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ORIGINAL ARTICLE

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Evaluation of antidotes for extravasation injury produced by 6-hydroxymethylacylfulvene (MGI 114), a novel cytotoxic antitumor agent, in an intradermal toxicity model in rats

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Abstract MGI 114 (HMAF; 6-hydroxymethylacylfulvene) is a cytotoxic drug currently in phase II human clinical trials. As with other anticancer agents, inadvertent drug extravasation may result in perivascular irritation and/or necrosis. In this study the degree of soft tissue injury produced by MGI 114 after intradermal administration to rats was quantified and four potential antidotes for extravasation injuries caused by MGI 114 were evaluated. Intradermal injections of MGI 114 (0.2 ml, concentrations 0.1, 0.5 or 1.0 mg/ml) and a positive control, doxorubicin (0.2 ml, concentration 2 mg/ml) were administered to male Fischer 344 rats in an experiment designed to establish a model for antidote evaluation. Dermal lesions at the injection sites were measured and quantitated as the total area under the lesion area-time curve (AUC). Physiological saline, sodium thiosulfate, dimethylsulfoxide (DMSO) and local cooling, were then compared as potential antidotes in this model. In the initial study, dermal lesions (erythema, ulcerations and eschar formation) occurred at the MGI 114- and doxorubicin-treated sites. The lesion area resulting from MGI 114 was dose-related and was greatest at approximately 5 days, with resolution by day 7–22. Doxorubicin-induced lesions were comparable in area to those induced by the highest dose of MGI 114, but persisted approximately twice as long. In the antidote study, sodium thiosulfate administration resulted in approximately 20% diminution of lesion area and AUC value when compared to untreated controls. Normal saline caused slight reductions in maximum lesion area, but had little effect on AUC values. Local cooling also caused a modest reduction in the maximum lesion area, but actually resulted in higher AUC values by prolonging eschar duration. DMSO provided near complete tissue protection from intradermal exposure to MGI 114. In this model MGI 114 and doxorubicin were found to produce similar soft tissue injuries, but MGI 114-induced lesions tended to show a more rapid resolution. Topical DMSO treatment was found to produce the most effective protection against MGI 114-induced local tissue irritation and necrosis.

Key words Acylfulvene · MGI 114 · HMAF · Extravasation · Antidote

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Introduction

The novel cytotoxic anticancer drug candidate 6-hydroxymethylacylfulvene (HMAF; MGI 114) is a semisynthetic derivative of the natural product mushroom toxin illudin S (Fig. 1). Illudins are isolated from mushrooms of the genus Omphalotus (*O. olearius* or *O. illudens*) or the closely related *Lampteromyces* (*L. japonicus*). Illudin S has been previously demonstrated to possess potent antitumor activity in vitro, but in vivo activity is limited by systemic toxicity [12]. Despite this limitation, illudin S and several derivatives known as acylfulvenes possess properties that make them unique as potential antitumor agents. These properties include alkylation and damage to DNA that appears to be more difficult to repair than damage produced by other alkylating agents, potent inhibition of DNA synthesis, cell cycle arrest at the G_1/S border, and promotion of apoptotic cell death [13–16, 23]. Acylfulvenes, including MGI 114, have therefore been synthesized as part of an effort to produce derivatives of illudin S that retain potent antitumor activity at doses tolerated by tumor-bearing animals [15, 17, 20]. The antitumor activity of MGI 114 in several

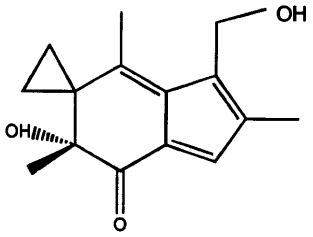

Fig. 1 Structure of 6-hydroxymethylacylfulvene (HMAF; MGI 114)

human solid tumor xenograft models in nude mice has been reported [2, 17, 19]. The compound is currently being administered intravenously to cancer patients with solid tumor malignancies in phase II clinical trials on a daily ×5 administration schedule.

Vesicant injuries resulting from extravasation of many anticancer drugs is a significant clinical concern [3]. Unpublished results of preclinical safety studies with MGI 114 have suggested that the compound is not a direct vascular irritant, but may produce local soft tissue lesions if unintentionally extravasated. Intradermal administration of anticancer drugs in rodents has been previously used as a model for assessing the vesicant effect of extravasated cytotoxic drugs and to evaluate the efficacy of potential methods or antidotes which may limit the local tissue response [5–10, 21]. In an initial dose-response study to better assess the extravasation injury potential of MGI 114, the local toxicities of three concentrations of MGI 114 were compared using doxorubicin as a positive control. In the second study, utilizing an MGI 114 concentration equivalent to the maximum concentration being used in clinical trials, the abilities of saline, sodium thiosulfate, dimethylsulfoxide (DMSO), and local cooling to ameliorate the vesicant effect of MGI 114 were compared.

