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Monitoring of urinary acrolein concentration in patients receiving cyclophosphamide and ifosphamide

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Abstract

Acrolein, the metabolite of cyclophosphamide and ifosphamide, irritates mucous membranes and is considered pathogenetically important in hemorrhagic cystitis. Increasing fluid intake or administering sodium 2-mercaptoethanesulfonate (mesna), a thiol compound, can reduce the risk of this complication. We measured urinary acrolein concentrations using headspace-solid-phase microextraction gas chromatography and mass spectrometry (headspace-SPME-GC–MS) in 19 patients receiving cyclophosphamide and ifosphamide (36 occasions). Peak acrolein concentrations occurred at 1–12 h (mean ± S.D., 5.0 ± 2.7) after starting therapy, ranging from 0.3 to 406.8 nM (39.7 ± 76.7), with varying patterns over time. Maintaining high urine volume was important for preventing increases in urinary acrolein concentration, as urinary acrolein concentration tended to rise as urine volume decreased. Urinalysis detected occult blood in three cases, but the patients had no clinical symptoms of hemorrhagic cystitis. In clinical trials involving cyclophosphamide and ifosphamide, monitoring of urinary acrolein concentration could indicate when to take heightened preventive measures against hemorrhagic cystitis.

Keywords: Acrolein; Cyclophosphamide; Ifosphamide; Hemorrhagic cystitis

1. Introduction

Cyclophosphamide and ifosphamide are alkylating agents widely used in treatment of various solid tumors. Mucosal irritation by acrolein (2-propenal), the metabolite of cyclophosphamide and ifosphamide, is believed to cause severe hemorrhagic cystitis. Urothelial damage is thought to occur from direct contact with acrolein, which causes edema, ulceration, neovascularization, hemorrhage, and necrosis [1–3]. The thiol compound, sodium 2-mercaptoethanesulfonate (mesna), has been found to inactivate acrolein (Fig. 1) [4–8]. In our previous study, mesna at urinary concentrations of 10 μM to 20 mM was effective in a dose-dependent fashion, completely inactivating urinary acrolein concentrations over 10 mM. In clinical observations using cyclophosphamide or ifosphamide with mesna, the urinary mesna concentration ranged from 4.7 μM to 45.8 mM (unpublished data), supporting clinical effectiveness [9]. Administration of mesna decreases incidence of hemorrhagic cystitis, in addition, intravenous hydration, diuretic medication, and frequent voiding or bladder catheterization with irrigation can reduce the risk [5].

We previously developed a headspace-solid-phase microextraction gas chromatography and mass spectrometry (headspace-SPME-GC–MS) method to measure acrolein, attaining high sensitivity within a short analysis time (15 min). By our method, acrolein was eluted on a gas chromatogram for 1.4–1.45 min and propionaldehyde, the internal standard, was eluted for 1.25–1.3 min. These were clearly differentiated by detection of their respective constituent ions [10].

In this study, we used this method to measure urinary acrolein concentrations in patients receiving cyclophosphamide and ifosphamide. We measured the free acrolein in the urine, since hemorrhagic cystitis occurs regardless of the administration of mesna. Our aim was to determine changes in urinary acrolein concentration during this therapy as well as the relationship between urinary acrolein concentration and occult blood in the urine.

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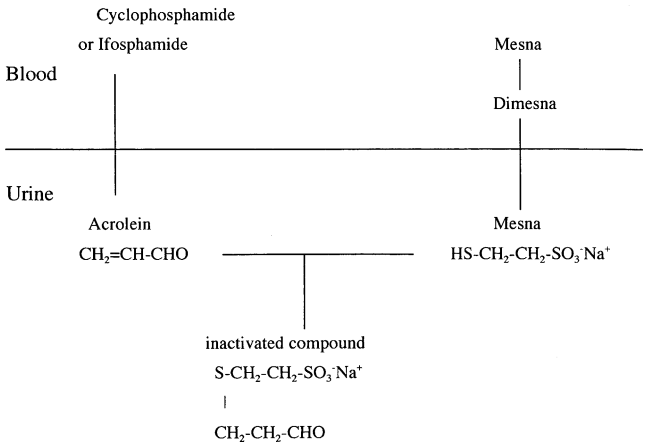

Fig. 1. Interaction between mesna and acrolein. Mesna combines with acrolein in the urine to form an inactivated compound.

