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Tissue distribution of transdermal toremifene

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Abstract *Purpose*: Toremifene is an orally administered triphenylethylene derivative with antiestrogenic activity that is primarily used in the treatment of patients with metastatic breast cancer. The purpose of this study was to evaluate the therapeutic advantage of local (transdermal) administration of toremifene in several animal models. Local (subcutaneous and skin) versus systemic concentrations of toremifene were evaluated serially following transdermal application of the drug. With high local concentrations and minimal distribution to other organs via the circulation, topical toremifene may deliver maximal therapeutic effects to local tissue while avoiding the side effects seen with systemic therapy. *Methods*: Three animal models (nude mice, baboons, and a horse) were used to examine topically administered toremifene for kinetic measurements. *Results*: In nude mice implanted subcutaneously with MDA-MB-231 human breast tumors, topical toremifene (2.5 mg/day × 5 days) produced greater than 50-fold higher tumor concentrations compared with intraperitoneal (i.p.) administration (1.0 mg/day × 5 days). Systemic distribution in plasma, uterus, and liver was lower following topical than following i.p. administration. In nude mice inoculated subcutaneously with estrogen receptor-positive (ER +) MCF-7 human breast cancer cells, topical toremifene and 4-hydroxytoremifene (4-OH) prevented tumor growth in the presence of estradiol. In four nontumor-bearing baboons that were given transdermal toremifene, relatively high distribution of drug was noted in normal breast tissue and fat, compared with undetectable serum concentrations. Finally, a new topical formulation of toremifene (a gel preparation for human use, Orion-Farmos, Finland) achieved high local tumor toremifene concentrations in a horse melanoma, with minimal systemic distribution. *Conclusions*: Transdermal toremifene can achieve high local tissue concentrations with minimal systemic distribution.

Key words Toremifene · Breast cancer · Transdermal · Antiestrogen

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Introduction

Toremifene is a triphenylethylene derivative with antiestrogenic activity in breast cancer [7, 22], as well as nonantiestrogenic inhibitory effects on melanocyte cell lines in vitro [17]. Toremifene also has chemosensitizing properties in doxorubicin-resistant cancer cells [1, 4, 16]. There are now accumulating data showing that antiestrogens have cytotoxic activity which may be associated with apoptosis in addition to antiestrogenic cytostatic effects [2, 3, 23].

The main side effects of antiestrogens are related to their partial estrogenic effects, especially on organs with high levels of hormonal receptors such as the uterus. With the long-term use of tamoxifen, the incidence of endometrial cancer increases, and the type of cancer that appears can be aggressive and associated with a poor prognosis [5]. The overall effectiveness of antiestrogens depends upon the balance of the given benefits and side effects. The therapeutic advantage of a drug may be increased by maximizing the efficacy and/or by reducing the side effects. Transdermal administration may fulfill this objective especially in early cancers of the skin and breast, which are accessible.

Materials and Methods

In vitro studies

Cell culture

MCF-7 cells were obtained from the American Type Culture Collection, Rockville, Maryland, and MDA-MB-231 cells were obtained from the late Dr. William McGuire. The cells were grown in improved minimum essential medium (IMEM) supplemented with 10% fetal bovine serum (FBS). Cells were cultured in Corning T-75 flasks and maintained at 37 °C in an atmosphere of 5% CO_2 and 95% air.

Growth inhibitory effects of toremifene on MCF-7 cells

The relationship between toremifene concentration and growth inhibition of estrogen receptor-positive ER + MCF-7 breast cancer cells was studied. The cells were plated in triplicate at 15,000 cells/ml in Corning T-25 flasks. The cells were allowed to attach overnight before exposure to toremifene at 0.1, 0.5, 1.0, 3.3, and 6.6 μ*M*. Toremifene was dissolved in sterile saline and ethanol (final ethanol concentration $< 0.1\%$). Control flasks were spiked with a sterile saline solution containing $< 0.1\%$ ethanol. Following a 6-day continuous exposure, the cells were harvested and counted using a hema-cytometer. During harvest, medium was aspirated, 1 ml 0.25% trypsin was added to each flask, and following detachment, 5 ml fresh IMEM was added to each flask. Cell viability was assessed by trypan blue dye exclusion, and in all cases was $> 95\%$. The inhibition curve was constructed by averaging the counts from each group of three and dividing by the control average.