Materials and methods

Male Fischer 344 rats (Harlan Sprague Dawley, Houston, Tx.), approximately 8–10 weeks old, were randomly assigned to treatment groups (five animals per group). The rats were housed individually in plastic cages equipped with cage liners and elevated steel wire mesh floors in environmentally controlled rooms. Intradermal injections of 0.2 ml were administered with a 27-gauge needle into the right flank area of the animals. MGI 114 was administered in a vehicle composed of 1% ethanol (USP) and 5% dextrose in water for injection (USP). Animals were observed daily for clinical signs of systemic toxicity. Body weights and food consumption were recorded weekly. Injection sites were observed on days 2, 4 and 6, and then twice weekly (until all visible skin lesions had resolved), and the results recorded. Hair loss, erythema, induration and/or ulcerations were measured with calipers and the area of involvement expressed as the product of the widest perpendicular widths [7]. Total area under the lesion area-time curve was determined for each animal and averaged for each group.

Dose-response study

Injection sites were prepared by shaving an area approximately 2 cm^2 24 h prior to MGI 114 administration. MGI 114 was administered by intradermal injection at a concentration of 0.1, 0.5 or 1.0 mg/ml. Based on solubility, the maximum concentration of MGI 114 to be administered in clinical trials using a 1% ethanol/5% dextrose in water vehicle is 1 mg/ml. A fourth group of positive control animals were given doxorubicin at the manufacturer's recommended concentration of 2.0 mg/ml in water.

Antidote study

Injection sites were prepared by shaving an area of approximately 2 cm^2 24 h prior to MGI 114 administration. Five groups were given intradermal injections of MGI 114 at a concentration of 1.0 mg/ml. No additional treatment was administered to the control group. Potential antidotes were administered immediately after MGI 114 administration to each of the remaining four groups. Saline for Injection USP (Baxter Healthcare, Deerfield, Ill.) or 0.14 M sodium thiosulfate (Sigma Chemical Co., St. Louis, Mo.) were administered as four 50-µl intradermal injections (one injection on each of the four sides of the bleb created by the MGI 114 injection). Care was taken to place these solutions several millimeters away from the MGI 114 bleb to prevent mixing and to prevent pressure expulsion of the HMAF from its injection site. DMSO (Sigma) was applied topically to the treatment site in three applications (0.2 ml each) over a 15-min interval. Local cooling was accomplished by application of ice directly over the treatment site for approximately 45 min after treatment. To facilitate the application of ice, rats were anesthetized with halothane and kept warm with a heating pad.

Results

There were no drug- or treatment-related clinical effects or body weight changes in either study, other than the intended development of local tissue reactions. All animals, except for one in each study, survived to their scheduled sacrifice dates. In the initial dose-response study, an MGI 114-treated animal (0.5 mg/ml) died on day 9. In the antidote study, an animal in the sodium thiosulfate group was killed in extremis on study day 6. Neither death was attributed to the drug or the antidote. The intradermal reactions to MGI 114 and/or doxorubicin were characterized by the local appearance of edema and/or erythema that frequently preceded development of ulceration and eschar formation that persisted for a variable period of time.

Dose-response study

Intradermal administration of MGI 114 at concentrations of 0.1, 0.5, and 1.0 mg/ml resulted in dose-related ulceration and eschar formation over the injection sites. The lesions attained maximal size or area on day 5 or 7, and then steadily regressed and resolved between days 7 and day 22 (Fig. 2). Table 1 lists mean maximum lesion area, day of maximum lesion area, duration, and area under the lesion area-time curve. Maximum lesion area was consistently observed on day 5 for all MGI 114 concentrations. Both the maximal lesion area and persistence of the lesion were directly related to the concentration of MGI 114 injected. Concentrations of 0.1, 0.5 and 1 mg/ml resulted in maximum mean lesion areas