2. Objective and study design

We studied 36 occasions from 19 patients (10 male, 9 female) who were admitted to our hospital for treatment of solid tumors or hematologic diseases during the years 1998–2001 (Table 1). The median age at the time of therapy was 8 years (range, 1–21). Specific diseases were as follows: rhabdomyosarcoma, 1; Ewing sarcoma, 4; primitive neuroectodermal tumor (PNET), 1; hepatocellular carcinoma, 1; osteosarcoma, 3; neuroblastoma, 6; retinoblastoma, 1; aplastic anemia, 1; and myelodysplastic syndrome (MDS), 1.

Doses of cyclophosphamide and ifosfamide were 1000–2100 and 1800–4000 mg/m^2, respectively. Patient 5 received single dose, whereas patients 4 and 10 received multiple doses. The drugs were administered intravenously over 1–6 h. Sodium 2-mercaptoethanesulfonate (mesna) was administered in all cases. The dose of mesna was about 60% of the ifosphamide dose, or 120% of the cyclophosphamide dose. These mesna dose were administered intravenously divided into 3 or 4 doses in patients receiving ifosphamide case, and divided into 3 or 4 doses with 24 h continuous infusion in patients receiving cyclophosphamide.

In one occasion (therapy), the urine sample was taken almost every 1 h after start of infusion. Over twelve samples were taken in one occasion. Some of the patients were catheterized, and the others were forced to void at a fixed time point as far as possible. Urinalysis and urine volume measurement were performed hourly. The urinary acrolein concentration was measured as soon as the urine was taken, since acrolein in urine is unstable. We measured urinary acrolein concentrations using headspace-solid-phase microextraction gas chromatography and mass spectrometry method that we previously devised.

3. Experimental

3.1. Sample preparation

After 0.5 ml of patient urine was transferred to a 10 ml glass vial, 50 μl of 10 nM propionaldehyde was added as an internal standard. The sample then was acidified to pH 2–4 with 2N H_2SO_4. As solutions of aldehydes are unstable, the propionaldehyde solution was freshly prepared. The vial was

Table 1
Patient background data and urinary acrolein concentrations

Patient	Occasions	Age at therapy (years)	Gender	Disease	Drug	Dose (mg/m^2)	Maximum concentration of acrolein (nM)	Time of maximum concentration (after dose)
1	1	5	Male	Rhabdomyosarcoma	CPM	1000	32.5	2.5
2	1	12	Male	Ewing sarcoma	IFO	2000	6.5	6
3	1	12	Female	Ewing sarcoma	IFO	1800	24.1	3
4	9	21	Female	PNET	CPM, IFO	2100–3000	3.5–111.3	1.0–8
5	4	3	Female	Ewing sarcoma	CPM	2100	18–406.8	5.5–8.0
6	1	12	Male	Hepatocellular carcinoma	IFO	1400	14.1	3
7	1	12	Male	Osteosarcoma	IFO	2300	14.2	8
8	1	10	Female	MDS	CPM	1600	9.4	3
9	1	11	Male	Neuroblastoma	IFO	4000	16.7	4
10	5	14	Female	Osteosarcoma	IFO	2000–2660	21.8–59.9	2.0–12
11	1	1	Male	Neuroblastoma	CPM	700	12.5	8
12	1	9	Male	Osteosarcoma	IFO	2000	40.1	2
13	1	4	Female	Neuroblastoma	IFO	2500	70.6	3
14	1	5	Male	Retinoblastoma	IFO	2000	9.6	3.5
15	1	3	Female	Neuroblastoma	CPM	1600	42.2	4
16	1	13	Male	Aplastic anemia	CPM	50[a]	14.2	3
17	1	2	Female	Neuroblastoma	CPM	70[a]	2.9	4
18	3	13	Female	Ewing sarcoma	CPM	70[a]	5.2–6.7	2.0–9
19	1	2	Male	Neuroblastoma	CPM	70[a]	0.3	7
Mean ± S.D.							39.7 ± 76.7	5.0 ± 2.7

Abbreviations: CPM, cyclophosphamide; IFO, ifosphamide; PNET, peripheral neuroectodermal tumor; MDS, myelodysplastic syndrome.
[a] Unit: mg/kg.

