Topical Chemotherapy of Intradermal Walker 256 Carcinosarcoma with Diaziquone and Doxorubicin in the Rat

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ABSTRACT

A model for metastatic skin cancer using intradermal injection of Walker 256 carcinosarcoma has been developed in the rat. Using this model, antitumor activity of topically applied doxorubicin and diaziquone in Vanicream, Plastibase, and dimethyl sulfoxide (DMSO) as vehicles was compared with intraperitoneal injection of the drugs at the same doses beginning 4 days after injection of tumor cells. Doxorubicin applied topically at 0.5 mg/day for 4 days in Vanicream or Plastibase exhibited no antitumor activity, while i.p. administered doxorubicin at 0.5 mg/day for 4 days inhibited tumor growth at day 20 by 66%. Diaziquone applied topically at 0.1 mg/day for 4 days in Vanicream, Plastibase, or DMSO inhibited tumor growth at day 20 by 66, 86, and 43%, respectively, and cured animals of the skin tumor at a dose of 0.5 mg/day. Diaziquone administered i.p. at 0.5 mg/day for 4 days was lethal to rats, and at 0.1 mg/day it produced 93% inhibition of tumor growth at day 20. Diaziquone applied topically at 0.1 mg/day for 4 days in Plastibase cured rats of advanced tumor when treatment was begun 12 days after injection of tumor cells. The area under the plasma radioactivity time curve over 5 h for a single 0.64-mg dose of topically applied [ring-14C]diaziquone in DMSO was 0.01% that of the same dose of [ring-14C]diaziquone administered i.p. in non-tumored rats. The decrease in WBC count following topical application of diaziquone at a dose of 0.1 mg/day for 4 days, compared to the same dose of diaziquone administered i.p., was 62% in Vanicream, 81% in Plastibase and 33% in DMSO. Topical diaziquone was non-toxic to normal skin in the rat and in the domestic pig. It is concluded that topical application of diaziquone offers a therapeutic advantage over systemic treatment for metastatic cancer of the skin.

INTRODUCTION

Metastatic carcinoma of the skin arises from adjacent or distant neoplasms and is a clinical problem occurring in about 4% of cancer patients (1–2). Women with breast cancer form the largest group in this heterogenous population. Many women who have had radical mastectomies for breast cancer will develop chest wall recurrences, and about 25% of women with recurrent breast cancer will develop skin involvement (3). In men 24% of skin metastases arise from cancer of the lung (4).

Radiotherapy can be useful in controlling localized metastases to the skin, for example, on the chest wall (5). In other patients systemic treatment, either cytotoxic chemotherapy or hormones, will be indicated because the presence of metastases at sites in addition to those to skin. Topical chemotherapy has the potential for achieving higher concentrations of drug in the immediate vicinity of tumors in the skin than can be achieved by systemic chemotherapy. For some patients a conservative approach using topical therapy might offer advantages if there is no clinical evidence of tumor at other sites. Patients who develop skin metastases later in the course of breast cancer following therapy with one or more drugs or who develop recurrence of local disease in a previously irradiated chest wall may have lesions which are poorly responsive to additional systemic drug treatment. These lesions frequently progress locally to necrotic masses (6) and in the extreme cases may extensively involve cutaneous lymphatics and encircle the entire thorax (carcinoma en curasse), resulting in respiratory impairment by mechanical restriction (7). Such patients might also benefit from topical chemotherapy.

There have been few studies with the topical administration of chemotherapeutic drugs apart from studies of 5-fluorouracil (8) and nitrogen mustard (9). Poor penetration of 5-fluorouracil into skin may limit its clinical usefulness (10). The use of more liposoluble chemotherapeutic drugs or vehicles that enhance penetration of the drugs into skin might improve the response to topically administered chemotherapy. We report the development of a model for metastatic skin cancer in rat using intradermal Walker 256 carcinosarcoma and the use of the model to study the activity of topical chemotherapeutic agents and vehicles that might be useful in the treatment of metastatic carcinoma to the skin in humans.

