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Lack of experimental vesicant activity for the anticancer agents cisplatin, melphalan, and mitoxantrone

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Summary. Cisplatin and L-PAM are DNA-crosslinking anticancer agents which have not been systematically studied for vesicant potential. Mitoxantrone is a new active anthracene-based, DNA intercalator which is undergoing widespread clinical testing for antitumor efficacy in man. These three agents were tested for vesicant activity in dehaired BALB/c mice given ID injections equivalent to human clinical doses. Neither cisplatin (up to 150 mg/m^2) nor L-PAM (up to 71 mg/m^2) produced any skin necrosis in the mice. The L-PAM solvent (acid/alcohol in propylene glycol) was ulcerogenic if injected undiluted. Mitoxantrone (up to 14 mg/m^2) was not ulcerogenic in the mice, although the skin site retained a blue drug discoloration for several weeks. It is concluded that in clinically relevant doses, cisplatin, L-PAM, and mitoxantrone are not vesicants.

Introduction

Severe and prolonged necrosis is a common sequala following the extravasation of vesicant anticancer drugs into soft tissues during IV administration [17]. Skin ulcers which develop after such accidents with the intercalator doxorubicin (Adriamycin) can require surgical debridement with full-thickness graft closure [18]. This potential for serious injury mandates extreme caution during IV administration if peripheral veins are used for vesicant drug injections. New indwelling central venous catheters or infusion ports may lessen this risk, but these systems require surgical insertion and careful maintenance [14]. The general list of vesicant anticancer agents includes the intercalating antibiotics doxorubicin and daunomycin [25], the alkylating agents mechlorethamine (nitrogen mustard) [5] and mitomycin C [4], and the vinca alkaloids such as vinblastine (Velban and vincristine (Oncovin [8]. There are several antineoplastic drugs which are not vesicants based on the current findings and clinical experience (Table 1). This group includes alkylating agents (e.g., cyclophosphamide), the antimetabolites, the epipodophyllotoxin derivatives (e.g., VP-16), and a number of miscellaneous agents, such as bleomycin (Blenoxane) and *L*-asparaginase (Elspar).

There are some standard and experimental drugs for which definitive studies of the vesicant potential are not available. This causes concern among practitioners about what would happen if an inadvertent drug extravasation should occur. Three agents in this group are the DNA-crosslinking drugs cisplatin (Platinol) and melphalan (L-PAM), and the DNA-intercalating anthracene derivative, mitoxanthrone. Cisplatin has been in clinical use for some time and at least two cases of mild local skin toxicity after extravasation have been reported [19, 20]. L-PAM injection [1] and mitoxantrone [7] are investigational anticancer drugs currently in clinical trial, yet detailed information about their vesicant potential is not available. In this report, the results of quantitative skin ulceration studies in an established mouse model are presented for each of these agents.

Materials and methods

An intradermal (ID) mouse model was used for these studies and has been reported in detail previously [11]. BALB/c female mice weighing 25–30 g (Jackson Laboratories, Bar Harbor, ME) are dehaired on their dorsum using applications of a topical depilatory agent. Potential vesicant agents are injected ID 24 h afterwards and the skin site is visually inspected daily for measurement of the widest perpendicular areas of induration, erythema, and ulceration. Drug doses used in this model are calculated to approximate maximal human clinical doses adjusted on a murine body surface area (BSA) basis [13]. When this method is used the model correlates well with clinical experience in that (a) known vesicants in man have proven to be vesicants in the mouse; (b) the converse applies for nonvesicants; and (c) a dose-response relationship exists for known vesicants given at BSA-adjusted ID doses in the mouse [9–12].

All drug doses were diluted to a final volume of 0.05 ml with: (a) 0.9% sodium chloride USP for cisplatin (supplied as Platinol, 10 mg vial, Bristol Laboratories, Syracuse, NY); (b) 5% dextrose in water for mitoxantrone (supplied as a 20 mg/10 ml solution for American Cyanamid Co., Pearl River, NJ); and (c) an acid-alcohol mixture for L-PAM (1 ml of a mixture of 0.047 ml 37% HCl, 0.943 ethanol with 9.0 ml of a potassium

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Table 1. Non-vesicant anticancer agents

