Cancer Chemother Pharmacol (1988) 22: 299–302

Cancer Chemotherapy and Pharmacology

Efficacy of sodium thiosulfate as a local antidote to mechlorethamine skin toxicity in the mouse*

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Summary. The highly vesicant nature of the alkylating anticancer agent mechlorethamine (HN_2, or nitrogen mustard) requires careful i.v. technique during its administration. Skin toxicity due to HN_2 extravasation is severe and typically prolonged over several months. Mouse skin toxicity studies were carried out to find a local antidote to decrease the severity of tissue damage by this agent. Intradermal (i.d.) HN_2 (0.005–0.5 mg) caused dose-dependent skin ulcers in the mouse. Isotonic sodium thiosulfate $Na_2S_2O_3$ (0.167 *M*) or hypertonic (0.34 *M*) $Na_2S_2O_3$ (0.05 ml) given immediately after HN_2 significantly reduced the mean HN_2 ulceration area and the total time of ulceration. Ineffective local HN_2 antidotes included hyaluronidase, hydrocortisone, and sodium chloride, all given i.d. Topical applications of DMSO, cold, and heat were also ineffective. Sodium thiosulfate is believed to chemically neutralize reactive mechlorethamine-alkylating species and thus decrease skin toxicity. Thiosulfate dosing studies showed that a molar excess of at least 200:1 ($Na_2S_2O_3$:HN_2) was required for significant antidotal activity. If thiosulfate treatment was delayed 4–24 h after HN_2, no antidotal effects were obtained. We conclude that sodium thiosulfate can decrease the severity of local tissue damage caused by HN_2. It should be considered the antidote of choice in the setting of clinical HN_2 extravasations.

Introduction

Mechlorethamine (HN_2, or nitrogen mustard) is a highly reactive alkylating anticancer agent that has been in clinical use for many years [6]. Although it has limited therapeutic applications due to its toxicity, it is still considered a standard agent in the treatment of Hodgkin's disease [7], non-Hodgkin's lymphomas, and mycosis fungoides, wherein HN_2 is used topically [16].

Mechlorethamine is also a well-known vesicant and produces severe tissue damage if inadvertently extravasated during i.v. administration [3]. The recommended method of administering nitrogen mustard has been a brief, rapidly flowing i.v. infusion [10]. This is believed to decrease the chance of extravasation and may lessen the severity of skin damage if such an event occurs. Extravasation of small amounts of mechlorethamine has been associated with local reactions such as tenderness, swelling, induration, erythema, and ulceration, which may then progress to tissue sloughing [2]. The drug manufacturer recommends the injection of isotonic (0.17 *M*) sodium thiosulfate ($Na_2S_2O_3$) and an ice compress for 6–12 h if nitrogen mustard is inadvertently extravasated [10]. This recommendation is based on experimental [8] and clinical studies showing systemic HN_2 antidotal effects for high-dose $Na_2S_2O_3$ [14].

However, documentation of the efficacy of local $Na_2S_2O_3$ and topical cooling for HN_2 extravasations was not available. Therefore, the following in vivo studies were carried out: (1) assessment of the topical skin toxicity of HN_2; (2) quantitative screening for local HN_2 antidotes; and (3) determination of effective local dose levels for any active antidotes.

Materials and methods

HN_2-induced skin toxicity was evaluated using an intradermal (i.d.) mouse skin ulceration model previously described in detail [3, 4]. Adult BALB/c female mice (Jackson Laboratories, Bar Harbor, Me) weighing 25–30 mg were dehaired on a 3 × 3 cm area of dorsum. After 24 h, mice were injected i.d. with HN_2 (Mustargen; Merck, Sharpe and Dohme, Philadelphia, Pa) in 0.89% sodium chloride United States Pharmacopeia (USP) (unpreserved). The drug was used within 15 min of mixing as directed by the manufacturer to reduce possible rapid decomposition in solution [10]. Local adjuvants (Table 1) were given i.d. or topically, immediately following HN_2, directly adjacent to the original injection site. Cold and heat were applied topically to unanesthetized mice immediately following HN_2 i.d. injection according to a standard protocol [5]. HN_2 was injected i.d. at five dose levels of 0.001 mg, 0.005 mg, 0.01 mg, 0.05 mg, and o.1 mg, each given in a 0.05-ml volume. These HN_2 doses represent molar concentrations of 0.01–10.4 m*M*.

