and normal cells to cytostatic drugs. Recent data indicate that the administration of mercaptoethane sulfonate (mesna), which is often combined with ifosfamide, markedly decreases the circulating concentration of total cysteine and could thereby influence the response of the organism to the cytotoxic effects of chemotherapy. The aim of the present study was to assess the effects of the combination of ifosfamide/mesna on sulfhydryl and disulfide homeostasis in tumor patients. Ifosfamide was infused into 14 patients with advanced sarcoma for 5 days at a dose of 2.4–3.2 g/m^2 per day together with mesna. The plasma concentrations of total mesna, cysteine, glutathione, and homocysteine were measured before and on days 1 and 6 of the first course of ifosfamide/mesna therapy and prior to the next course of chemotherapy, and the urinary excretion of cysteine and mesna was monitored daily using a high-performance liquid chromatography (HPLC) method. Ifosfamide/mesna resulted in a marked depletion of circulating total cysteine, i.e., cysteine, cystine, and cysteine mixed disulfides [from 245 ± 36 to 50 ± 14 nmol/ml (mean ± 95% CI) on day 6], total glutathione (from 6.9 ± 1.1 to 2.5 ± 1.1 nmol/ml), and total homocysteine (from 12.3 ± 2.1 to 1.4 ± 1.1 nmol/ml). The values returned to baseline levels prior to the next course of chemotherapy. The urinary excretion of cysteine increased significantly from 0.28 to 1.82 mmol/day on the 1st day, whereupon it returned toward baseline. An average of 62% ± 6% of the delivered dose of mesna was recovered in urine. The combination of ifosfamide/mesna results in depletion of circulating total cysteine, glutathione, and homocysteine. This marked derangement of sulfhydryl and disulfide homeostasis could modulate the efficacy and toxicity of ifosfamide/mesna therapy.

Key words Ifosfamide · Mesna · Cysteine · Glutathione · Homocysteine

Introduction

2-Mercaptoethane sulfonate (mesna) is combined with high doses of oxazaphosphorines in the therapy of a variety of solid tumors so as to prevent hemorrhagic cystitis [4]. Mesna, a sulfhydryl that is not taken up by most cells, is thought to react with toxic metabolites of oxazaphosphorines in urine and not to affect thiol homeostasis [7, 14]. However, recent data from our laboratory show that single doses of oral and, particularly, intravenous mesna transiently decrease circulating cystine and cysteine mixed disulfides [16]. These data indicate that mesna reduces cystine in plasma to its thiol and that the resulting cysteine either is excreted in the urine as mesna-cysteine mixed disulfide or is taken up by cells. Mesna, which itself remains in the extracellular compartment, may thus transiently increase the intracellular concentration of cysteine. An increase in intracellular cysteine, the availability of which is rate-limiting for glutathione synthesis, may result in an increased concentration of glutathione, which detoxifies electrophilic metabolites of oxazaphosphorines. This initial increase in intracellular thiols is likely to be followed by a progressive depletion of thiols due to consumption by reactive metabolites of oxazaphosphorines and by a decreased formation of thiol adducts of metabolites of these cytostatic agents. Since the sulfhydryl status of cells is a critically important determinant of the response of tumoral and normal cells to alkylating drugs, the effects of mesna on thiols could influence the therapeutic efficacy and toxicity of oxazaphosphorines such as ifosfamide [3, 12].

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The effects of a continuous infusion of mesna and ifosfamide, a widely used chemotherapeutic regimen, on thiol and disulfide homeostasis are not known. Therefore, the circulating concentrations of total cysteine, glutathione, and homocysteine and the loss of cysteine in urine was investigated in 14 tumor-bearing patients during a 5-day infusion of the 2 compounds.