MATERIALS AND METHODS

Drugs and Vehicles. Doxorubicin was purchased from Adria Laboratories, Columbus, OH and contained 50 mg lactose for every 10 mg doxorubicin. Diaziquone, 2,5-bis[(1-aziridinyl)-3,6-diazo-1,4-cyclohexadiene-1,4-diyl]bis(carbamic acid) and [ring-14C]diaziquone (specific activity, 13.63 μCi/mmol) was generously supplied by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. The radiochemical purity of the [ring-14C]diaziquone was determined to be 99% by high-performance liquid chromatography. Drugs were applied in Vanicream (Pharmaceutical Specialties, Rochester, MN), an oil in water emulsion vanishing cream used for prescription compounding and dry skin care, in Plastibase (Squibb Laboratories, New Brunswick, NJ), a plasticized hydrocarbon gel ointment base used for prescription compounding, or in dimethyl sulfoxide (Burdick and Jackson, Muskegon, MI). The maximum dose of drug that could be applied was determined by the maximum amount of drug that could be mixed with Vanicream or Plastibase or dissolved in dimethyl sulfoxide, which for all of the vehicles, was 5 mg/ml with both doxorubicin and diaziquone, and by the amount of vehicle that could be spread over a 4-cm² area of skin, which was 0.1 ml. This gave a maximum dose of drug per animal of 0.5 mg/day. Drug was applied daily for 4 days. Lower doses of diaziquone of 0.1 mg/day and 0.02 mg/day applied in 0.1 ml vehicle were also used. Drug was prepared fresh each day in the vehicles at the appropriate concentrations.

Development of a Rat Model for Metastatic Skin Cancer. Groups

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2 To whom requests for reprints should be addressed.

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of 6 female Sprague-Dawley rats weighing 150–200 g (colony 205, Harlan Sprague-Dawley, Madison, WI) were used for all studies and housed individually in raised stainless steel cages. Food and water were provided ad libitum. Walker 256 carcinosarcoma cells, in the ascites form, were obtained from the National Cancer Institute Breast Cancer Animal and Human Tumor Bank, Mason Research Institute, Worcester, MA and carried in the ascites form in the peritoneal cavity of rats. Animals were given injections i.p. with 10^6 Walker 256 carcinosarcoma cells (passage 5 to 19), and tumor cells were harvested 6 days later (11). Prior to harvesting of the cells, the animal was anesthetized with diethyl ether, and the abdominal area was washed with 70% ethanol. Ascite fluid was removed with a 6-ml syringe, and blood cells were lysed with a solution of 0.83% ammonium chloride:0.1% potassium carbonate:0.0037% EDTA. Tumor cells were collected by centrifugation at 2000 x g for 10 min and resuspended in 0.9% NaCl. The resulting cell suspension consisted predominantly of single tumor cells. Counts of viable cells were determined in a hemocytometer using Trypan blue exclusion (12) and averaged 95%. Each animal yielded approximately 6 x 10^6 tumor cells.

Experimental animals were lightly anesthetized with intraperitoneal pentobarbital (30 mg/kg), and an area on the back of the neck was shaved prior to intradermal injection with 0.1 ml of Walker 256 carcinosarcoma cell suspension containing 10^6 tumor cells. To inject the tumor cells into the animals, the skin on the back of the neck was rolled between the finger and thumb, and the tumor cells were injected through a 19-gauge needle inserted at an angle of 25° so that injections were made above the panniculus carnosus, the muscle layer of the skin. The tumor grew rapidly in all injected animals and in most cases formed a palpable lump 2–3 days after injection. Tumor diameter was measured with calipers as the mean of the perpendicular diameters. Animals whose tumor diameter was not 5 ±1 mm on day 4 after tumor cell injection were excluded (12) and averaged 95%. Each animal yielded approximately 6 x 10^6 tumor cells.