Skin lesions produced by i.d. HN_2 were measured daily by a single observer using a micrometer. Induration, erythema, and ulceration were used to assess skin toxicity according to a standard grading methodology [7]. Perpendic-

* Supported in part by Public Health Service Grants CA-31078, CA-23074, and CA-17094 from the National Cancer Institute; National Institute of Health, Department of Health and Human Services, Bethesda, MD

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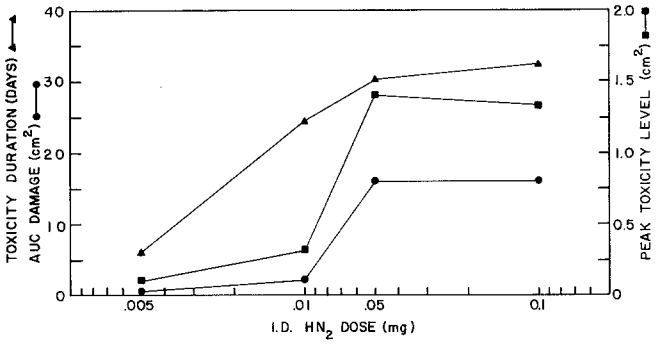

Fig. 1. The semilogarithmic dose-response patterns for HN_2-induced skin toxicity in BALB/c mice. Each point represents the mean of five determinations. The three parameters followed include: (1) the duration of ulveration in days (▲——▲): (2) the integrated area under the ulcer size × time curve in cm^2 × days (●——●); and (3) peak ulcer size in cm^2 (■——■)

ular measurements of the widest diameters at the ulcer site were recorded for all three parameters.

The area under the curve for ulceration (AUC = cm^2 days), peak lesion size (cm^2), and total time of ulceration (days) were the parameters used in statistically evaluating the extent and severity of i.d. HN_2 toxicity. Statistical tests included an initial analysis of variance (ANOVA) with subsequent intergroup multiple range comparisons (Least-Significant-Difference Method, LSD) [11].

Results

HN_2-induced swelling and edema were apparent within 30 min of i.d. injection in the mice. Swelling did not disappear until at least 48 h postadministration, and the animals appeared irritable and hyperactive for several days.

In Fig. 1, the dose-response relationship for skin ulceration for the five i.d. H_2 doses is illustrated. Skin ulceration peaked after 24 h to 5 days, depending on the dose of HN_2 injected. Figure 1 shows that i.d. HN_2 doses of 0.05 mg produced maximal skin ulcers. At 0.1 mg HN_2 (approximately 14 mg/m^2), death occurred in 80% of the animals 4–6 days after injection. Because of these excessive toxic effects, local adjuvants were only tested against HN_2 doses of 0.01 and 0.05 mg (1.4–7.1 mg/m^2).

The effects of local adjuvants on skin toxicity after these HN_2 doses are summarized in Table 1. These results show that with a constant volume of 0.05 ml, only hypertonic sodium thiosulfate (0.34 *M*) significantly reduced the mean HN_2 ulceration AUC, the peak lesion size, and the total time of skin ulceration ($P < 0.05$ by the LSD test). In contrast, some of the local adjuvants such as hyluronidase, hydrocortisone, and DMSO actually increased HN_2 ulceration by a statistically significant amount ($P < 0.05$ by LSD analysis).

The results in Table 1 clearly show a marked antidotal efficacy from 0.34 *M* $Na_2S_2O_3$ given in 0.05 ml volume. This regimen reduced the mean HN_2-induced ulceration AUC to approximately one-fourth that of the equivalent saline-treated group. This result was significantly better than that observed following the application of all other local adjuvants ($P < 0.05$ by the LSD test). Delaying the i.d. injection of $Na_2S_2O_3$ by 4–24 h was not helpful, and these later i.d. injections appeared to slightly increase HN_2-induced skin ulcers at both HN_2 dose levels (0.01 and 0.05 mg).

In contrast to the manufacturer's recommendation [10], the addition of topical cooling to $Na_2S_2O_3$ did not enhance wound healing from HN_2. Similarly, mild topical heating of HN_2 lesions was also ineffective. Both topical temperature manipulations actually appeared to increase skin toxicity, but this was statistically significant only for topical heating.

Table 2 compares the antidotal dose response to different volumes of isotonic (0.17 *M*) $Na_2S_2O_3$ or isotonic saline. The results show that following a 0.01 mg and 0.05 mg i.d. HN_2 injection, at least 0.3 ml isotonic $Na_2S_2O_3$ was required to significantly reduce (i.e., halve) the HN_2-induced

Table 1. Efficacy of local adjuvants to 0.05 mg and 0.01 mg i.d. HN_2-induced skin ulceration

