Protection against Adriamycin-induced Skin Necrosis in the Rat by Dimethyl Sulfoxide and \( \alpha \)-Tocopherol

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ABSTRACT

Extravasation of Adriamycin during i.v. infusion can cause serious local complications. We have used a rat skin model to study the protection afforded by dimethyl sulfoxide and \( \alpha \)-tocopherol (vitamin E) against Adriamycin-induced skin necrosis. Topical daily application of 1 ml dimethyl sulfoxide for 2 days produced a small decrease in ulcer diameter of up to 11% at 2 weeks. Topical daily applications of 1 ml 10% \( \alpha \)-tocopherol succinate in dimethyl sulfoxide for 2 days produced a marked decrease in ulcer diameter at 2 weeks of up to 68%. Daily topical application of 1 ml 10% \( \alpha \)-tocopherol succinate in dimethyl sulfoxide for 7 days offered no greater protection than 2-day application. \( \alpha \)-Tocopherol acetate appeared to have activity slightly less than that of \( \alpha \)-tocopherol succinate in reducing ulcer size, and both compounds were considerably more active than was \( \alpha \)-tocopherol alcohol. Administration of \( \alpha \)-tocopherol succinate or \( \alpha \)-tocopherol acetate i.p. had no significant effect upon ulcer diameter. Topically applied dimethyl sulfoxide and \( \alpha \)-tocopherol may provide an effective way of treating accidentally extravasated Adriamycin in cancer patients.

INTRODUCTION

Extravasation of anticancer drugs during i.v. infusion can cause tissue damage. The local effects of Adriamycin are particularly severe. Estimates of the frequency of Adriamycin extravasation range from 0.5% to over 6% (3, 19, 37, 39). Extravasation of Adriamycin into soft tissue produces tissue necrosis which increases in severity over several weeks and results in very slowly healing ulcers. These indolent ulcers remain a source of severe pain or functional impairment for many months. In severe cases, the lesion may extend to deep structures such as underlying tendon and bone, resulting in loss of joint mobility (5, 12, 15, 18, 22, 29, 32, 42). There have been no controlled studies to determine the best method for the treatment of extravasation of anticancer drugs (16). Adriamycin extravasation is commonly treated by hot compresses (16) and by infiltration of sodium bicarbonate (4, 16, 42) and corticosteroids (3, 16, 29). Hot compresses produce vasodilation and may promote absorption of Adriamycin (16). Sodium bicarbonate has been suggested to decrease the solubility and binding of Adriamycin to DNA (4, 42) and corticosteroids, to reduce local inflammation (16). For more severe cases, early surgical débridement followed by full-thickness skin grafting and/or flap coverage has been recommended (5, 16, 18, 29).

Animal models have been used to study the histopathogenesis of Adriamycin-induced skin necrosis (20, 33, 34) and to study nonsurgical methods of treatment (10, 11, 26). Cohen (10) found that corticosteroids had no effect on Adriamycin-induced skin ulcers in mice. High doses of hydrocortisone delayed skin ulceration but only for a few days. Dorr et al. (11) reported that i.d. \( \alpha \) hydrocortisone reduced ulceration but only in mice receiving low doses of Adriamycin. Bartokowski-Dodds and Daniels (4) have presented data recently suggesting that sodium bicarbonate infiltration might provide some benefit against Adriamycin-induced ulceration in the rat. They point out, however, that sodium bicarbonate can itself produce tissue necrosis. Other workers found that sodium bicarbonate offered no protection against Adriamycin ulceration in rabbit (26) and mouse (11).

The mechanism by which Adriamycin produces its antitumor and cytotoxic effects is not known with certainty. Adriamycin can be reduced enzymatically (28) to form a short-lived semi-quinone free radical which, in the presence of molecular oxygen, undergoes oxidation-reduction cycling to form the superoxide anion radical (14) and then other species of reactive oxygen, including the cytotoxic hydroxyl radical (13). We reasoned that, if the skin cytotoxicity of Adriamycin was due to the formation of free radicals, it might be possible to prevent the effects of accidentally extravasated Adriamycin using \( \alpha \)-tocopherol (vitamin E), a naturally occurring free radical scavenging agent (9), and dimethyl sulfoxide, a hydroxyl radical scavenging agent (9). Dimethyl sulfoxide would have the additional advantage of acting as a vehicle for \( \alpha \)-tocopherol and enhance its penetration into the skin when applied topically (17). The results of this study show that topically applied dimethyl sulfoxide and \( \alpha \)-tocopherol may protect against Adriamycin-induced skin necrosis in the rat.