Drug Administration. Topical drug applications were begun 4 days after tumor cell injection to an area of skin 4 cm^2 overlying the tumor on the back of the neck and were continued daily for 4 days. In some studies drug was applied to animals with advanced tumor for 4 days beginning 12 days after injection of tumor cells. Drug dissolved in 0.1 ml vehicle or vehicle alone was applied from a syringe and was spread over the skin with a flat spatula. Animals were lightly anesthetized each day prior to drug administration using intraperitoneal pentobarbital (30 mg/kg). Because the drug could be wiped off the skin by the animal rolling around the floor of the cage during recovery from the anesthetic, the drugs were applied under a non-occlusive dressing formed from a 4 cm^2 Band Aid porous dressing with a non-medicated pad fixed to the skin with 4 stainless steel surgical clips. The dressing and clips were removed 24 h after the last drug treatment. Tumor growth was measured every second day. In some studies drugs were injected intraperitoneally at the same doses as applied topically. For intraperitoneal injection diaziquone was dissolved in 0.1 ml 50% N,N-dimethylacetamide in 0.9% NaCl, and doxorubicin was dissolved in 0.1 ml 0.9% NaCl (the normal vehicles for systemic administration).

Systemic Toxicity. The systemic toxicity of diaziquone administered topically and given by intraperitoneal injection was determined in groups of 4 non-tumored rats by measuring total WBC counts. Diaziquone (0.1 mg/day) in vehicle or vehicle alone was applied to a 4 cm^2 area on the back of the neck of each animal, and treatment was continued daily for a total of 4 days. An equivalent amount of diaziquone dissolved in 0.1 ml 50% N,N-dimethylacetamide in 0.9% NaCl or 0.1 ml of the vehicle alone was administered by intraperitoneal injection daily for 4 days to another group of rats. Blood samples were taken for determination of total WBC counts on days, 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, and 22. WBC counts were determined on 0.02 ml of blood using a Coulter Counter Model ZT (Coulter Electronics, Inc., Hialeah, FL) after a 1:500 dilution of blood with Isoton II and hemolysis with Zap-O-Globin II (Coulter Diagnostics, Hialeah, FL).

Local Skin Toxicity. All rats were observed for evidence of local skin toxicity following topical administration of drugs. In addition, local skin toxicity studies were conducted in pigs which have a skin structure similar to that of humans (12). Two 18-kg cross-bred female pigs were obtained from the Mayo Clinic, Animal Breeding Station. The pigs were housed individually in raised stainless steel cages, and food and water were provided ad libitum. Prior to drug treatment the back of each pig was shaved, and a 5-mm full thickness skin biopsy was taken from each animal under local anesthesia with 0.1 ml 2% Lidocaine (Eilkins-Sinn, Cherry Hill, NJ). Diaziquone, 0.5, 0.1 and 0.02 mg/day in 0.1 ml Vani
cream, Plastibase, or dimethyl sulfoxide was applied to a 4-cm^2 area at 12 different sites on the pig skin for a total of 4 days. Full thickness skin biopsies, 5 mm in diameter, were taken from the treated areas on days 1, 4, and 11. Each skin biopsy was prepared for histological examination by fixing in formalin, embedding in paraffin, and staining with hematoxylin and eosin.

Systemic Absorption of Topical Diaziquone. A single dose of [ring-14C]diaziquone, 7.5 μCi, 0.64 mg, dissolved in 0.1 ml dimethyl sulfoxide was applied to a 4-cm^2 shaved area of skin of 3 non-tumored rats. A similar number of rats received the same dose of [ring-14C]diaziquone dissolved in 50% N,N-dimethylacetamide in 0.9% NaCl by intraperitoneal injection. Blood, 0.1 ml, was collected into heparinized capillary tubes at various times up to 5 h by nicking the tail vein with a scalp knife, and plasma prepared by rapidly centrifuging the blood for 10 min at 2000 x g. The radioactivity in 0.05-ml aliquots of plasma was determined by liquid scintillation counting. Results were expressed as diaziquone equivalents.

Statistics. Groups of data were analyzed for statistical significance using Student’s t-test (14).