Adjuvant ID antidote (in 0.05 ml for drug solutions)	Total AUC (cm_2 days)		Peak level (cm_2)		Duration of ulceration (days)	
	0.05 mg*	0.01 mg	0.05 mg	0.01 mg	0.05 mg	0.01 mg
$Na_2S_2O_3$ (0.34 M)	–**	–**	–**	0	–**	–**
$Na_2S_2O_3$ (0.17 M)	0	0	0	0	0	0
$Na_2S_2O_3$ (0.34 M) (4 h post-HN_2)	+	0	+	0	0	0
$Na_2S_2O_3$ (0.34 M) (24 h post-HN_2)	+	0	0	0	0	0
Sodium chloride[b]	0	0	0	0	0	0
Hyaluronidase[c]	+	0	0	0	0	0
Hydrocortisone[d]	0	0	+	0	0	0
Heat 20 min	0	+**	+**	+**	0	0
Cold 20 min + $Na_2S_2O_3$ 0.34 M	0	NT	0	NT	0	NT
Heat 20 min + $Na_2S_2O_3$ 0.34 M	0	NT	0	NT	0	NT
DMSO (Topical)[e]	+**	+**	+	+	+	0

[a] From 10% USP solution, Torrigian Laboratories, Queens Village, NY
[b] 0.89% (unpreserved)
[c] Wydasee, Wyeth Laboratories, Pittsburgh, Pa
[d] Solu Cortef, Upjohn Laboratories, Kalamazoo, Mich
[e] 99%, reagent grade, J. T. Baker
* Dose of adjuvant given i.d. in 0.05 ml volume
** Indicates statistically significant difference by the LSD test ($P < 0.05$)
0, No significant change from control; +, increased skin ulcer control ($P < 0.05$); decreased skin ulcer over control ($P < 0.05$); NT, not tested

Table 2. Dose and volume studies of antidotal activity for isotonic $Na_2S_2O_3$ (0.17 *M*) or saline (0.89% NaCl) against HN_2 ulceration

HN_2 Dose mg/0.05 ml	Volume of saline or isotonic (0.17 *M*) $Na_2S_2O_3$ injected i.d. (ml)	Skin ulcers ($n = 5$)		
		Total AUC (cm^2 days)	Peak level size (cm^2)	Duration of ulceration (days)
0.01	none (control)	2.15	0.33	24.6
0.01	0.05 ($Na_2S_2O_3$)	2.82	0.32	15.6
0.01	0.1 ($Na_2S_2O_3$)	1.69	0.27	17.8
0.01	0.3 ($Na_2S_2O_3$)	1.09*	0.14*	12.0
0.01	0.05 (NaCl)	1.96	0.31	18.0
0.01	0.1 (NaCl)	3.93	0.43	17.8
0.01	0.3 (NaCl)	5.34*	0.49*	20.6
0.05	none (control)	16.14	1.42	30.2
0.05	0.05 ($Na_2S_2O_3$)	13.71	1.03	25.2
0.05	0.1 ($Na_2S_2O_3$)	12.94	0.94	15.6
0.05	0.3 ($Na_2S_2O_3$)	7.26*	0.67*	20.3
0.05	0.05 (NaCl)	12.51	1.12	26.0
0.05	0.1 (NaCl)	18.06	1.29	26.4
0.05	0.3 (NaCl)	20.85	1.45	22.4

* Indicates a treatment significantly different from all other treatments at that dose level ($P < 0.05$ by ANOVA and LSD tests)

ulcers. This dose of $Na_2S_2O_3$ represents a molar excess of at least 200:1 ($Na_2S_2O_3$:HN_2). In contrast, the i.d. injection of comparable volumes of saline was ineffective and at high volumes actually enhanced skin ulcer size. Thus, the antidotal advantage of the large-volume $Na_2S_2O_3$ solution involved the delivery of more active thiol.

Discussion

The current studies were carried out to screen for potential antidotes to nitrogen mustard skin toxicity. Our results show that $Na_2S_2O_3$ injected immediately into an HN_2 lesion is most effective at preventing subsequent skin ulceration. The manufacturer recommends that if extravasation of the HN_2 occurs, 10–20 ml isotonic, 0.17 *M* (or 1/6 *M*) sodium thiosulfate should be used locally at the site. However, data documenting the efficacy of this treatment and its proper design were not previously available. In comparing the different concentrations of sodium thiosulfate used in our study, we found that there is indeed a significant difference in the mean size of HN_2 ulcers following treatment with 0.17 *M* or 0.34 *M* $Na_2S_2O_3$. The severity of an HN_2 lesion was much lower with the higher sodium thiosulfate solution. Thus, it appears that $Na_2S_2O_3$ does decrease local skin toxicity caused by HN_2 if a sufficient dose is given immediately after HN_2 extravasation. The mechanism of this antidotal effect is not known with certainty but probably involves the nucleophilic attack of ionized sulfur with the highly reactive or "activated" ethylenimmonium form of HN_2 [12].