Classification	Agent
Alkylating agents	Cyclophosphamide (Cytoxan)
DNA Crosslinking Agents	Thiotepa
	Cisplatin #
	Melphalan (L-PAM) #
Antimetabolites	Cytarabine (ARA C)
	Fluorouracil (5 FU) *
	Merthotrexate (MTX)
	Mercatopurine (6 MP)
DNA-intercalatiang agents	Mitoxantrone #
Epipodophyllotoxins	Etoposide (VP-16) **
	Teniposide (VM-26) **
Hormonal agents	Estrogens
	Glucocorticosteroids
	Progestins
	Antiestrogens
Miscellaneous	Asparaginase
	Bleomycin (Blenoxane) *

Result from the current study
* Occasionally causes mild to moderate phlebitis
** Occasional phlebitis may be related to commerical drug solvent [10]

phosphate/propylene glycol buffer for a 100-mg vial of durg supplied by Burroughs-Wellcome Co., Research Triangle Park, NC). Subsequent dilutions of this L-PAM solution were made with 0.9% sodium chloride USP. Cisplatin ID doses were 0.05 mg, 0.1 mg, 0.5 mg, 0.75, 1.0 mg, and 2.0 mg (corresponding to human doses of approximately 7–290 mg/m^2 BSA). Concentrations >1.0 mg/ml of cisplatin were injected as an aqueous suspension. This was necessitated by the solubility limits of cisplatin and the need to maintain a constant volume for injection in the mouse. L-PAM was given at ID doses of 0.05 mg, 0.14 mg, 0.28 mg, and 0.5 mg (corresponding to clinical doses of approximately 7–71 mg/m^2 BSA). The L-PAM diluent was separately evaluated at a dose of 0.05 ml ID. Mitoxantrone was given ID at doses of 0.01, 0.05 mg, and 0.1 mg (corresponding to clinical doses of approximately 1.4 to 14 mg/m^2 BSA). Each treatment group contained five mice, and all animals were followed until all visible skin lesions were completely resolved.

Results

Cisplatin studies

Cisplatin produced only a mild induration in 60% of mice given ID doses greater than 0.5 mg (approximately 70 mg/m^2). There was no erythema or ulceration seen even at the 2.0-mg dose (approximately 290 mg/m^2). This dose, which is higher than that used in cancer patients, killed all five mice within 72 h of ID injection. The area of cisplatin injection appeared blanched immediately after drug injection and remained so up to the time of death of the mice. There was no accumulation of blood or fluid into these skin sites as has been noted after extravasation of classic DNA alkylators such as mechlorethamine (nitrogen mustard) [5].

Melphalan studies

L-PAM similarly produced no evidence of local skin toxicity at doses of 0.28 mg (40 mg/m^2). At this dose, only three of five mice demonstrated induration and mild ulceration. No erythema was seen at any L-PAM dose level. The higher dose of 0.5 mg did not produce any greater induration or ulceration. This dose of L-PAM, which killed four of five mice, is equivalent to 71 mg/m^2 in human cancer patients. In contrast to L-PAM solutions diluted in saline, the investigational solvent system when injected ID undiluted produced significant ulceration. A mean peak ulcer of 0.07 cm^2 was evident within 1–3 days after ID injection of 0.05 ml undiluted L-PAM solvent (containing acidified alcohol and propylene glycol). These lesions completely resolved within 10–14 days. When this solvent was diluted into saline equivalent to the L-PAM-containing treatments and injected ID at 0.05 ml no skin toxicity was produced.

Mitoxantrone studies

Mitoxantrone also produced no skin toxicity at the lowest dose tested (0.01 mg, or approximately 1.4 mg/m^2). At 0.05 mg (7 mg/m^2) consistent induration was produced, which was most pronounced on the first day following ID injection. This area of induration reached an average peak size of 1.73 cm^2. Erythema was also produced by this regimen, and it peaked (0.39 cm^2) on day 9. In only one of five mice was there any evidence of ulceration at the 0.05 mg dose. This peaked at 0.32 cm^2 on day 6. At the higher dose of 0.1 mg ID (equivalent to 14 mg/m^2 in cancer patients) mitoxantrone produced an intense blue discoloration of the skin and immediate induration, which peaked on day 1 at 3.92 cm^2. Only two of five mice had erythema surrounding the injection site. The skin site in two mice was blanched 5 days after injection, but no subsequent ulceration occurred even though the skin remained intensely blue for up to 6 weeks after ID injection. The skin color and texture had returned to normal with complete hair regrowth over the site within 10–11 weeks after ID injection. The highest dose of mitoxantrone caused no animal deaths.

