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Inactivation of acrolein by sodium 2-mercaptoethanesulfonate using headspace-solid-phase microextraction gas chromatography and mass spectrometry

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Abstract

Acrolein, the metabolite of cyclophosphamide and ifosphamide, is an irritant of mucous membranes and seems to play an important role in hemorrhagic cystitis. Several methods are available to reduce the risk of hemorrhagic cystitis. Mesna is a regional detoxificant which inactivates acrolein. However, the interaction of mesna and acrolein has never been reported because no available method can detect acrolein. In this study, we measured acrolein to evaluate the effect of mesna in urine or phosphate-buffered saline using a headspace-solid-phase microextraction gas chromatography and mass spectrometry method which we had previously established. We also investigated the effect of mesna at different conditions of pH. Mesna was effective in a dose-dependent (10 μ*M* to 20 m*M*) fashion in both urine and phosphate-buffered saline and completely inactivated acrolein at concentrations over 10 m*M*. Furthermore, mesna was more effective in alkaline conditions than in acid.
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Keywords: Sodium 2-mercaptoethanesulfonate (Mesna); Acrolein

1. Introduction

Alkylating agents such as cyclophosphamide and ifosphamide are important drugs for chemotherapy. Acrolein (2-propenal), the metabolite of cyclophosphamide and ifosphamide, is an irritant of mucous membranes and seems to play an important role in urotoxicity [1]. The thiol compound, sodium 2-mercaptoethanesulfonate (mesna), has been proven to inactivate acrolein and is routinely given to patients receiving cyclophosphamide or ifosphamide. When mesna is given as prophylaxis, the incidence of hemorrhagic cystitis is decreased (Fig. 1) [2–6].

There are some recent reports on the analysis of acrolein or mesna [7–10]. However, there are no reports describing the interaction between acrolein and mesna. We have previously established a head-

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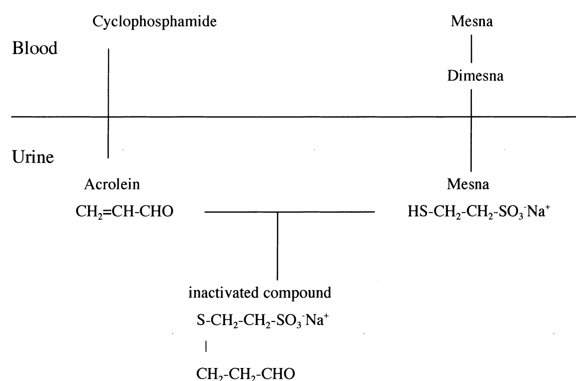

Fig. 1. Interaction between mesna and acrolein. Mesna combines with acrolein and forms an inactivated compound in urine.

space-solid-phase microextraction gas chromatography and mass spectrometry (headspace-SPME–GC–MS) method to measure acrolein [11]. By our method, acrolein was eluted on a gas chromatogram for 1.4–1.45 min and propionaldehyde (internal standard) was eluted for 1.25–1.3 min (Fig. 2). These were clearly differentiated by their molecular ions. In this study, we measured acrolein with various concentrations of mesna in urine or phosphate-buffered saline using the headspace-SPME–GC–MS method in order to investigate the effect of mesna. We also investigated the effect of mesna at different pH conditions.

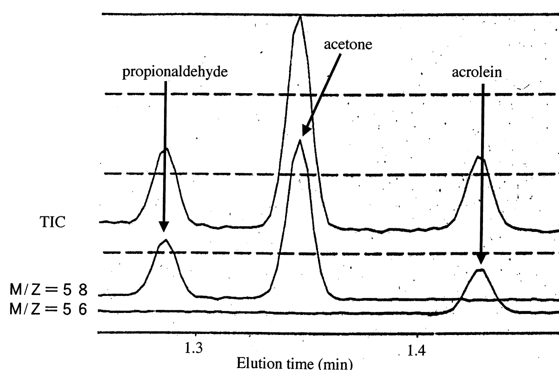

Fig. 2. SIM chromatogram for acrolein (10 nM) and propionaldehyde (10 nM) in urine. Targeted ions: m/z 56.05 for acrolein and m/z 58.05 for propionaldehyde (internal standard).