RESULTS

Walker 256 Carcinosarcoma Skin Tumor Model. Tumor grew in all animals injected intradermally with Walker 256 carcinosarcoma tumor cells. The growth of the tumor was rapid and linear for up to 12 days, at which time the tumor had broken through the skin to form a blood-encrusted necrotic mass. Tumor growth slowed, and the tumor reached its maximum size by 15–20 days after tumor cell injection. Microscopic examination of the subcutaneous injection site revealed that the early tumor occupied the interface between dermis and muscle. Pleomorphic cells with large vesicular nuclei and prominent nucleoli formed a single aggregate mass, and numerous mitoses were present. Enlarging tumors contained numerous vascular channels. Eventually the tumor infiltrated adjacent fat and dermis, destroying hair appendages and epidermis, and central tumor necrosis occurred. Non-treated tumor-bearing animals began to die, in most studies about day 25, but this varied from study to study. Animals dying from tumor showed extensive tumor infiltration of the lungs and liver on autopsy.

Antitumor Activity of Diaziquone. Diaziquone applied topically for 4 days resulted in a dose-dependent inhibition of the growth of intradermal Walker 256 carcinosarcoma. Growth curves for control and drug-treated Walker 256 carcinosarcoma are shown in Chart 1 where, for clarity, bars denoting standard errors of the means have been omitted. Standard errors of means are shown in Table 1 for tumor diameter on day 10, when in non-treated animals the tumor was still rapidly growing, and day 20, when in non-treated animals the tumor had reached a maximum size, which was about 1% of the body surface area of the animal. Also shown in Table 1 is the number of surviving animals in each group.

Diaziquone consistently showed the greatest antitumor activity...
against intradermal Walker 256 carcinosarcoma when applied in Plastibase, slightly less activity when applied in Vanicream, and the least, although still marked, activity when applied in dimethyl sulfoxide. Complete tumor regression was seen with diaziquone at 0.5 mg/day in all vehicles by day 18 and by day 31 for diaziquone at 0.1 mg/day in Plastibase and Vanicream. No significant effect on tumor growth was seen with topical diaziquone at 0.02 mg/day in any of the vehicles. Animals showing complete regression of tumor did not regrow tumor in the skin within 60 days. The skin overlying the area where the tumor had appeared in these animals was normal upon histological examination and showed no microscopic sign of tumor. However, the lungs and livers of approximately 50% of these animals showed tumor infiltration on autopsy. None of the vehicles by themselves had any significant effect upon tumor growth (results not shown).

Diaziquone applied topically in Plastibase was toxic at the two higher doses; 4 animals died, apparently of diaziquone toxicity with diarrhea, at 0.5 mg/day, and 1 animal died at 0.1 mg/day. Only 1 animal died when diaziquone was applied topically in Vanicream, at 0.5 mg/day, while there were no deaths at lower doses of diaziquone in Vanicream. There were no deaths at any dose of diaziquone in dimethyl sulfoxide. A few animals died under anesthesia during drug administration, and they were excluded from the study.

The effect of intraperitoneally administered diaziquone against intradermal Walker 256 carcinosarcoma at doses similar to those applied topically is shown in Table 2. Diaziquone, 0.5 mg/day for 4 days, was lethal to all animals, and death occurred within 5 days of commencement of treatment. Diaziquone, 0.1 mg/day for 4 days, produced almost complete regression of tumor, while a dose of 0.02 mg/day produced some slowing of tumor growth but no decrease in the final tumor diameter.

Antitumor Activity of Diaziquone against Advanced Tumor. The antitumor activity of topical and intraperitoneal diaziquone against advanced intradermal Walker 256 carcinosarcoma was studied in rats 12 days after tumor cell injection. Diaziquone, 0.5 mg/day applied topically in Plastibase or administered intraperitoneally in 50% N,N-dimethylacetamide in 0.9% saline for 4 days, resulted in death of all animals within 5 days. Death of the animals on topical application of diaziquone in Plastibase was presumably due to absorption of drug through the area of skin broken down by tumor. Diaziquone applied topically (Chart 2) or administered intraperitoneally at 0.1 mg/day for 4 days caused regression of the tumor, which had completely disappeared by days 34 and 32, respectively, in all animals leaving apparently normal skin. The tumor did not grow back by day 60. Diaziquone applied topically or administered intraperitoneally at 0.02 mg/day produced only a small nonsignificant decrease in tumor diameter.