Patients and methods

A total of 14 patients, including 5 women and 9 men whose median age was 48 years (range, 20–68 years), with advanced soft-tissue sarcoma were studied. All patients had a creatinine clearance of >60 ml/min transaminase levels of less than 2 times the upper limit of normal, a hemoglobin value of >10 g/l, a leukocyte count of >3.5 ml 10^9/l, and a thrombocyte count of >100 × 10^9/l. All patients gave informed consent to participate in the study, which had been approved by the local ethics committee. The patients received a continuous infusion of ifosfamide at a dose of 2.4–3.2 g/m^2 per day over 5 days. Mesna was given together with ifosfamide by continuous infusion over the same period at a dose of 1.9–2.8 g/m^2 per day, i.e. 80% of the ifosfamide dose as determined on a weight-to-weight basis, with an additional 24-h infusion being given on day 6.

Blood samples were obtained prior to therapy and after 24 and 144 h on therapy. An additional blood sample was obtained prior to the start of a second course of chemotherapy at 1 month after the first course. Blood was collected into heparinized tubes and immediately centrifuged. Urine was collected in 24-h portions during the study. Plasma and urine were stored at –20° C until analysis by high-performance liquid chromatography (HPLC).

Analytical methods

Total mesna, glutathione, homocysteine, and cysteine, i.e., free sulfhydryls, disulfides, and small and protein mixed disulfides, were measured after reduction of plasma and urine samples with sodium borohydride and derivatization of the resulting sulfhydryls with monobromobimane by HPLC with fluorometric detection as previously described [1, 15]. Free sulfhydryls disappear rapidly in plasma ex vivo by formation of disulfides and protein mixed disulfides and can be assayed only if plasma samples are derivatized immediately after acquisition of the blood sample. When blood samples are processed within 3 min of collection, the concentrations of free and total cysteine measured in the plasma of healthy volunteers amount to 8.9 ± 3.5 (mean ± SD) and 289 ± 50 nmol/ml, respectively [1].

Data analysis

The results are given as mean values ± 95% confidence intervals (CI). Statistical differences were evaluated by analysis of variance followed by Scheffé's test for multiple comparisons.

Results

The concentrations of mesna determined in plasma after 1 and 6 days of infusion are shown in Fig. 1. No mesna was detected prior to the start of the infusion or prior to the second course of ifosfamide treatment. There was no statistically significant correlation between plasma levels and the dose of mesna infused per minute in the narrow range of doses used. The urinary excretion of mesna, i.e., free mesna, dimesna, and mesna mixed disulfides, averaged

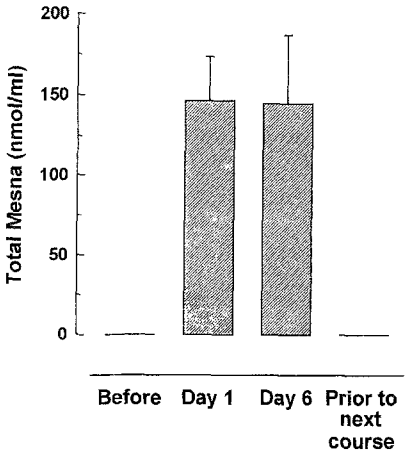
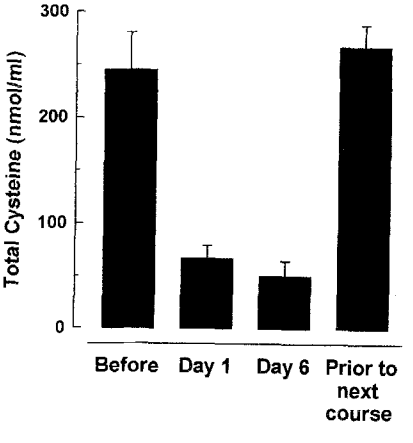


Fig. 1 Concentrations of mesna in plasma as determined before (baseline) and at 1 and 6 days after the beginning of a continuous infusion of ifosfamide plus mesna and prior to the next course of chemotherapy in 14 patients receiving ifosfamide/mesna. Data represent mean values ± 95% CI

14.2 ± 2.0 mmol/day. The excretion did not change significantly with time, and 62% ± 6% of the delivered dose of mesna was recovered in urine.

