

Extravasation injury potential of CI-980, a novel synthetic mitotic inhibitor

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Received 3 December 1992/Accepted 12 March 1993

Abstract. Severe soft-tissue ulceration is known to result from inadvertent extravasation of a number of anticancer drugs, including tubulin-binding vinca alkaloids, during intravenous administration. CI-980 is a novel anticancer drug candidate that also inhibits mitosis by binding to tubulin. Intradermal administration of CI-980 to mice at doses of 0.02 and 0.05 mg produced ulcerative lesions in 3/5 and 2/5 animals, respectively, that were significantly smaller than those produced in all animals at vinblastine doses of 0.05 and 0.1 mg. Ulcerative lesions resulting from CI-980 treatment were also less persistent, resolving in 2–8 days versus 7–16 days following vinblastine administration. As based on the common dose of 0.05 mg, CI-980 appears to have significantly less vesicant activity than vinblastine.

Introduction

Because of the vesicant potential of many anticancer drugs, inadvertent extravasation during intravenous administration remains a significant concern of oncologists [5]. Anticancer drugs with known vesicant potential include the DNA intercalators doxorubicin [18], daunorubicin [2], and actinomycin D [17]; the alkylating agents nitrogen mustard [4] and mitomycin C [1]; and the tubulin-binding vinca alkaloids vinblastine [14], vincristine [15], and vindesine [8]. Dorr et al. [9] have developed a mouse model of extravasation injury for anticancer drugs. Utilizing intradermal administration to mimic extravasation, the model has been used to characterize the vesicant potential of a wide number of anticancer drugs [7, 12, 19] and to test the efficacy of a variety of antidotal treatments for established vesicants [6, 7, 9–11].

The tubulin-binding agent CI-980, ethyl(*S*)-(5-amino-1,2-dihydro-2-methyl-3-phenylpyrido[3,4-*b*]pyrazin-7-yl) carbamate 2-hydroxysulfonate (1:1), is a novel synthetic mitotic inhibitor [3] undergoing clinical trials as an anticancer drug candidate. The present intradermal toxicity study in mice was conducted to assess the extravasation injury potential of CI-980 in comparison with vinblastine, a tubulin-binding vinca alkaloid with established activity in the model [7].

Materials and methods

Adult male BALB/c mice (Charles River Laboratories, Portage, MI) weighing 20–24 g were used. Animals were individually housed in stainless-steel wire-mesh cages in environmentally controlled rooms on a 12-h light/dark cycle with access to water and food ad libitum (Purina, St. Louis, Mo.). Approximately 24 h prior to compound administration, hair was removed from the mouse dorsum using topical applications of the depilatory agent Neet as per Dorr et al. [9]. D5W vehicle (Dextrose, 5% in Water for Injection, USP), CI-980 (Warner-Lambert Co., Morris Plains, N.J.), and vinblastine sulfate (Sigma Chemical Co., St. Louis, Mo.) were given as single 50- μ l intradermal injections to five animals per group. Because of its potential systemic toxicity at higher doses, CI-980 was given at total doses of 0.01, 0.02, and 0.05 mg. Vinblastine sulfate was injected at doses of 0.05 and 0.1 mg as previously tested by Dorr et al. [7]. Animals were observed daily during the study for signs of drug toxicity. Each day on which cutaneous ulcers were evident, lesion size was quantitated by micrometer measurement of the two largest perpendicular diameters [9]. Areas under the lesion size-time curve for individual animals were calculated. The study was terminated and the animals were euthanized by CO₂ asphyxiation when all primary injection-site ulcers had healed.

Dose-related differences in mean lesion size and areas under the lesion size-time curve were independently analyzed within a one-tailed analysis of variance (ANOVA) framework for CI-980 and vinblastine. A two-tailed comparison of CI-980 and vinblastine at the common dose of 0.05 mg was also performed. Differences were considered to be statistically significant at $P < 0.05$.

Results

Except for skin lesions near the injection site, animals given vinblastine had no other drug-related clinical sign of

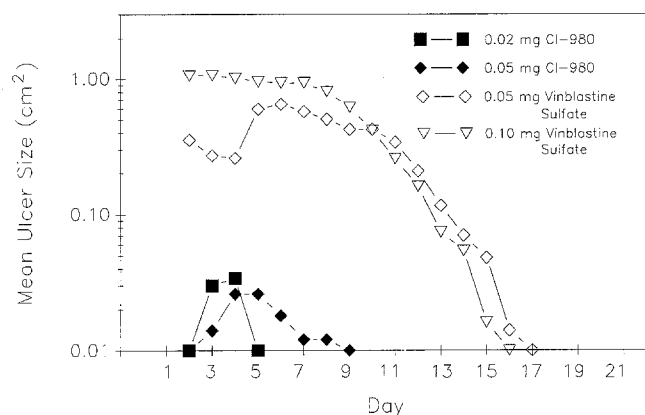


Fig. 1. Mean ulcerative lesion size over time. Each point represents the mean value for five animals per group

toxicity. In contrast, animals given 0.05 mg CI-980 exhibited clinical signs indicating systemic toxicity. These included urine staining of the ventral abdomen, alopecia, hypoactivity, and slight body-weight loss (0.4 ± 0.2 g) in the 1st week after dosing versus a weight gain of 1.2 ± 0.4 g in the vehicle control group.