2. Methods

2.1. Reagents

Acrolein was purchased from Aldrich (Milwaukee, WI, USA). Propionaldehyde and mesna were purchased from Nacalai Tesque (Kyoto, Japan). All other reagents used were of the highest grade.

2.2. Sample preparation

A volume of 0.5 ml phosphate-buffered saline (0.5 M) or human urine was spiked with 50 μl of 10 nM propionaldehyde (internal standard), 50 μl of various concentrations (1 to 10 000 nM) of acrolein, and 50 μl of various concentrations of mesna (0.1 to 20 000 μM). Prior to assay, the urine was stored at 4 °C. Standard solutions of aldehydes were unstable, so they were freshly prepared. The stock urine sample was stored in a freezer until it was spiked.

We examined two different reaction times of mesna. Method 1: a volume of 0.5 ml phosphate-buffered saline (0.5 M) or human urine was transferred to a glass vial (10-ml volume) and spiked with 50 μl of 10 nM propionaldehyde (internal standard) and 50 μl of acrolein. Immediately after the addition of 50 μl of mesna, 2 N H_2SO_4 was added to acidify urine at pH 2 to 4. The vial was tightly sealed with a butyl rubber septum and an aluminum cap, and then heated for 5 min at 60 °C to vaporize acrolein and propionaldehyde. Method 2: a volume of 0.5 ml phosphate-buffered saline (0.5 M) or human urine was transferred to a glass vial (10-ml volume) and spiked with propionaldehyde, acrolein, and mesna as above. The vial was sealed only with a butyl rubber septum and put into the incubator at 37 °C for 30 min. Thereafter, 2 N H_2SO_4 was added and the vial was tightly sealed with an aluminum cap and heated for 5 min at 60 °C.

2.3. Instrumentation

A Shimadzu GC17A-QP5000 gas chromatograph–mass spectrometer (Kyoto, Japan) with electron impact mode was used. A DB-WAX capillary column (30 m×0.32 mm, film thickness 0.5 μm, J&W Scientific, Folsom, CA, USA) was installed. Helium

was used as the carrier gas at a flow-rate of 2.0 ml/min and a pressure of 40 kPa.

Quantitative data were obtained by selective ion monitoring (SIM) at m/z 56.05 for acrolein and 58.05 for propionaldehyde (internal standard).

2.4. SPME method and GC–MS analysis

The SPME holder for manual sampling, a 65-μm Carbowax–divinylbenzene fiber, was purchased from Supelco (Bellefonte, PA, USA).

The SPME fiber was exposed to the headspace at extraction temperature of 60 °C for 45 s, and then inserted into the GC injector port (150 °C) for thermal desorption of the extracted analytes in splitless mode (0.5-min splitless time). Thereafter, we changed to the split mode. The splitting ratio was 1:10. The desorption time was 0.3 min and the column temperature was 70 °C.

In phosphate-buffered saline assays, we used three solutions of different pH (4.0, 6.7, and 8.0). In urine assays, we used the same urine specimens (pH 7.0).

3. Results

The chelating effect of mesna was evaluated by the ratio of the acrolein concentration with mesna relative to the acrolein concentration without mesna; 0% shows complete inhibition, and 100% shows no effect.

Fig. 3 shows the result of assays in urine (method 1). The acrolein concentrations were 10, 100, 1000 and 10 000 n*M*. At all concentrations, the mesna was dose-dependently effective. There was little effect at mesna concentrations below 10 μ*M*, and mesna revealed complete inhibition at 20 000 μ*M*.

Mesna was also dose-dependently effective in phosphate-buffered saline at pH 6.7, as well as in urine, at acrolein concentrations of 10, 100, 1000, and 10 000 n*M* (Fig. 4). There was little effect at mesna concentrations below 10 μ*M*, and mesna revealed complete inhibition at 10 000 μ*M*.

Fig. 5 shows the two different reaction times with acrolein concentrations of 100 n*M* and 1000 n*M*. At both concentrations, method 2 (30 min incubation) showed more effectiveness compared to method 1.