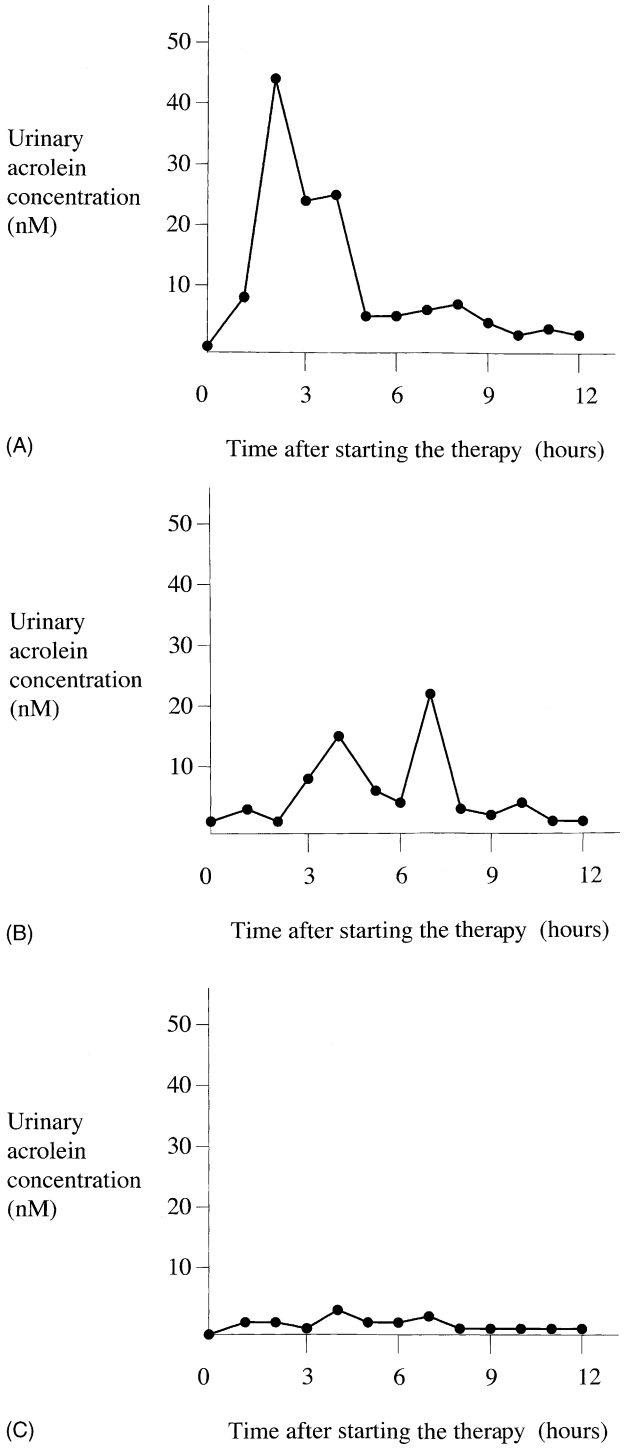

Fig. 2. Changes of urinary acrolein concentrations after starting therapy in three different cases: (A) patient 10, occasion 5; (B) patient 10, occasion 3 and (C) patient 17.

Table 2
Three cases of occult blood in urine

Patient	Occasions	Acrolein concentration at occult blood (nM)	Time after start of therapy (h)
4	1	18.32	7
5	1	260.38	8
10	4	37.21	1

sealed tightly with a butyl rubber septum and an aluminum cap, and then heated for 5 min at 35 °C to vaporize acrolein and propionaldehyde.

3.2. Instrumentation

A GC17A-QP5000 gas chromatograph-mass spectrometer (Shimadzu, 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) 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.

3.3. SPME method and GC–MS analysis

The SPME holder for manual sampling, a 65 μm carbowax/divinylbenzene fiber, was purchased from Supelco (Bellefonte, PA).

The SPME fiber was exposed to the headspace at an extraction temperature of 35 °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). We then 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. The retention time of acrolein was 1.4–1.45 min and that of propionaldehyde was 1.25–1.3 min.