Activity of Doxorubicin. Doxorubicin applied topically at 0.5 mg/day for 4 days in Vanicream or in dimethyl sulfoxide had no significant effect upon the growth of intradermal Walker 256 carcinosarcoma. Doxorubicin at the same dose administered intraperitoneally caused slowing of tumor growth (Table 3).

Systemic Toxicity of Topical Diaziquone. The effect of topical diaziquone, 0.1 mg/day, for 4 days upon total WBC counts was studied in non-tumored animals for all of the drug vehicle combinations and for vehicle alone and was compared to the same dose of diaziquone administered intraperitoneally. The time course of the response of total WBC counts to topical diaziquone in Vanicream and intraperitoneal diaziquone is shown in Chart 3. WBC nadirs for all of the treatments are shown in Table 4. The small drop in total WBC counts in non-treated and vehicle alone animals may represent a response to repeated blood sampling. Intraperitoneal diaziquone gave a WBC nadir of 2,129 ± 267/μl on day 8, which had not returned to pretreatment values by day 22. With all topical treatments WBC counts had returned to pretreatment values by day 22.

Systemic Absorption of Topical Diaziquone. The systemic absorption of a single dose of topically applied [ring-14C]diaziquone was studied in non-tumored animals. The peak plasma concentration of radioactivity following topical administration of 0.64 mg diaziquone in 0.1 ml dimethyl sulfoxide was 22 ng diaziquone equivalents/ml, while the peak plasma concentration of radioactivity following intraperitoneal administration of the same dose of diaziquone was 3.7 μg diaziquone equivalents/ml (Chart 4). The area under the radioactivity-time curve up to 5 h for topicaly applied diaziquone in dimethyl sulfoxide was 0.01%
TOPICAL CHEMOTHERAPY WITH DIAZIQUONE

Table 1
Antitumor activity of topical diaziquone against intradermal W256 carcinosarcoma

Diaziquone was applied topically for 4 days to a 4 cm² area of skin overlying the tumor, beginning 4 days after injection of 10⁶ W256 tumor cells i.d. The drug was applied in 0.1 ml Vanicream, Plastibase, and dimethyl sulfoxide. All animals, including controls, received i.p. pentobarbital (30 mg/kg) each day for 4 days to facilitate application of drug. There were originally 6 animals in each group, but some animals died under drug treatment and were excluded from study.

<table>
<thead>
<tr>
<th>Dose level (mg/day)</th>
<th>No. of animals alive at Day 10</th>
<th>Tumor diameter on Day 10 (mm)</th>
<th>No. of animals alive at Day 20</th>
<th>Tumor diameter on Day 20 (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6/6</td>
<td>18.6 ± 0.7²</td>
<td>6/6</td>
<td>0.0²</td>
</tr>
<tr>
<td>0.5 mg DQ² in Vanicream</td>
<td>6/6</td>
<td>6.1 ± 1.1¹</td>
<td>5/6</td>
<td>0.0 ± 0.0²</td>
</tr>
<tr>
<td>0.5 mg DQ in Plastibase</td>
<td>5/6</td>
<td>4.4 ± 0.7¹</td>
<td>2/6</td>
<td>0.0 ± 0.0²</td>
</tr>
<tr>
<td>0.5 mg DQ in DMSO</td>
<td>5/5</td>
<td>5.5 ± 0.9²</td>
<td>5/5</td>
<td>1.0 ± 0.9²</td>
</tr>
<tr>
<td>Control</td>
<td>5/5</td>
<td>15.9 ± 0.8</td>
<td>5/5</td>
<td>21.5 ± 3.2</td>
</tr>
<tr>
<td>0.1 mg DQ in Vanicream</td>
<td>4/4</td>
<td>12.5 ± 0.8²</td>
<td>4/4</td>
<td>7.3 ± 2.3²</td>
</tr>
<tr>
<td>0.1 mg DQ in Plastibase</td>
<td>6/6</td>
<td>6.6 ± 0.9²</td>
<td>5/6</td>
<td>2.9 ± 1.5²</td>
</tr>
<tr>
<td>0.1 mg DQ in DMSO</td>
<td>6/6</td>
<td>11.4 ± 1.0¹</td>
<td>6/6</td>
<td>12.3 ± 4.6²</td>
</tr>
<tr>
<td>Control</td>
<td>5/5</td>
<td>11.8 ± 1.2</td>
<td>5/5</td>
<td>15.2 ± 2.8</td>
</tr>
<tr>
<td>0.02 mg DQ in Vanicream</td>
<td>4/4</td>
<td>13.0 ± 0.9²</td>
<td>4/4</td>
<td>14.6 ± 2.7</td>
</tr>
<tr>
<td>0.02 mg DQ in Plastibase</td>
<td>3/3</td>
<td>9.2 ± 1.4</td>
<td>3/3</td>
<td>10.0 ± 5.0</td>
</tr>
<tr>
<td>0.02 mg DQ in DMSO</td>
<td>5/5</td>
<td>13.6 ± 1.3</td>
<td>5/5</td>
<td>18.2 ± 4.9</td>
</tr>
</tbody>
</table>