The plasma concentrations of total cysteine are shown in Fig. 2. There was a striking, statistically significant (P <0.001) decrease in total cysteine in plasma after 1 day, which persisted for the duration of the infusion of ifosfamide/mesna. There was no statistically significant correlation between the depletion of circulating cysteine and the dose of mesna and ifosfamide, respectively, in the narrow range of doses given. Just before the start of the

Fig. 2 Concentrations of total cysteine, i.e., cysteine, cystine, and cysteine mixed disulfides, in plasma as determined before (baseline) and at 1 and 6 days after the beginning of a constant infusion of ifosfamide plus mesna and prior to the next course of chemotherapy in 14 patients receiving ifosfamide/mesna at a dose of 2.4–3.2 g/m^2 per day. Data represent mean values ± 95% CI. The values obtained on days 1 and 6 are significantly (P <0.001) different from those obtained at baseline and prior to the subsequent course of chemotherapy. There is no statistically significant difference between the concentrations determined at baseline and those measured prior to the subsequent course of chemotherapy



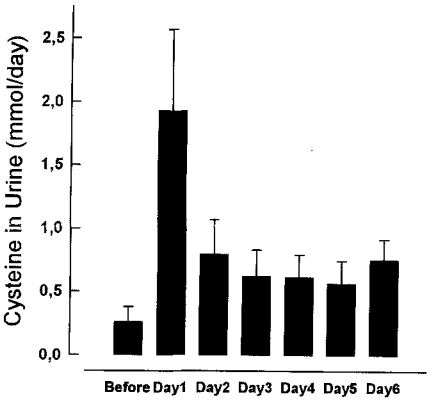
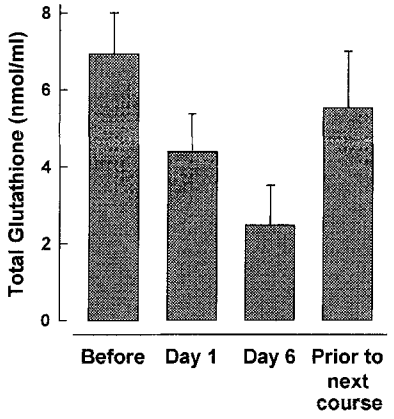


Fig. 3 Urinary excretion of total cysteine, i.e., cysteine, cystine, and cysteine mixed disulfides, during a continuous infusion of ifosfamide plus mesna of 6 days' duration. The excretion of cysteine observed on the 1st day is significantly ($P < 0.001$) higher than that seen prior to treatment. Data represent mean values ± 95% CI

second course of chemotherapy, i.e., 1 month after the first course, the plasma concentrations of total cysteine returned to baseline levels. The urinary excretion of cysteine increased significantly ($P < 0.001$) on the 1st day of mesna/ifosfamide therapy. It then returned to values not significantly different from baseline levels for the remainder of the study (Fig. 3).

The depletion of total cysteine in plasma was associated with a delayed but equally statistically significant ($P < 0.001$) decrease in the circulating concentrations of total glutathione and homocysteine (Figs. 4, 5). Prior to the subsequent treatment cycle the concentrations of glutathione and homocysteine tended to be lower than the baseline values, but the differences were not statistically significant.

Fig. 4 Concentrations of total glutathione in plasma as measured before (baseline) and at 1 and 6 days after the beginning of a continuous infusion of ifosfamide plus mesna and prior to the next course of chemotherapy in 14 patients receiving ifosfamide/mesna at a dose of 2.4–3.2 g/m^2 per day. Data represent mean values ± 95% CI. The values obtained on days 1 and 6 are significantly ($P < 0.001$) different from those obtained at baseline and prior to the subsequent course of chemotherapy. There is no statistically significant difference between the concentrations determined at baseline and those measured prior to the subsequent course of chemotherapy