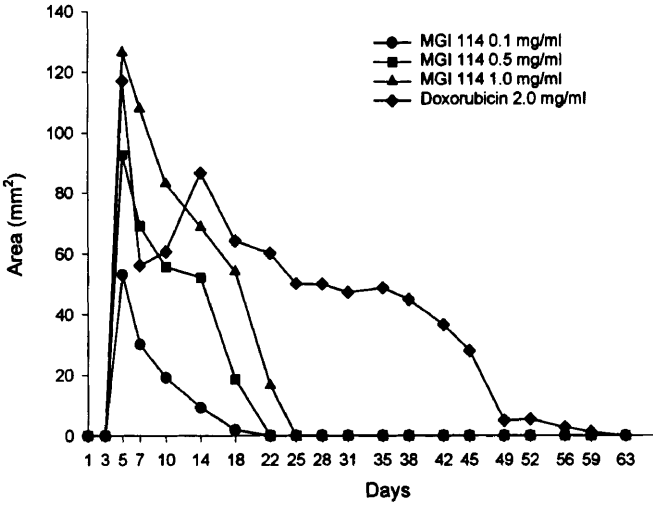

Fig. 2 Change over time of the mean area of lesions induced by various doses of MGI 114 or doxorubicin

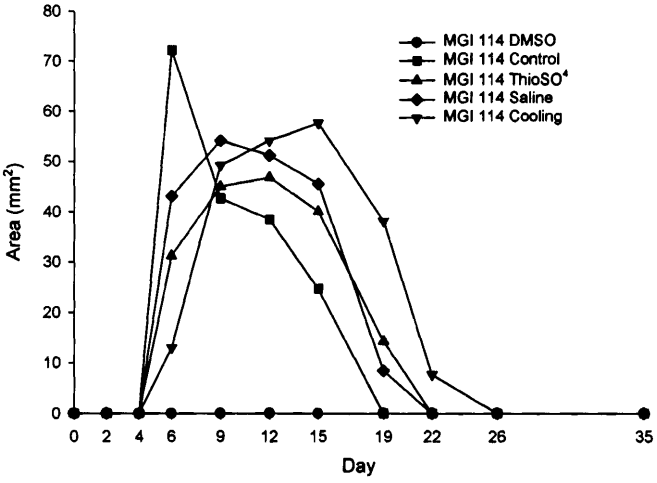

Fig. 3 Effects of different antidotes on the mean MGI 114-induced lesion area over time

of 53.3, 92.7, and 126.5 mm^2 and an average duration of 9, 13, and 16 days, respectively.

Doxorubicin also produced lesions at the injection sites that attained a maximum area on day 5 (Fig. 2). Between days 5 and 25 the lesions decreased in area to approximately one-half of their maximal area and then remained essentially unchanged until approximately day 42 when eschar regression started to occur. The eschars were sloughed between days 49 and 59. Doxorubicin lesions attained maximum area (approximately 117 mm^2) by approximately day 5, but persisted approximately 20 days longer than the MGI 114-induced lesions with an area under the lesion area-time curve almost twice that from high-dose MGI 114 (2466 vs 1363 mm^2 days, respectively).

Antidote study

Based on the results of the initial dose-response study, an MGI 114 concentration of 1.0 mg/ml was selected for use in the subsequent study to assess the effects of different potential antidotes. The intradermal injection of MGI 114 (0.2 ml of a 1-mg/ml solution) in the control animals of the second study resulted in tissue damage and eschar formation of comparable severity and duration to that observed in the preliminary study (Fig. 3, Table 2). The topical administration of DMSO provided complete protection against eschar formation. Erythema in the area of dosing was evident on day 4 in all rats, with discoloration/induration occurring in one or more rats between days 2 and 22 and an area of hair loss in two rats between days 19 and 26. Sodium thiosulfate (10%), administered peripherally to the MGI 114 injection sites, resulted in an apparent reduction in both the peak mean eschar area and total AUC for eschar formation when compared to untreated MGI 114-induced lesions. The peak mean eschar area and the AUC were approximately 35% and 20%, respectively, less than the positive controls. There was a large area of erythema in all rats on days 2 and 4 and a large area of discoloration/induration surrounding the ulcerative lesions or eschars in all rats on days 6 and/or 9. The relatively small reduction in tissue irritation and damage, based on eschar formation, is probably of little clinical significance and evidence that sodium thiosulfate, as used in this study, may not be an effective antidote for extravasated MGI 114. Group mean lesion area (including standard deviations), days to maximum area, duration and AUC are shown in Table 2.