a Mean ± SE.
b This was the only group in which control animals had begun to die of tumor by day 20.
c DQ, diaziquone; DMSO, dimethyl sulfoxide.
d P < 0.01 compared to the appropriate non-treated control.
e Animal died with diarrhea, apparently from diaziquone toxicity.
f P < 0.05 compared to the appropriate non-treated control.

Table 2
Antitumor activity of intraperitoneal diaziquone against intradermal W256 carcinosarcoma

Diaziquone was applied intraperitoneally for 4 days, beginning 4 days after injection of tumor cells. Non-treated animals received vehicle alone. No phenobarbital was used in these studies.

<table>
<thead>
<tr>
<th>Dose level (mg/day)</th>
<th>No. of animals alive at Day 11</th>
<th>Tumor diameter on Day 10 (mm)</th>
<th>No. of animals alive at Day 20</th>
<th>Tumor diameter on Day 20 (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6/6</td>
<td>13.7 ± 1.2²</td>
<td>6/6</td>
<td>15.1 ± 2.7</td>
</tr>
<tr>
<td>0.5</td>
<td>5/6</td>
<td>0.0 ± 0.0²</td>
<td>0/6</td>
<td>0.0 ± 0.0²</td>
</tr>
<tr>
<td>0.1</td>
<td>6/6</td>
<td>0.6 ± 0.4²</td>
<td>6/6</td>
<td>1.1 ± 1.1³</td>
</tr>
<tr>
<td>0.02</td>
<td>6/6</td>
<td>9.1 ± 1.5³</td>
<td>6/6</td>
<td>14.4 ± 3.3</td>
</tr>
</tbody>
</table>

a Mean ± SE.
b Animals died with diarrhea, apparently from diaziquone toxicity.
c P < 0.01.
d P < 0.05.

The area under the radioactivity time curve for intraperitoneally administered diaziquone.

Toxicity of Diaziquone to Skin. There was no damage to the skin of rats by the topical diaziquone treatments in any of the studies. In animals in which the tumor had completely regressed at 60 days the skin appeared to be histologically normal. Studies of diaziquone skin toxicity were also conducted in pig, which approaches fairly closely the morphological and functional characteristics of human skin (13). Diaziquone at 0.5, 0.1, and 0.02 mg/day for 4 days in Vanicream, Plastibase, or dimethyl sulfoxide had no apparent adverse effects on the skin of pig. There was no erythema, drying, or sloughing of the skin. Histological examination showed the epidermis and dermis to be intact. No dyskeratosis or parakeratosis was evident. Dermal connective tissue, vasculature, and appendageal structures were normal. No inflammatory infiltrates were present.