4. Results

Table 1 presents results from 19 patients (36 occasions, 13–20 samples/one occasion). In most samples, the urinary

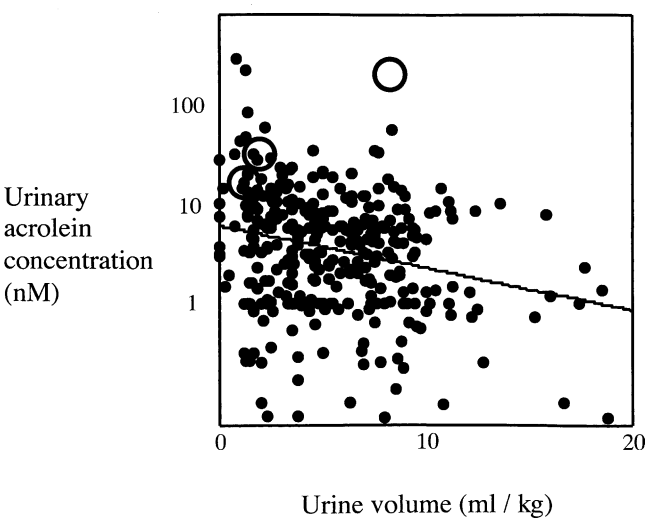

Fig. 3. Relationship between urine volume and urinary acrolein concentration ($r = 0.249$). The large open circles indicate the three samples with hematuria.

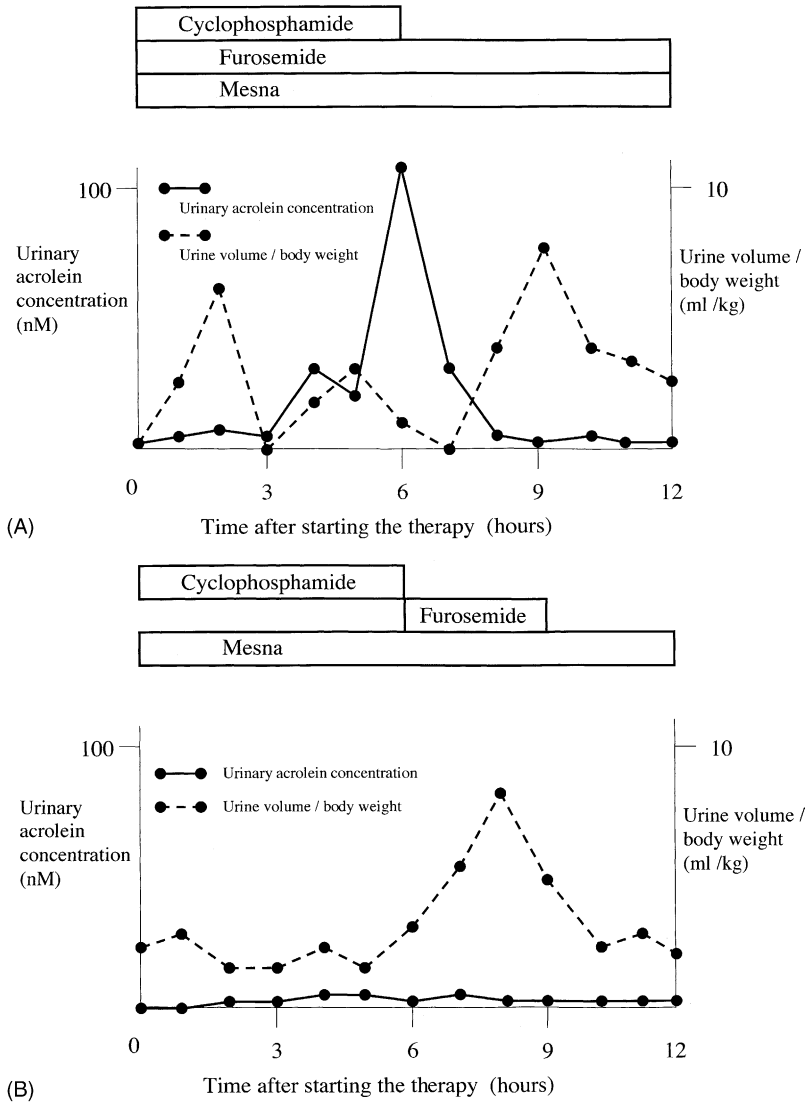

Fig. 4. Urinary acrolein concentrations in the same patients on different days with the same therapy: (A) anuria and elevated urinary acrolein concentration occurred on one day (patient 4, occasion 3) and (B) anuria or oliguria did not occur on another day and urinary acrolein concentration maintained low (patient 4, occasion 9).

acrolein concentration showed a gradual increase after starting therapy, reaching a maximum and then gradually decreasing to zero in a few hours (Fig. 2A). In some samples, the concentration of acrolein showed a second increase (Fig. 2B). In other samples, the amount of acrolein was slight, remaining slight above zero (Fig. 2C). Thus, a variety of patterns was evident in changes of urinary acrolein concentration over time.