HPLC analysis of toremifene

Toremifene concentrations in all specimens were quantitated by a high-performance liquid chromatography (HPLC) system as previously described [13]. Briefly, tissue samples were placed in preweighed extraction tubes (16×100 mm) and internal standard (nafoxidine HCl, 200 ng) was added. Samples were homogenized, if necessary, extracted with 6 ml 2% butanol in hexane, vortexed for 1 min, centrifuged for 10 min at 1000 *g*, and the organic layer was evaporated to dryness under nitrogen. Samples were then reconstituted in 200 μl methanol, transferred to an Infrasil quartz cuvette, and irradiated for 1 min with high-intensity ultraviolet light (254 nm). The activated samples were removed from the cuvette and injected onto the HPLC column. The fluorescence of photochemically activated compounds was detected with an Applied Biosystems 980 Fluorescence Detector set at an excitation wavelength of 266 nm. Retention times and peak heights were recorded with a Spectraphysics 4100 integrator. The extraction efficiencies of toremifene and its major metabolites from tissue and cell specimens have been found to be very similar to that of plasma ($> 80\%$) [19]. All standard curves for toremifene, 4-hydroxytoremifene, and *N*-desmethyltoremifene were prepared in 1.0 ml stock human plasma, and were extracted using the procedure described above. The correlation coefficient for each curve was > 0.985. To ensure linearity, standard points were repeated regularly. All samples not immediately analyzed after extraction were stored at -20 °C until used.

Cellular accumulation of toremifene by MCF-7 cells

MCF-7 cells were plated in triplicate at a density of 40,000/ml in Corning T-75 flasks and incubated for 4 days before the application of toremifene at 3.3 μ*M* for 15 min, 30 min, 1 h, 2 h, and 4 h. Cell confluency at the time of drug exposure was 75%. The toremifene solution was prepared as described above. At each time-point, the drug-containing medium was quickly aspirated, and 4 ml ice-cold phosphate-buffered saline (PBS) was added. After the cells were thoroughly washed, the wash solution was aspirated and 2 ml fresh PBS was added. The cells were then removed from each flask using a plastic scraper. The volumes were recorded, and the cells were counted using a hemacytometer. All cells were then extracted for toremifene and assayed by HPLC as described above.

To determine the number of MCF-7 cells per milligram, three Corning T-150 flasks were inoculated with MCF-7 cells and the cells allowed to grow to 75% confluency. The cells were then trypsinized, counted with a hemacytometer, added to individual preweighed centrifuge tubes, and then centrifuged at 2000 *g* for 10 min. All excess fluid was carefully removed by suction, and the tubes were weighed again. The average number of cells per milligram was then calculated.

Mouse studies

Female athymic BALB-C/nu/nu nude mice (Harlan Sprague Dawley, Indianapolis, Ind.) were kept in sterile plastic cages in a temperature-controlled room on a 12-h/12-h light/dark schedule with food and water *ad libitum*. All mouse experiments were carried out at the University of Texas Health Science Center at San Antonio under a protocol approved by the Institutional Animal Care and Use Committee. All mice were sacrificed by cervical dislocation.

Kinetic study

A total of six mice were inoculated with approximately 5 million MDA-MB-231 cells (200 μl) subcutaneously on their right front flank using a 22-gauge needle. Three mice received topical administration of toremifene and the remaining three received intraperitoneal (i.p.) administration. When tumors reached an adequate size (25 mm^2) topical toremifene was administered by micropipette in a mixture of DMSO, ethanol, and normal saline at a dose of 2.5 mg/day for 5 days. Toremifene citrate was suspended in peanut oil for i.p. administration. Mice were injected with toremifene 1.0 mg/day for 5 days i.p. using a 25-gauge needle. All mice were sacrificed on the 5th day of treatment 2 h after the final dose. Blood specimens were collected under diethyl ether anesthesia from axillary vessels by capillary pipette and placed into heparinized centrifuge tubes. Plasma was separated from whole blood and the volume recorded. The plasma volumes recovered ranged from 100 to 500 μl. The whole tumors, brains, livers, and uteri were collected in separate tubes and the weights of each were recorded. Tissue weights ranged from 25 mg (uterus) to 1.5 g (liver). Samples were placed in

extraction tubes and spiked with an internal standard, nafoxidine. The concentrations of toremifene and its metabolites were quantitated in each group by HPLC as described above and then compared.