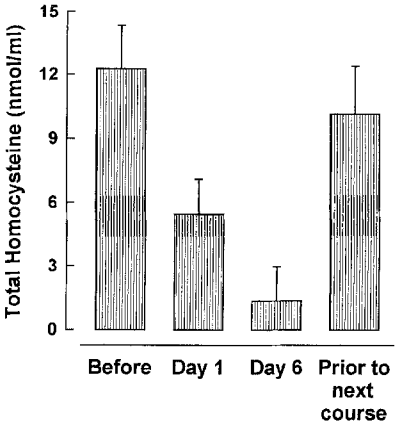


Fig. 5 Concentration of total homocysteine in plasma as determined before (baseline) and at 1 and 6 days after the beginning of a continuous infusion of ifosfamide plus mesna and prior to the next course of chemotherapy in 14 patients receiving ifosfamide/mesna at a dose of 2.4–3.2 g/m^2 per day. Data represent mean values ± 95% CI. The values obtained on days 1 and 6 are significantly ($P < 0.001$) different from those obtained at baseline and prior to the subsequent course of chemotherapy. There is no statistically significant difference between the concentrations determined at baseline and those measured prior to the subsequent course of chemotherapy

Discussion

The present data demonstrate that the combined infusion of ifosfamide and mesna results in a marked depletion of circulating total cysteine, glutathione, and homocysteine, i.e., the free sulfhydryls, disulfides, and mixed disulfides. Approximately 35% of the total glutathione and more than 95% of the total cysteine and homocysteine measured in plasma occur in the form of disulfides and mixed disulfides [11]. Since disulfides would not be expected to react with reactive metabolites of ifosfamide such as acrolein or chloroacetaldehyde, it is unlikely that the observed depletion of total sulfhydryls is due to ifosfamide alone. Rather, the decrease in circulating total cysteine, glutathione, and homocysteine must be explained by the combination of ifosfamide with mesna.

We have recently shown that a single intravenous dose of 1.2 g (7.3 mmol) mesna results in a transient 5-fold increase in the plasma concentration of free cysteine followed by a decrease of over 50% in total circulating cysteine [16]. Mesna reacts with circulating cystine, thereby generating free cysteine and mesna-cysteine mixed disulfides (Fig. 6). These mixed disulfides account for the transient increase in the urinary excretion of cysteine that is also seen after the administration of mesna alone [5, 16]. As illustrated in Fig. 3, the urinary excretion of cysteine decreased from day 2 onward, most likely because circulating total cysteine was depleted and fewer mesna-cysteine mixed disulfides were formed at this time than on day 1.

In contrast to cystine, cysteine is readily taken up by most cells [8]. The reduction of circulating cystine to cysteine by mesna may therefore be expected to result in a shift of extracellular cysteine to the intracellular compart-

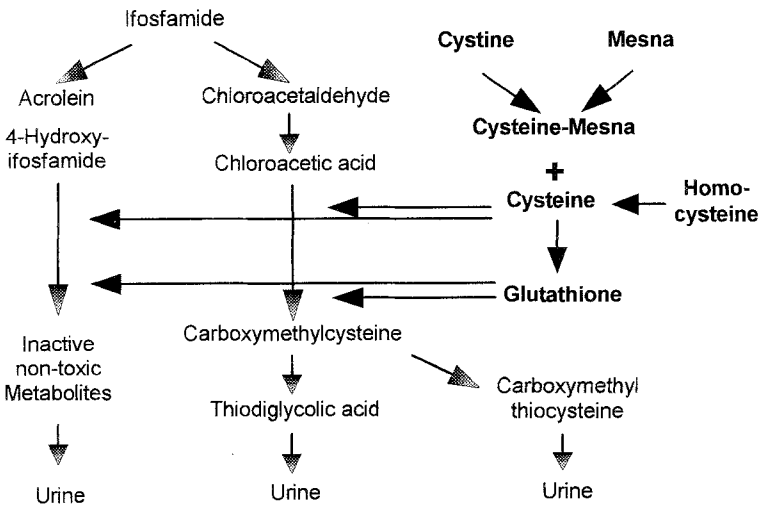


Fig. 6 Proposed interaction of ifosfamide/mesna with endogenous sulfhydryls. Mesna reacts with cystine in plasma to generate mesna-cysteine and cysteine, which is utilized for the detoxification of reactive metabolites of ifosfamide. As a consequence of the resulting depletion of cysteine, more homocysteine is utilized for the synthesis of cysteine and less cysteine is available for the synthesis of glutathione