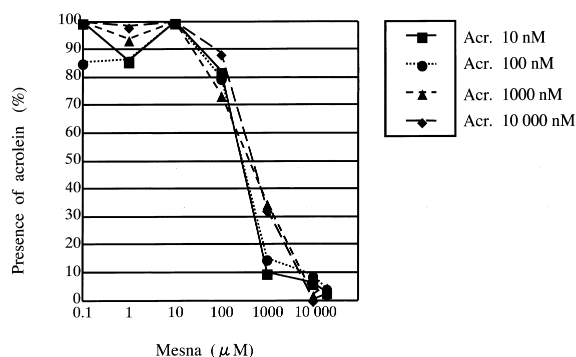

Fig. 3. The effect of mesna in urine. Acrolein concentrations are 10, 100, 1000 and 10 000 n*M*. At all concentrations of acrolein, there is a slight change below 100 μ*M* of mesna and there is a rapid decrease over 100 μ*M*.

Mesna completely abolished acrolein at concentrations of 100 or 1000 μ*M*.

The chelating effect of mesna (100 μ*M*) was estimated at three different pH conditions in phosphate-buffered saline (pH 4.0, 6.7, and 8.0) (Fig. 6). There was no inhibition at pH 4.0. Mesna was more effective at pH 8.0 compared to pH 6.7. Mesna demonstrated more effectiveness under alkaline conditions than acid.

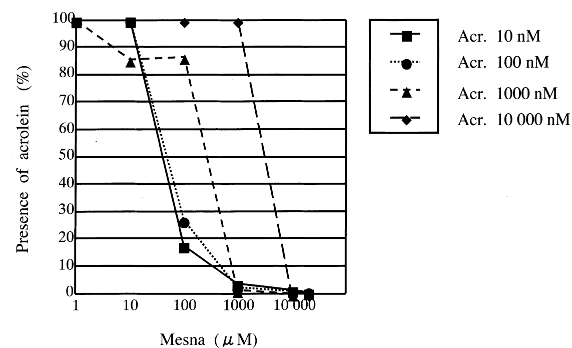

Fig. 4. The effect of mesna in phosphate-buffered saline (pH 6.7). Acrolein concentrations are 10, 100, 1000 and 10 000 n*M*. For 10 and 100 n*M* of acrolein concentration: there is no change below 10 μ*M* of mesna and there is a marked decrease over 10 μ*M*. For 1000 n*M* of acrolein: there is a slight change below 100 μ*M* of mesna and there is a marked decrease over 100 μ*M*. For 10 000 n*M* of acrolein concentration: there is no change below 1000 μ*M* of mesna and there is a marked decrease over 1000 μ*M*.