Topical prevention study

Twelve mice were inoculated subcutaneously with 5 million (injection volume 200 μl) ER + MCF-7 human breast cancer cells on their right flank using a 22-gauge needle and then divided into three groups of four: control, transdermal toremifene, and transdermal 4-OH toremifene. Prior to injection, cells were harvested by scraping, centrifuged at 1000 *g* for 8 min, and concentrated to a density of 5 million/200 μl in culture medium. Each mouse was implanted subcutaneously 24 h prior to inoculation with a 0.25-mg 21-day time-release estradiol pellet (Innovative Research of America, Toledo, Ohio) on the center of its back. The topical solutions were prepared at a concentration of 0.5 mg/20 μl in a mixture of DMSO, ethanol, and sterile normal saline. The mice were treated daily by micropipette for 15 days with 20 μl of the appropriate solution. The control mice received an equal volume of blank solution. Tumor growth was measured during the treatment period to assess the growth preventive effects of topical toremifene and 4-OH toremifene. On day 15 after initiation of treatment, when measurable tumors were detected in the control mice, all mice were sacrificed and then photographed.

Baboon studies

All baboons (*Papio* sp.) were purchased from the Southwest Foundation for Biomedical Research, San Antonio, Texas. During the studies, the baboons were housed in individual metal cages. They were fed commercial monkey chow, fresh fruit daily, water *ad libitum*, and cared for in accordance with the Guide for the Care and Use of Laboratory Animals, National Institutes of Health publication no. 86–23, revised 1985. All procedures were performed under ketamine anesthesia.

Kinetic study

Four adult baboons, two males and two females, were used in this study. The animals were 5–12 years old, and weighed 11.7–21.0 kg. The chest area was shaved, and 500 mg of toremifene base in 4.0 ml pure DMSO was applied to an area of 36 cm^2 involving both mamillas. The administration took 90–120 min. When the treatment areas dried satisfactorily, the breast skin was thoroughly cleansed with alcohol and Betadine. A 10-ml blood specimen from a femoral vein was taken 15 min posttreatment along with 6-mm punch biopsies from six sites: (1) from skin near the nipple at the 6 o'clock position; (2) from subcutaneous tissue under the first biopsy site; (3) from peripheral breast skin at the 5 o'clock position; (4) from subcutaneous tissue under the third biopsy site; (5) from peripheral skin at the 11 o'clock position; and (6) from subcutaneous tissue under the fifth biopsy site. An additional 10-ml blood specimen was collected 60 min after discontinuation of therapy. Biopsies were repeated at 24 h and then 7 days after topical application. All biopsies were collected in preweighed tubes and immediately extracted as described above. All tissues collected averaged 25 mg in weight. Toremifene concentrations in these specimens were analyzed by HPLC and compared. A 1-ml aliquot of each plasma specimen was used for the analysis.

Pharmacokinetic study in a baboon with lymphoma: a case report

Topical toremifene therapy of a 15-year-old, 25-kg, female baboon with a spontaneous non-Hodgkin's lymphoma was evaluated. She had no significant health problems until an inguinal lymphadenopathy was detected. Enlarged lymph nodes with diameters of 1–2 cm were evident in both groins. The enlarged node in the left groin was biopsied for histology. Microscopically, there was neoplastic lymphoid cell proliferation in the medullary cords with extension into adjacent paracortical areas. The cells localized along medullary cords and tended to expand into adjacent sinuses. The cells were moderate to large, vesicular with irregular nuclei and were numerous. Unstained sections were evaluated immunohistochemically using the avidin-biotin-peroxidase complex method [14]. The lymphoid neoplasm was found to be a diffuse, large-cell non-Hodgkin's lymphoma.

ER and progesterone receptors (PgR) were measured in tumor tissue with an immunoperoxidase staining technique using the H226 antibody for ER and the B-39 antibody for PgR (both provided by Dr. C. Green, University of Chicago, Chicago, Ill.), and a standard streptavidin-biotin-peroxidase detection system [8, 11, 12].