The antidotal activity of $Na_2S_2O_3$ has also been shown in other animal models. Hatiboglu et al. [8] have shown that nitrogen mustard given i.a. in dogs was efficiently antagonized by an i.v. $Na_2S_2O_3$ infusion. These investigators have suggested that sodium thiosulfate could directly neutralize the drug and thereby prevent systemic toxicity. Bonadonna and Karnofsky [1] have also described extensive animal and clinical studies using $Na_2S_2O_3$ as a systemic antidote to HN_2 or its chlorimine picryl sulfonate derivative. They found that in rodents and rabbits 1 g/kg $Na_2S_2O_3$ given i.v. afforded only minimal protection against HN_2-induced lethality. However, 200 mg/kg $Na_2S_2O_3$ given immediately before a daily HN_2 injection of 0.4 mg/kg efficiently blocked HN_2-induced myelotoxicities in man. This $Na_2S_2O_3$ regimen facilitated the administration of HN_2 doses up to 2–9 times the usual maximal tolerated dose. Of interest, nausea and vomiting symptoms from HN_2 were not reduced by the $Na_2S_2O_3$ injections.

Our results further demonstrate that the antidotal effects of sodium thiosulfate are not mediated by simple dilution, since large volumes of isotonic sodium chloride actually augmented HN_2-induced skin ulceration in the mice. In contrast, equal volumes of isotonic 0.17 *M* $Na_2S_2O_3$ produced progressively smaller HN_2 lesions.

Owen et al. [13] have recently described a clinical case involving an accidental i.m. injection of 30 mg mechlorethamine (0.3 mg/kg) into the gluteal muscle of a 55-year-old psoriasis patient. Radiating pain at the injection site was immediate, but the error was not discovered for 5 h. At that time, five separate 5-ml i.m. injections of 0.17 *M* sodium thiosulfate were given and an ice pack was placed at the site for an additional 12 h. There was no subsequent necrosis in this patient. From our studies, this prolonged delay in $Na_2S_2O_3$ administration would have obviated any potential antifotal benefit. Systemic toxicity studies in mice have similarly shown that $Na_2S_2O_3$ was ineffective when the interval between HN_2 (given first) and the thiol was greater than 15 min [8]. We evaluated only the longer intervals of 4 and 24 h, thus, the last effective time in which i.d. $Na_2S_2O_3$ can be given is not known but is probably quite short.

Sodium thiosulfate also appears to be well tolerated when given systemically in man. Studies with i.v. doses of 12–25 g/m^2 have been safely given to patients with normal cardiac and renal function [9, 14, 15]. Our studies suggest that this safety index may also extend to local injections into mouse skin. The i.d. injections of hypertonic 0.34 *M* $Na_2S_2O_3$ produced no apparent skin toxicity in the mice. While mouse skin is substantially different from human skin, similar safe, local use of $Na_2S_2O_3$ may be inferred from the single clinical case report [13].

Our controlled dosing and volume studies additionally suggest that a large molar excess of $Na_2S_2O_3$ must be given to reduce HN_2 lesions. This ratio was at least 200:1 in mice given both 0.05 and 0.01 mg i.d. HN_2. Since the formula molecular weights of $Na_2S_2O_3$ (158.1) and HN_2 (192.5) are similar, it may be possible to use comparable milligram dose conversions of the two drugs in managing clinical HN_2 extravasations. For instance, if 1.0 mg HN_2 is extravasated, at least 200 mg $Na_2S_2O_3$ (an approximate 200-fold molar excess) may be required to produce significant antidotal effects. If isotonic $Na_2S_2O_3$ (0.17 *M*) is used as the local antidote, at least 2 ml would be needed to deliver this 200-fold molar excess of $Na_2S_2O_3$. Isotonic $Na_2S_2O_3$ solutions may be easily prepared using 4 ml standard 10% (w/v) USP commercial sodium thiosulfate preparation combined with 6 ml sterile water for injection USP [10].

In summary, we have demonstrated that nitrogen mustard produces dose-dependent skin ulcers in mice. These skin lesions can be significantly reduced if large molar excesses of i.d. $Na_2S_2O_3$ are given immediately following HN_2. Until firm clinical data are available, we recommend that clinical extravasations of HN_2 be treated immediately with (0.17 *M*) $Na_2S_2O_3$ in at least 200-fold molar excess. A large volume of the 0.17 *M* solution is recommended for clinical use due to its isotonic osmolarity with blood [10]. This solution should be injected directly into the area of HN_2 infiltration in a volume that will ensure direct contact with all of the extravasated HN_2 solution. The application of topical heating or cooling, which were not effective in our mouse model, are not recommended for clinical HN_2 extravasations. Our data suggest that it is important to treat HN_2 extravasations immediately with $Na_2S_2O_3$, since it may not be possible to prevent severe skin ulceration if therapy is delayed.

Acknowledgements. The authors would like to thank Mr. James Liddil for his expert technical assistance.

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Received December 9, 1986/Accepted March 7, 1988