MATERIALS AND METHODS

Adriamycin-induced Skin Ulceration. The rat skin model developed by Rudolph et al. (34) was used to study the ulcerogenic activity of Adriamycin and the effect of \( \alpha \)-tocopherol and dimethyl sulfoxide as modifiers of activity. A total of 200 male Sprague-Dawley rats (Sprague-Dawley, Madison, Wis.) weighing between 200 and 250 g was used in the study. Rodents differ from humans in having a thin muscle layer, the panniculus carnosus, which provides nourishment, intimately adherent to the skin. Drugs must be placed above the panniculus carnosus to be in direct contact with the skin. If care is taken to inject the drug i.d. above the panniculus carnosus, uniform dose-dependent skin necrosis can be produced (11, 34). Rats were anesthetized with pentobarbital (50 mg/kg i.p.), and both flanks were shaved with electric hair clippers. The same concentration of Adriamycin solution, 2 mg Adriamycin per ml 0.9% NaCl solution, was injected i.d. on both flanks through a 27-gauge needle. The concentration of Adriamycin chosen was that reported by Rudolph et al. (34) to produce maximum ulceration. 

1 The abbreviation used is: i.d., intradermal(ly).

2 To whom requests for reprints should be addressed.

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size in the rat; the volume injected was varied in different studies. Modifier solutions were applied immediately with cotton swabs to the skin surface above the site of Adriamycin injection on the right flank only. Modifier solutions: 1 ml 90% dimethyl sulfoxide* 1 ml dimethyl sulfoxide containing 10% (w/v) α-tocopherol acetate, 10% (w/v) α-tocopherol succinate, or 10% (v/v) α-tocopherol alcohol; 0.1 ml α-tocopherol alcohol; 1 ml lanolin-white petrolatum (50% w/w); or 1 ml lanolin-white petrolatum containing 10% (w/w) α-tocopherol acetate or α-tocopherol succinate. Application was repeated daily for 2 or 7 days. Two groups of rats were given injections i.p. with α-tocopherol acetate or α-tocopherol succinate, 100 mg/day in corn oil, for 2 and 7 days. The size of the ulcer produced by Adriamycin was measured every 7 days as the mean of 2 perpendicular diameters. An initial erythematous reaction overlying an area of induration was followed by ulceration at 7 days. No attempt was made to measure erythema or induration, which were obscured by regrowth of hair after 2 or 3 weeks.

Four separate studies were performed, each with a slightly different format. The first study was to investigate the time dependence of ulceration with different volumes of Adriamycin solution and the effects of 2-day topical application of dimethyl sulfoxide or 10% α-tocopherol succinate in dimethyl sulfoxide in reducing ulcer diameter. The results of this study are shown in Charts 1 and 2. The second study, which forms the basis for Chart 3, used a single dose of Adriamycin, 300 µl of 2 mg of Adriamycin per ml, and examined the effects of 7-day topical application of dimethyl sulfoxide or 10% α-tocopherol succinate in dimethyl sulfoxide in reducing ulcer diameter. The third study used the same dose of Adriamycin and compared the effects of dimethylsulfoxide and lanolin-white petrolatum as vehicles for the 2- and 7-day topical applications of α-tocopherol acetate and α-tocopherol succinate. The results of this study are shown in Table 1. The effects of i.p. administration of α-tocopherol acetate and α-tocopherol succinate in reducing ulcer diameter were also examined in this study. The fourth study used a single dose of Adriamycin and compared the effects on ulcer diameter of direct topical application for 2 and 7 days of 0.1 ml α-tocopherol alcohol, which is liquid at room temperature, with the effects of the same amount of α-tocopherol alcohol dissolved in dimethyl sulfoxide. The results of this study are shown in Table 2.