Skin lesions observed in the study included erythema as well as ulceration. However, only ulcerative lesions were quantitated by micrometer measurement. No skin lesion was observed in the vehicle control or 0.01-mg CI-980 groups. The total incidence of skin lesions, including both ulcerative lesions and erythema, was 4/5 mice at 0.02 or 0.05 mg CI-980 and 5/5 animals at both vinblastine doses.

The incidence, severity, and persistence of ulcerative lesions produced by CI-980 and vinblastine are summarized in Table 1. When present, ulcerative lesions in CI-980-treated animals were small punctate lesions at the injection site. In contrast, both vinblastine doses produced more widely spread ulcerative lesions that were not uniformly centered around the injection site. In one case, more than one discrete ulcerated lesion was observed. Ulcerative lesions that appeared in two animals at 0.05 mg vinblastine 8 or 10 days after the primary lesion had healed were considered to be secondary and were not included in the calculation or statistical analysis of lesion size or other descriptive statistics.

In characterizing the mouse intradermal model for assessing the extravasation injury potential of anticancer drugs, Dorr et al. [9] reported that the best representation of dose-response data for a single agent was the area under the lesion size-time curve (AUC) for ulcerative lesions. The group mean AUC values calculated for this study are also included in Table 1. The group mean lesion size over time is graphically depicted in Fig. 1.

There were clear differences in intradermal toxicity between CI-980 and vinblastine with respect to incidence, maximal lesion size, persistence of lesions, and AUC values. These differences were statistically significant at the common dose of 0.05 mg. A dose response for lesion induction was clearly demonstrated for CI-980, as 0.01 mg did not produce lesions. There was no distinguishable difference between CI-980 doses of 0.02 and 0.05 mg. Despite interanimal variability in the severity of the response to vinblastine, mean maximal lesion size and mean AUC values were higher at 0.1 mg than at 0.05 mg.

Discussion

A mouse intradermal model of extravasation injury was used for the direct comparison of two anticancer drugs that share tubulin binding as a cytotoxic mechanism of action. The results suggest that CI-980 has significantly lower potential for producing soft-tissue injury when it has extravasated than does vinblastine at comparable doses.

The doses of the two drugs that were tested were limited to a single common dose because of the potential systemic toxicity of CI-980 at doses exceeding 0.05 mg and a previous study in which the maximal mean lesion size in the mouse model at 0.05 mg vinblastine sulfate [7] approached the lower limits of response desirable in a positive control group. In a single intravenous dose-lethality study of CI-980 in CF-1 mice, deaths were seen within 8 days of administration, with calculated LD₁₀ and LD₅₀ values being 3.9 and 4.5 mg/kg, respectively [16]. Some clinical indications of systemic toxicity were in fact observed in the present study at 0.05 mg CI-980. The total doses of both drugs given in this study (approximately 2–2.5 mg/kg CI-980 and 4–5 mg/kg vinblastine) represent similar fractions of their respective lethal parenteral doses in mice, as the

Table 1. Ulcerative skin lesions in mice following intradermal CI-980 or vinblastine sulfate administration

Treatment	Dose (mg)	Incidence	Ulcerative lesions		Onset (day)	Resolution (day)	AUC (cm ² days) ^a
			Maximal size (cm ²)	Mean maximal size (cm ²)			
Vehicle control	–	0/5	0	0	–	–	0
CI-980	0.01	0/5	0	0	–	–	0
CI-980	0.02	3/5	0.15	$0.036 \pm 0.064^*$	2	4–6	$0.055 \pm 0.090^*$
CI-980	0.05	2/5	0.12	$0.026 \pm 0.053^{*,**}$	2–3	6–10	$0.114 \pm 0.244^{*,**}$
Vinblastine sulfate	0.05	5/5	1.30	$0.818 \pm 0.359^*$	2	9–18	$5.030 \pm 3.589^*$
Vinblastine sulfate	0.10	5/5	2.46	$1.132 \pm 1.026^*$	2	10–17	$7.817 \pm 7.685^*$

^a Data represent mean values \pm SD ($n = 5$ animals/group)

* Significantly different from control ($P < 0.05$)

** Significantly different from 0.05 mg vinblastine sulfate ($P < 0.05$)

reported intraperitoneal single-dose LD₁₀ value for vinblastine is 2.7 mg/kg in the Swiss Webster mouse [13] and the reported intravenous single-dose LD₅₀ value is 10 mg/kg in the ICR mouse [20].

Enhanced systemic distribution of intradermally injected CI-980 relative to vinblastine could explain the lesser degree of localized dermal toxicity observed for CI-980 in this model. Promoting greater systemic distribution of extravasated drug is in fact the basis for clinical treatment of vinca alkaloid skin toxicity with hyaluronidase [5]. The mouse intradermal model has been used to validate this therapeutic approach by demonstrating that [³H]-vinblastine sulfate was more rapidly cleared from intradermal injection sites following hyaluronidase treatment [7]. This study also identified hyaluronidase treatment as the most effective antidote for extravasated vincristine and vindesine [7].

Despite the lower vesicant activity found for CI-980 as compared with vinblastine in this model, the two higher doses of CI-980 did produce minor ulcerative skin lesions. In light of a similar cytotoxic mechanism of action and the experimental effectiveness of hyaluronidase treatment for intradermally injected vinca alkaloids, hyaluronidase could be considered for the clinical management of extravasated CI-980.

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