Urinary acrolein concentrations peaked at times between 1 and 12 h after starting therapy (mean ± S.D., 5.0 ± 2.7). Maximum urinary acrolein concentrations ranged from 0.3 to 406.8 nM (mean ± S.D., 39.7 ± 76.7 nM). Occult blood was detected by urinalysis in three samples, but the patients had no clinical symptoms of hemorrhagic cystitis. Their urinary acrolein concentrations were 18.3, 37.2, and 260.4 nM (Table 2).

The relationship between the urine volume and urinary acrolein concentration is shown in Fig. 3. Urinary acrolein concentration tended to increase as urine volume decreased ($r = 0.249$).

5. Discussion

Hemorrhagic cystitis is a serious side effect unique to cyclophosphamide and ifosphamide. Symptoms may range from mild dysuria and frequency to severe hemorrhage resulting from bladder epithelial damage. The reported incidence of this complication ranges from 5 to 10% for cyclophosphamide and from 20 to 40% for ifosphamide. This toxic effect is dose-related and appears to be caused by activated metabolites and by biologically active by-products,

such as acrolein. The incidence and severity of such chemical cystitis can be lessened by aggressive hydration and frequent emptying of the bladder, and by concurrent administration of sodium 2-mercaptoethanesulfonate (mesna). However, the incidence of hemorrhagic cystitis is 5% even with administration of mesna [11], and the urinary concentration of acrolein likely to cause hemorrhagic cystitis has not been determined. In this study, we measured urinary acrolein concentrations in patients receiving cyclophosphamide and ifosphamide, with special attention to changes of urinary acrolein concentration during the therapy, since high urinary acrolein concentration is likely to closely associated with hemorrhagic cystitis.

The urinary acrolein concentration reached a maximum a few hours after starting therapy. The few hours near this maximum point presumably represent the most dangerous interval for occurrence of hemorrhagic cystitis. At this time, care must be taken to maintain sufficient constant urinary flow to reduce the acrolein concentration. However, changes in the urinary acrolein concentrations showed varying patterns (Fig. 2), so urinary acrolein concentrations should be monitored.

Fig. 4 demonstrates measurements from the same patient on different days of therapy. The urinary acrolein concentration varied despite no change in therapy. Anuria as well as a high urinary acrolein concentration occurred on one day (Fig. 4A), while with greater urinary output on another day, the urinary acrolein concentration was low (Fig. 4B). This illustrates the importance of maintaining urine volume.

Urinalysis showed occult blood in only three samples. These samples had acrolein concentrations above the correlation curve in Fig. 3, but were not readily discriminated from those in non-hematuric samples. These observations were not sufficient to prove an association between urinary acrolein concentration and hemorrhagic cystitis, even though minor hematuria was used as a surrogate marker for risk of hemorrhagic cystitis, since an actual occurrence could be serious or fatal. In clinical trials, involving alkylating agents, if occult blood is shown by urinalysis or a urinary acrolein concentration exceeds those seen in hematuric cases, we increase intravenous hydration or administer additional mesna to prevent hemorrhagic cystitis. Since adopting this protocol, we have had no cases of hemorrhagic cystitis.

The total analysis time needed to quantitate urinary acrolein using headspace-SPME-GC–MS was only 15 min. In therapy using cyclophosphamide and ifosphamide, monitoring of urinary acrolein concentrations would be a practical aid in prevention of hemorrhagic cystitis.

Our study has a limitation. We did not examine the difference of urinary acrolein concentration between cyclophosphamide and ifosphamide. We did not investigate the mesna dose or the method of administration. Further study is needed to clarify the relationship between mesna and the prevention of hemorrhagic cystitis. We hope to refine our protocol for prevention.

6. Conclusion

We measured urinary acrolein concentrations using headspace-solid-phase microextraction gas chromatography and mass spectrometry in patients receiving cyclophosphamide and ifosphamide. A diversity of patterns was seen in the course of urinary acrolein concentrations. The urinary acrolein concentration tended to increase as urine volume decreased. Urinary acrolein concentration has shown to be an index of hydration with diuretic therapy, and also should be useful in prevention of hemorrhagic cystitis.

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