**Drugs and Chemicals.** Adriamycin was supplied by the Division of Drug Treatment, National Cancer Institute, Bethesda, Md. Dimethyl sulfoxide (distilled in glass) was obtained from Burdick & Jackson Laboratories, Inc., Muskegon, Mich. D-α-Tocopherol alcohol (mixed isomers from vegetable oil), D-α-tocopherol acetate (natural vitamin E acetate), and D-α-tocopherol succinate (natural vitamin E succinate) were obtained from Sigma Chemical Co., St. Louis, Mo.

**RESULTS**

Injection of Adriamycin i.d. produced necrotic skin ulcers in the rat. Mean ulcer diameter increased with increasing amount of Adriamycin (Chart 1). Mean ulcer diameter reached a maximum at 2 weeks and was maintained for at least a further 2 weeks (Chart 2). By this time, rats had begun to die, particularly at the higher doses of Adriamycin. For this reason, the number of surviving animals is shown for each point on Charts 1 and 2. In rats alive at 2 weeks after Adriamycin treatment but which received no other treatment, there was no significant difference (p > 0.05, paired t test) in the average size of the ulcers on the left and right flanks of the animal with different amounts of Adriamycin (Chart 1). Two-day topical application of dimethyl sulfoxide and 10% α-tocopherol succinate in dimethyl sulfoxide to the right flank of the animal following i.d. injection of Adriamycin produced a decrease in 2-week mean ulcer diameter on that flank, compared to the mean ulcer diameter on the right flank of control rats when dose was ignored (p < 0.001, paired t test). When looking within each dose level, the 2-week mean ulcer diameters of surviving rats in the dimethyl sulfoxide group were not statistically different from the 2-week mean ulcer diameters of the control (p > 0.05 in all 4 grouped t tests). There was a significant difference in the mean ulcer diameter of the 10% α-tocopherol succinate in dimethyl sul-

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**Table 1**

**Effect of α-tocopherol esters and vehicle on Adriamycin-induced skin necrosis in rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Untreated left flank</th>
<th>Treated right flank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13</td>
<td>6.3 ± 0.5</td>
</tr>
<tr>
<td>7-day treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dimethyl sulfoxide</td>
<td>9</td>
<td>7.6 ± 0.9</td>
</tr>
<tr>
<td>Dimethyl sulfoxide-α-tocopherol</td>
<td>8</td>
<td>8.1 ± 0.8</td>
</tr>
<tr>
<td>succinate</td>
<td></td>
<td>3.9 ± 0.7</td>
</tr>
<tr>
<td>Lanolin</td>
<td>5</td>
<td>4.1 ± 1.5</td>
</tr>
<tr>
<td>Lanolin-α-tocopherol succinate</td>
<td>5</td>
<td>6.2 ± 1.3</td>
</tr>
<tr>
<td>i.p. α-tocopherol succinate</td>
<td>5</td>
<td>6.6 ± 0.7</td>
</tr>
<tr>
<td>2-day treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dimethyl sulfoxide</td>
<td>5</td>
<td>8.0 ± 0.3</td>
</tr>
<tr>
<td>Dimethyl sulfoxide-α-tocopherol</td>
<td>5</td>
<td>7.7 ± 0.5</td>
</tr>
<tr>
<td>acetate</td>
<td></td>
<td>5.0 ± 0.8</td>
</tr>
<tr>
<td>Lanolin</td>
<td>4</td>
<td>8.6 ± 1.2</td>
</tr>
<tr>
<td>Lanolin-α-tocopherol acetate</td>
<td>5</td>
<td>8.6 ± 0.5</td>
</tr>
<tr>
<td>i.p. α-tocopherol acetate</td>
<td>5</td>
<td>8.5 ± 0.8</td>
</tr>
</tbody>
</table>