DISCUSSION

The choice of chemotherapeutic agents for topical application for treatment of skin cancers in humans has, to date, been largely empirical, based upon a lack of toxicity to normal skin (15). Little attention has been directed toward the penetration of drug into the skin (16). 5-Fluorouracil is the chemotherapeutic agent most frequently administered by the topical route and is used for treatment of solar keratosis (8). 5-Fluorouracil is poorly absorbed by skin (17) and probably fails to penetrate to the base of the hair follicle (10). Thus, topical application of 5-fluorouracil can lead to the appearance of tumor control with superficial inhibition of tumor growth, while deeper regions of the tumor continue to grow, and disease can reoccur when treatment stops (10, 18).

The present study was designed using combinations of drugs and vehicle that were likely to show appreciable penetration of drug into the skin. The drugs used were chosen on two criteria.
TOPICAL CHEMOTHERAPY WITH DIAZIQUONE

Antitumor activity of topical and intraperitoneal doxorubicin against intradermal W256 carcinosarcoma

Doxorubicin was applied topically or injected intraperitoneally for 4 days, beginning 4 days after i.d. injection of 10^6 W256 tumor cells. Doxorubicin-treated and control animals in the topical treatment group received i.p. pentobarbital (30 mg/kg) each day for 4 days. Pentobarbital was not used in the study with intraperitoneal doxorubicin.

Dose level (mg/day) | No. of animals alive at Day 10 | Tumor diameter on Day 10 (mm) | No. of animals alive at Day 20 | Tumor diameter on Day 20 (mm)
---|---|---|---|---
Control | 6/6 | 15.3 ± 0.6 | 5/6 | 22.0 ± 2.7
Topical treatment | 0.5 mg DOX in Vanicream | 5/5 | 16.2 ± 0.5 | 5/5 | 26.4 ± 2.5
0.5 mg DOX in DMSO | 5/5 | 16.2 ± 1.7 | 5/5 | 22.2 ± 3.1
Intraperitoneal administration | 0.9% NaCl control | 6/6 | 12.9 ± 0.1 | 6/6 | 26.8 ± 1.9
0.5 mg DOX in 0.9% NaCl | 6/6 | 7.8 ± 0.8 | 5/6 | 9.1 ± 1.7

* Mean ± SE.
* DOX, doxorubicin; DMSO, dimethyl sulfoxide.

Doxorubicin applied topically in Vanicream or in dimethyl sulfoxide for 4 consecutive days had no antitumor activity against intradermal Walker 256 carcinosarcoma, and these studies were not pursued. Diaziquone applied topically in Vanicream, Plastibase, or dimethyl sulfoxide daily for 4 consecutive days resulted in regression of intradermal Walker 256 tumor in all treated animals. Of the vehicles studied, Plastibase imparted the greatest antitumor activity to diaziquone, Vanicream had slightly less antitumor activity, and dimethyl sulfoxide had the least antitumor activity. By day 18 tumor had disappeared from the skin of animals receiving diaziquone, 0.5 mg/day, in all vehicles, and by day 31 tumor had disappeared from the skin of animals receiving diaziquone, 0.1 mg/day, in Vanicream and Plastibase. Topical diaziquone at a dose of 0.02 mg/day in any of the vehicles showed no significant inhibition of tumor growth. Drug-treated animals exhibiting complete tumor regression that were allowed to survive to 60 days showed no regrowth of tumor in the skin. Topical and intraperitoneally administered diaziquone at 0.1 mg/day showed antitumor activity against advanced (12 day) intradermal Walker 256 carcinosarcoma and caused complete tumor regression by days 34 and 32, respectively, with regrowth of normal skin. Higher doses of diaziquone of 0.5 mg/day could not be used against advanced tumor because of severe toxicity of diaziquone. This was presumably due to increased absorption of diaziquone through an area where the skin had broken down due to ulceration caused by the tumor.