The administration of saline or topical cooling resulted in no appreciable diminution of severity of MGI 114-induced lesions. Saline and local cooling, when compared to the positive controls, reduced the peak mean eschar area by approximately 20%. Saline reduced the AUC of the eschar by approximately 5% while local cooling actually increased the AUC by approximately

Table 1 Ulcerative skin lesions in rats following intradermal MGI 114 or doxorubicin administration (*SD* standard deviation)

Treatment	Dose (mg/ml)	Incidence	Maximum lesion area (mm^2)		Day of maximum mean lesion area	Lesion duration (days)		AUC (mm^2 days)
			Mean	SD		Mean	SD	
MGI 114	0.1	5/5	53.3	18.1	5	9	4	294
	0.5	5/5	92.7	53.2	5	13	3	836
	1.0	5/5	126.5	56.5	5	16	2	1363
Doxorubicin	2.0	5/5	117.1	61.3	5	47	56	2466

Table 2 Ulcerative skin lesions in rats following intradermal injection of MGI 114 at a concentration of 1 mg/ml followed by experimental antidotes (*SD* standard deviation)

Antidote	Lesion incidence	Maximum lesion area (mm^2)		Day of maximum mean lesion area	Lesion duration (days)		AUC (mm^2 days)
		Mean	SD		Mean	SD	
None	5/5	72.1	21.4	6	8.4	1.34	1223
DMSO	0/5	–		–	–		–
Sodium thiosulfate	4/4	46.7	7.9	12	10	2.0	958
Normal saline	5/5	54.1	27.2	9	9.8	1.74	1160
Local cooling	5/5	57.5	12.4	15	9.6	3.51	1388

10%. While cooling decreased the maximum area of the eschar, it apparently delayed the onset of peak eschar area.

Discussion

The ulcerative lesions formed in these studies following intradermal administration of MGI 114 resemble lesions described for other vesicant cytotoxic drugs in rodent models of extravasation injury [7, 21]. Since eschar formation, epidermal cell necrosis and ulceration are active processes in wound formation and healing, the extent of eschar formation (i.e. eschar lesion area) has been used as an index of the severity of skin lesions and as a basis for comparing the potency of different drugs and treatment regimens for inducing skin injury.

In the preliminary dose-response study MGI 114 produced ulcerative lesions with eschar formation at the injection sites at all concentrations. An additional group of animals was given a standard vesicant dose of doxorubicin to serve as a positive control. The highest concentrations of both MGI 114 and doxorubicin were the highest concentrations currently recommended for clinical use. The high concentration of MGI 114 produced a maximum eschar area that was similar to, or slightly greater than, that of doxorubicin. However, healing of the skin lesion, as measured by the persistence of the eschar and the AUC, was considerably faster in MGI 114-treated animals than in doxorubicin-treated animals.

Although different animal models have been known to produce variable results regarding the relative effectiveness of individual antidotes for extravasation injury [3], the vesicant potential of MGI 114 in the initial dose-response study warranted a further investigation of potential antidotes. Because MGI 114 has been shown to alkylate DNA in sensitive tumor cell types [11, 18], potential antidotes were selected based on recommended treatments for extravasations of other alkylating or DNA-interactive cytotoxic agents [3]. Sodium thiosulfate has been previously demonstrated to have protective effects against mitomycin C and mechlorethamine vesicant injury in animal models [9, 10] and in clinical use [3]. Topical DMSO treatment has been reported to be protective against anthracycline-induced dermal toxicity in rats [22], but not mice [4]. DMSO is also the most effective antidote for mitomycin C dermal toxicity in mice [9]. Accordingly, topical DMSO treatments are recommended for both anthracycline [1, 3] and mitomycin C clinical extravasations [3]. Local cooling has been shown to have protective activity against doxorubicin-induced dermal toxicity in mice [8] and is recommended for clinical extravasations [3]. Normal saline injections have similarly been shown to have some protective activity against vinblastine-induced dermal toxicity in mice, although hyaluronidase treatment is more effective [5]. Hyaluronidase injections are also effective against vinorelbine-induced dermal toxicity in mice, where normal saline injections are not effective [6]. Hyaluronidase injections are therefore the recommended clinical antidote for vinca alkaloid extravasations [3].

Similar to the results of a previous study in mice with mitomycin C-induced dermal toxicity [9], topical DMSO applications were very effective in blocking development of ulcerative lesions following intradermal injection of MGI 114 in rats. In contrast, sodium thiosulfate, normal saline, and local cooling provided little protection from lesion formation and primarily acted to delay the development of the lesion compared to untreated controls. It is unknown whether additional injections of saline or sodium thiosulfate at later time-points or extending the time-frame of local cooling may have provided an additional protective effect. However, given the profound protective effect of topical DMSO applications in this rat model of extravasation injury, topical DMSO treatment would appear to be the treatment of choice for known extravasations of this experimental agent in clinical trials.

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