**Table 2**

**Effect of α-tocopherol alcohol on Adriamycin-induced skin necrosis in rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Untreated left flank</th>
<th>Treated right flank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>7.0 ± 0.6</td>
</tr>
<tr>
<td>2-day treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Tocopherol alcohol</td>
<td>6</td>
<td>6.7 ± 0.8</td>
</tr>
<tr>
<td>α-Tocopherol alcohol-dimethyl</td>
<td>6</td>
<td>8.4 ± 1.1</td>
</tr>
<tr>
<td>sulfoxide</td>
<td></td>
<td>5.2 ± 1.0</td>
</tr>
<tr>
<td>α-Tocopherol succinate-dimethyl</td>
<td>5</td>
<td>10.3 ± 1.4</td>
</tr>
<tr>
<td>sulfoxide</td>
<td></td>
<td>2.8 ± 1.4</td>
</tr>
<tr>
<td>7-day treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Tocopherol alcohol</td>
<td>5</td>
<td>6.3 ± 1.0</td>
</tr>
<tr>
<td>α-Tocopherol alcohol-dimethyl</td>
<td>5</td>
<td>8.0 ± 1.4</td>
</tr>
<tr>
<td>sulfoxide</td>
<td></td>
<td>5.8 ± 1.1</td>
</tr>
<tr>
<td>α-Tocopherol succinate-dimethyl</td>
<td>5</td>
<td>8.7 ± 0.6</td>
</tr>
<tr>
<td>sulfoxide</td>
<td></td>
<td>4.0 ± 0.9</td>
</tr>
</tbody>
</table>

* n, number of animals.

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* Dimethyl sulfoxide equilibrated with room air at 20° which has an equilibrium moisture content of 10%, hereafter referred to simply as dimethyl sulfoxide.
A maximum decrease of 68% was seen with 400 μl Adriamycin for 2 days, by 21%, and by topical application of 10% α-tocopherol succinate in dimethyl sulfoxide for 7 days, by 38%. Neither lanolin-white petrolatum applied topicaly for 2 or 7 days, lanolin-white petrolatum containing 10% α-tocopherol acetate applied for 2 days, or lanolin-white petrolatum containing 10% α-tocopherol succinate applied for 7 days produced any significant decrease in mean ulcer diameter. α-Tocopherol succinate and α-tocopherol acetate injected i.p. at doses similar to those applied topically had no significant effect in decreasing mean ulcer diameter.

Unlike α-tocopherol acetate or succinate which are solids, α-tocopherol alcohol is liquid at room temperature and can be applied directly to skin. However, when applied topically for 2 or 7 days, α-tocopherol alcohol by itself had no significant effect upon 2-week mean ulcer diameter (Table 2). The same

The effect of 7 daily topical applications of 1 ml dimethyl sulfoxide or 1 ml 10% α-tocopherol succinate in dimethyl sulfoxide on ulcer diameter is shown in Chart 3. In this experiment, rats received 300 μl of 2 mg of Adriamycin per ml of 0.9% NaCl solution on each flank. The weekly mean ulcer diameters on the right flank to which dimethyl sulfoxide was applied were all less than the weekly mean ulcer diameters on the control right flank, but these reductions were not statistically significant (p > 0.08, grouped t test). Ten % α-tocopherol succinate in dimethyl sulfoxide significantly reduced mean weekly ulcer diameters on the right flank to which it was applied (p < 0.05, grouped t test) by a maximum of 68% at 4 weeks. In this study, neither treatment had any significant effect upon mean ulcer diameter on the nontreated left flank.

Table 1 summarizes the effects of esters of α-tocopherol in different vehicles on ulcer diameter at 2 weeks following i.d. injection of 300 μl of 2 mg of Adriamycin per ml of 0.9% NaCl solution. None of the topical applications significantly lessened average ulcer diameter on the nontreated left flank. Topical application of dimethyl sulfoxide for 2 and 7 days produced a significant decrease in mean ulcer diameter when compared to the control right flank with decreases of 11 and 32%, respectively. Mean ulcer diameter was significantly reduced by topical application of 10% α-tocopherol acetate in dimethyl sulfoxide for 2 days, by 21%, and by topical application of 10% α-tocopherol succinate in dimethyl sulfoxide for 7 days, by 38%.
amount of α-tocopherol alcohol in dimethyl sulfoxide produced a small but significant decrease in 2-week mean ulcer diameter after 2 days, although not after 7-day topical application. The effect of 10% α-tocopherol alcohol in dimethyl sulfoxide in decreasing mean ulcer diameter was not much different from the effect of dimethyl sulfoxide alone seen in some other studies. Ten % α-tocopherol alcohol in dimethyl sulfoxide is clearly much less effective in decreasing 2-week mean ulcer diameter than is 10% α-tocopherol succinate in dimethyl sulfoxide, which was included in this study as a positive control.