ment that could contribute to the depletion of total cysteine in plasma. The resulting increase in intracellular cysteine may vary from cell type to cell type, depending on the capacity to take up cysteine, and tumor cells may be affected differently from normal cells. An increase in intracellular cysteine can provide cells with the cysteine necessary to detoxify metabolites of ifosfamide. Large quantities of cysteine are utilized for the formation of thiodiglycolic acid, a metabolite of carboxymethylcysteine that is formed from cysteine and ifosfamide-derived chloroacetic acid. Additional cysteine and glutathione, respectively, are utilized for the formation of carboxymethylthiocysteine from carboxymethylcysteine [13] and the detoxification of acrolein and 4-hydroxy-ifosfamide [4]. Once the extracellular pool of total cysteine is depleted, the intracellular availability of cysteine is likely to decrease as well. Consequently, the formation of thiodiglycolic acid decreases markedly after 2 days of a continuous infusion of ifosfamide and mesna (unpublished observation). Since the concentration of glutathione in plasma is determined by efflux of the tripeptide from intracellular sources, the gradual decrease in the extracellular concentration of glutathione could reflect a corresponding decrease in intracellular glutathione secondary to the decreased availability of cysteine and consumption of glutathione via detoxification of reactive metabolites of ifosfamide. This interpretation is consistent with the reported decrease in glutathione measured in peripheral blood mononuclear cells of a single patient following a short infusion of ifosfamide and mesna [10]. Like glutathione, the concentration of total homocysteine decreased during the infusion of ifosfamide and mesna. Homocysteine can also react with metabolites of ifosfamide and, in addition, its concentration in plasma may be expected to decrease in a situation of cysteine depletion, where more methionine is utilized for the synthesis of cysteine via homocysteine and cystathionine.

The design of the present study does not allow us to assess the extent to which the combination of ifosfamide and mesna furthers the imbalance in thiol homeostasis that might be produced by single-agent therapy. For ethical considerations, it was not possible to study a population treated with the present ifosfamide regimen without uroprotection or a population receiving a continuous infusion of mesna alone over 6 days. A single dose of mesna results in a decrease in circulating total cysteine [16], but the effects of a prolonged infusion are not known. Metabolites of ifosfamide decrease intracellular glutathione in vitro [10], but no data are available regarding the effects of prolonged infusion of ifosfamide alone on total cysteine, glutathione, and homocysteine in vivo.

The recovery of mesna in urine amounted to 62% of the delivered dose, which is similar to the recovery seen after a single dose of mesna without coadministration of ifosfamide [15]. Since adducts of mesna with reactive metabolites of ifosfamide such as acrolein would not be expected to yield free mesna upon incubation with sodium borohydride, this suggests that little mesna is actually consumed for the direct detoxification of ifosfamide metabolites. Indeed, adducts of mesna with metabolites of ifosfamide have not been identified in vivo. However, by reducing cystine to cysteine, mesna may indirectly provide the actual detoxifying species.

In conclusion, the present data show a marked derangement of sulfhydryl and disulfide homeostasis in patients treated with ifosfamide and mesna. Ifosfamide/mesna therapy results in a profound and sustained depletion of circulating total cysteine, glutathione, and homocysteine. Preclinical and clinical studies have shown that the therapeutic index of ifosfamide is increased by fractionation of the delivered dose of ifosfamide/mesna over several days [9]. The decrease in disulfides and mixed disulfides in plasma and, as a probable consequence, the subsequent decrease in intracellular glutathione occurring after the first 24 h of ifosfamide/mesna therapy could possibly explain this observation, since deprivation of protective sulfhydryls renders cells more susceptible to the cytotoxic effects of active ifosfamide metabolites. On the other hand, depletion of sulfhydryls might also decrease the detoxification of metabolites responsible for systemic toxicity such as ifosfamide-associated encephalopathy, which typically occurs at a time when sulfur-containing amino acids are depleted [2, 6].

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