Discussion

The results of this study reveal that the anticancer agents cisplatin, L-PAM, and mitoxantrone have little or no vesicant activity in an experimental mouse model. This conclusion is supported by observations showing that none of the drugs caused consistent ulceration at doses representative of human clinical doses adjusted on a BSA basis. With this method of dose conversion between mice and humans, previous studies have shown a positive correlation of anticancer drug-induced skin ulceration in our mouse model and the known vesicant activity of the agents in patients [9–12]. This relationship has been demonstrated clearly for several standard anticancer drugs, including the anthracycline antibiotic doxorubicin (Adriamycin) [11], for the vinca alkaloids [10], and for the alkylating agents mechlorethamine and mitomycin C. These vesicants are known to cause severe skin and soft tissue necrosis following inadvertent extravasations in clinical practice [4, 5].

In addition to experimental findings, clinical case reports, while nonquantitative, are helpful in establishing the vesicant potential of an anticancer agent. Two case reports of cisplatin-induced skin necrosis after inadvertent

extravasation are described. In one instance cellulitis and fibrosis were seen after interstitial leakage of a small amount of a concentrated cisplatin solution (0.42 mg/ml) into the hand [20]. This resolved without incident. In the other case, a large amount of a concentrated cisplatin solution (75 mg/100 ml) was infused into the dorsum of the hand of a disoriented patient. Pain, swelling, and inflammation developed over a 2-week period, and subsequently a large, full-thickness skin ulcer developed which required surgical debridement [19]. These cases appear to represent isolated experiences, since in phase I–III clinical trials cisplatin was not found to be ulcerogenic when extravasated [24]. It has also been successfully used IP without evidence of chemical peritonitis [15]. The results of our animal studies similarly suggest that cisplatin is not a significant vesicant, since lethal ID doses were required to produce a small degree of local skin ulceration. The use of an ID cisplatin suspension in these animal studies should not limit the clinical conclusion of nonvesicant potential. This is because the lethality seen with the high doses clearly documented the drugs bioavailability from the suspension. Additionally, this drug is typically infused in a large volume of fluid. Thus, it is very unlikely that ulcerogenic amounts of the drug could ever extravasate unnoticed in the clinic.

The parenteral formulation of L-PAM has been available for clinical trials for several years in both Europe and the United States, but extravasation reactions with necrosis have not been described. In our study, the diluent in this formulation was toxic in mouse skin if injected undiluted. This local toxicity may contribute to the rare, severe generalized hypersensitivity reactions described with this drug (2.4% among 425 patients) [6]. From the results of the current studies, however, L-PAM does not appear to produce localized necrotic reactions in mice given large clinical equivalents of the drug directly into the skin. In addition, L-PAM has not produced significant local toxicity in regional drug delivery trials using high drug doses given by the IP route [16]. However, L-PAM has caused severe local soft tissue necrosis when administered by isolated limb perfusion, but only in the presence of regional hyperthermia [22].

Mitoxantrone is a new anthracene derivative which interacts with DNA by intercalation and electrostatic binding properties [21, 23]. It has a similar spectrum of clinical activity to the anthracycline antibiotic doxorubicin [26]. In ongoing clinical trials, mitoxantrone extravasations have not produced skin necrosis although a blue discoloration of the skin may be apparent [1, 2, 27, 28]. This is identical to the findings of the current experimental studies in mice. The clinically tolerable dose of mitoxantrone for solid tumor theapy is in the range of 12–14 mg/m^2 given IV every 3–4 weeks [27]. The same BSA-adjusted dose range was evaluated in the current animal study and did not produce significant skin toxicity. It thus appears that mitoxantrone is not a vesicant. This is in marked contrast to the severe local toxicities produced in our own mouse model and in cancer patients by its anthracene congener, bisantrene [12]. This latter agent is also active in acute leukemia and advanced breast cancer, but it causes severe and often dose-limiting local toxicities in patients. The marked contrast between these two anthracene derivatives suggests that structural modifications in this series, can produce significant alterations in toxicity without the loss of clinical antitumor activity.

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