DISCUSSION

Topical application of 10% α-tocopherol succinate in dimethyl sulfoxide for 2 days significantly reduced the mean size of Adriamycin-induced necrotic skin ulcers in the rat up to 68%. There appeared to be no further advantage of the 7-day compared to the 2-day course of application. Dimethyl sulfoxide applied topically may itself reduce the size of skin ulcers, but this effect was less marked than with α-tocopherol succinate or α-tocopherol acetate in dimethyl sulfoxide and was not always statistically significant. A significant 32% decrease in mean ulcer size was produced by 7-day topical application of dimethyl sulfoxide. Ten % α-tocopherol acetate in dimethyl sulfoxide appeared to have activity slightly less than that of 10% α-tocopherol succinate in dimethyl sulfoxide in reducing ulcer diameter. Both 10% α-tocopherol acetate and 10% α-tocopherol succinate in dimethyl sulfoxide exhibited greater activity in reducing ulcer diameter than did α-tocopherol alcohol, which being liquid could be applied directly to the skin. By itself, α-tocopherol alcohol exhibited no activity in reducing ulcer diameter and, in dimethyl sulfoxide, showed minimal activity probably not much greater than dimethyl sulfoxide alone. Dimethyl sulfoxide and α-tocopherol were effective only when applied locally and had no reproducible systemic activity against Adriamycin-induced skin necrosis. Lanolin-white petrolatum could not substitute for dimethyl sulfoxide as a vehicle for topical application of α-tocopherol succinate or acetate. Dimethyl sulfoxide rapidly penetrates the skin without damaging the stratum corneum (17) and acts as a penetrant-carrier for other drugs. It probably enhances the penetration of α-tocopherol into the skin. Dimethyl sulfoxide might itself prevent Adriamycin-induced skin ulceration by acting as a hydroxyl radical scavenging agent (9). It could also aid the dispersion of Adriamycin from i.d. sites. Dimethyl sulfoxide is a vasodilator (1) and has anti-inflammatory and mild antibacterial activity (17, 41). All of these properties might help to prevent ulceration. α-Tocopherol is a well-known radical scavenging agent and antioxidant (21). α-Tocopherol pretreatment has been reported to protect mice and other animals against acute Adriamycin cardiomyopathy (24, 25, 36, 40), although it does not protect animals against chronic Adriamycin cardiomyopathy (6). The protective effect of α-tocopherol has been ascribed to its ability to inhibit lipid peroxidation in the heart (25). α-Tocopherol might protect against Adriamycin skin ulceration by a similar mechanism.

Dimethyl sulfoxide and α-tocopherol are nontoxic when applied topically. Dimethyl sulfoxide is exceptionally nontoxic (30). There were early reports of lenticular changes in experimental animals receiving dimethyl sulfoxide (31), but these changes have never been seen in humans (8). Dimethyl sulfoxide has been applied topically to human volunteers at doses up to 9 ml/day for 6 months with no adverse effects apart from an initial stinging sensation and a transient erythema and scaling of the skin (7, 17). The unique breath odor and garlic taste associated with cutaneous application of dimethyl sulfoxide was annoying to some subjects. α-Tocopherol is a constituent of proprietary skin preparations and has been reported to protect human skin against photosensitization erythema (27) and to enhance the regenerative capacity of skin burn wounds (23). α-Tocopherol in doses up to 1600 units daily is relatively free from side effects in humans (2).

In summary, this report shows that a combination of α-tocopherol succinate in dimethyl sulfoxide applied topically offers effective protection against skin necrosis produced by i.d. Adriamycin in the rat. Immediate liberal application of 10% (w/v) α-tocopherol succinate in 90% dimethyl sulfoxide with a repeat application the following day might form a convenient treatment for accidentally extravasated Adriamycin in cancer patients.

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