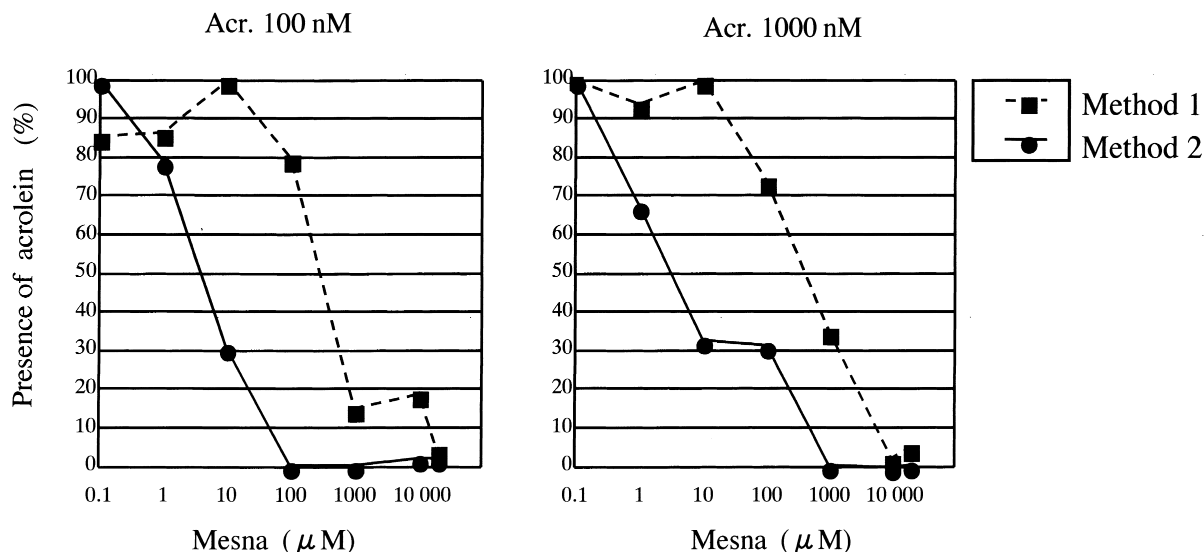

Fig. 5. Effect of mesna in two different reaction times. Left, acrolein concentration at 100 n*M*, there is a slight change below 10 μ*M* of mesna and there is a marked decrease over 10 μ*M* in method 1. There is an obvious decrease over 0.1 μ*M* of mesna and complete inhibition over 100 μ*M* of mesna in method 2. Right, acrolein concentration at 1000 n*M*, there is a slight change below 10 μ*M* of mesna and there is a marked decrease over 10 μ*M* in method 1. There is an obvious decrease over 0.1 μ*M* of mesna and complete inhibition over 1000 μ*M* of mesna in method 2.

4. Discussion

Cyclophosphamide and ifosphamide cause hemorrhagic cystitis due to contact with acrolein, the metabolite of cyclophosphamide and ifosphamide, which causes sloughing, thinning, and inflammation of the epithelium. If no means of prevention are taken, the incidence of hemorrhagic cystitis is 40 to 60%. Several methods reduce the risk of hemorrhagic cystitis. Mesna, bladder catheterization with irrigation, and hydration with diuretics are used [3]. Mesna serves as a regional detoxificant that has proved to inactivate acrolein sufficiently [12]. The interaction between acrolein and mesna in urine results in an inactive compound, and mesna given as prophylaxis for cyclophosphamide or ifosphamide markedly reduces the incidence of hemorrhagic cystitis. However, the best method of administering mesna is unknown, since there is no method to measure acrolein.

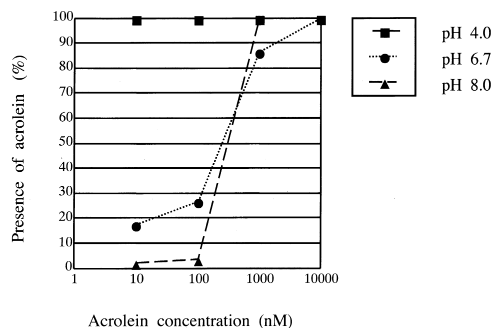

Fig. 6. The effect of mesna at three different conditions of pH (4.0, 6.7, and 8.0) in phosphate-buffered saline (mesna concentration at 100 μ*M*). At pH 4.0 there is no change at all in acrolein concentrations. At pH 6.7 and 8.0 there is a marked increase over 100 n*M* of acrolein.

In this study, we investigated the effect of mesna by measuring acrolein concentration. Mesna worked in a dose-dependent manner in both urine and phosphate-buffered saline (10 μ*M* to 20 m*M*). In clinical cases using cyclophosphamide or ifosphamide, the concentration of mesna in urine is 4.7 μ*M* to 45.8 m*M*, and the concentration of acrolein in urine is 0.3 to 406.8 n*M* (unpublished data). It was proved that mesna inactivates acrolein at the concentration in clinical practice according to the result of this study. Furthermore, it should be noted that

mesna was much more effective in alkaline conditions. We used phosphate-buffered saline to investigate the effect of mesna in different conditions of pH, since in general, the pH of urine is 6.0 to 8.0. This is the first report on the effect of mesna at different pH conditions. Keeping the urine alkaline will provide greater inactivation of acrolein when administering mesna for prevention of hemorrhagic cystitis. It may be helpful to measure the pH of urine after chemotherapy for safety management. We examined two reaction times of mesna in this study. The reaction time of method 2 was set at 30 min, since the interval to urination after hydration is about 30 to 60 min, and method 2 showed more effectiveness than method 1.

5. Conclusion

We investigated the effect of mesna from acrolein quantitation using a headspace-solid-phase microextraction gas chromatography and mass spectrometry method. It was proved that mesna was effective in a dose-dependent (10 μM to 20 mM) fashion in both urine and phosphate-buffered saline. It is consistent with the fact that mesna reduces the incidence of hemorrhagic cystitis due to acrolein in clinical cases. Furthermore, it was found that mesna was more effective in alkaline conditions.

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