As well as being the most active against tumors, diaziquone in Plastibase exhibited the most toxicity to the animals. Four of 6 animals died, presumably from toxic effects of the drug, at the 0.5 mg/day dose of diaziquone, and 1 animal died at the 0.1 mg/day dose. Diaziquone in Plastibase also produced the greatest systemic toxicity of any of the topical drug/vehicle combinations, with a WBC nadir of 2,792 ± 163/µl compared to a nadir of 2,129 ± 267/µl with the same dose of diaziquone given intraperitoneally. All of the topical diaziquone preparations showed systemic toxicity with a decrease of total WBC counts, despite the fact that very little topically applied diaziquone was absorbed.
TOPICAL CHEMOTHERAPY WITH DIAZIQUONE

Table 4
Effect of topical and intraperitoneal diaziquone on WBC counts
Diaziquone 0.1 mg/day for 4 days was administered topically in Vanicream, Plastibase, or dimethyl sulfoxide or intraperitoneally in 50% N,N-dimethylacetamide in 0.9% NaCl to non-treated animals. WBC nadirs are mean values for 4 rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pretreatment</th>
<th>Nadir</th>
<th>Day of nadir</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>13,600 ± 367</td>
<td>9,088 ± 746</td>
<td>12</td>
</tr>
<tr>
<td>Topical treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vanicream</td>
<td>11,590 ± 907</td>
<td>8,332 ± 663</td>
<td>22</td>
</tr>
<tr>
<td>Plastibase</td>
<td>12,915 ± 353</td>
<td>7,209 ± 420</td>
<td>8</td>
</tr>
<tr>
<td>DMSO</td>
<td>6,832 ± 685</td>
<td>6,468 ± 499</td>
<td>10</td>
</tr>
<tr>
<td>DQ in Vanicream</td>
<td>12,417 ± 700</td>
<td>4,458 ± 146</td>
<td>10</td>
</tr>
<tr>
<td>DQ in Plastibase</td>
<td>12,938 ± 718</td>
<td>2,792 ± 165</td>
<td>8</td>
</tr>
<tr>
<td>DQ in DMSO</td>
<td>9,959 ± 364</td>
<td>4,847 ± 82</td>
<td>8</td>
</tr>
<tr>
<td>Intraperitoneal treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMA</td>
<td>10,899 ± 354</td>
<td>8,571 ± 829</td>
<td>10</td>
</tr>
<tr>
<td>DQ in DMA</td>
<td>11,335 ± 544</td>
<td>2,129 ± 267</td>
<td>8</td>
</tr>
</tbody>
</table>

* Mean ± SE.
* P < 0.05.
* P < 0.01 compared to pretreatment values.
* DMSO, dimethyl sulfoxide; DQ, diaziquone; DMA, N,N-dimethylacetamide.

The domestic pig approaches fairly closely the morphological and functional characteristics of human skin and has been used widely in dermatological research (9). In the present study when diaziquone was applied topically to pig skin for 4 consecutive days, the skin looked histologically normal and showed no erythema or inflammation, either during or after diaziquone treatment. This was true for all doses of diaziquone and for all vehicles.

The results of the study show that topical application of diaziquone offers a therapeutic advantage over systemically administered diaziquone for the treatment of intradermal Walker 256 carcinosarcoma. Diaziquone at 0.5 mg/day applied topically produced complete regression of tumor, while the same dose of diaziquone administered intraperitoneally was lethal to rat. Diaziquone at 0.1 mg/day applied topically or administered intraperitoneally produced complete regression of tumor, but topical diaziquone produced a drop in total WBC counts only 60% of that of intraperitoneal diaziquone. Vanicream appears to offer the best therapeutic activity with the least systemic toxicity of the vehicles used for topical application of diaziquone in the study. The consistency of Vanicream also make it the easiest preparation to prepare and apply to the skin. Diaziquone in Vanicream might be a useful combination to use for topical application in patients with localized metastatic or advanced refractory metastatic skin cancer. Human skin is less permeable to many drugs than rat skin (25), but this should not affect the therapeutic advantage of topical application of diaziquone if it is used at a correct dose. Walker 256 carcinosarcoma appears to be a very sensitive tumor to diaziquone, and it remains to be seen whether human metastatic tumors in the skin will respond in the same way. The study has shown, however, that topical application of lipid-soluble anticancer drugs in the appropriate vehicle might be a way to treat some forms of human metastatic skin tumors.

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