The hemoglobin concentration in the blood was 13.5 g/dl, WBC count was 5.4×10^3, and the platelet count was 411×10^3. The blood chemistry was normal. Chest computed tomography showed consolidation or atelectasis along the periphery of the right lung, possibly due to animal positioning and prominent pulmonary arteries; no other significant abnormality was found.

Initially, 400 mg toremifene dissolved in DMSO and methyl cellulose was administered topically to the right groin tumor for 2 days, followed by 200 mg topically for 3 days. The sizes of both the right and left lymphomas were measured daily up to day 9. On day 9, the right inguinal tumor was removed for histological examination along with three peritumoral fat specimens. One blood specimen was also collected. Over an 18-day period (days 14, 16, 17, 18, 22, 24, 25, 28, 29, 30, 31, 32, 33), 11 1-g doses of toremifene were then given orally. Corresponding blood specimens were also collected. On days 22, 24, 28, 29, 30, 31, 32, and 33, six 200-mg doses of topical toremifene were applied in addition to the oral dosing. On day 25, the left inguinal tumor was removed for histological examination along with three peritumoral fat specimens. Afterward, the newly regrown right inguinal lymphoma received topical treatment on the days oral therapy was given. On day 36, the remainder of the right inguinal tumor was removed together with three peritumoral fat specimens. Tissue for light microscopic examination was preserved in neutral buffered 10% formalin, processed, cut, and stained conventionally with hematoxylin and eosin. Lymph nodes were taken for cell culture and frozen in liquid nitrogen for possible future immunocytochemical evaluation.

Horse study

A male appaloosa horse diagnosed with malignant melanoma was housed by the University of California Veterinary Medical Teaching Hospital, Davis, California. The horse had multiple subcutaneous lesions of the head, shoulder, hip, anus, and tail. A lesion on the left temple measuring approximately 25 cm^2 was selected for transdermal application of a new gel preparation of toremifene (Orion-Farmos). The horse was sedated with xylazine, and the lesion was shaved and scrubbed with Betadine. On day 1, after collecting a 2-mm pretreatment punch biopsy, 5 g of the 1% gel (equal to 50 mg pure toremifene citrate) was applied to the entire shaved surface of the lesion, avoiding the initial biopsy site. The horse was restrained for 30 min to allow the gel to penetrate and was then returned to its stall. Additional biopsies of the same lesion were collected for HPLC analysis 24 and 48 h following the initial treatment. On days 3–7, 5 g of transdermal gel were applied at the same time each day, again avoiding biopsy sites. The fourth and final biopsy was taken 24 h following the sixth dose. The application area

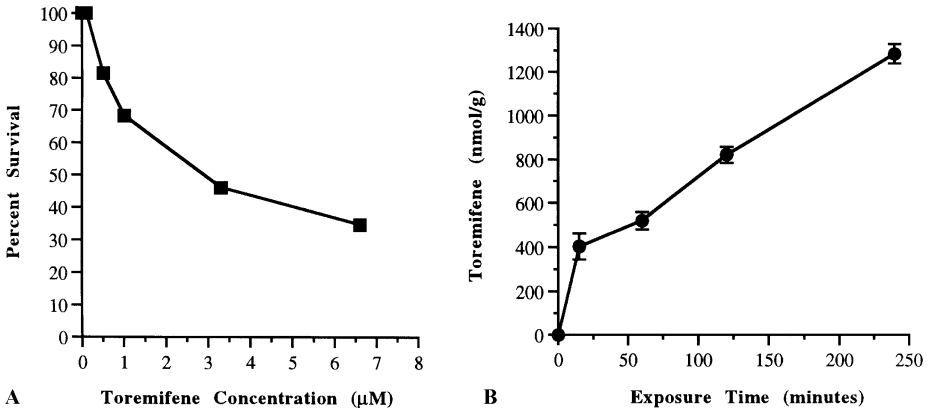

Fig. 1 **A** In vitro growth inhibition of MCF-7 human breast cancer cells by toremifene following a 6-day continuous exposure. Percent survival expressed as percent of control. **B** Cellular accumulation of toremifene in MCF-7 human breast cancer cells following 15 min, 30 min, 1 h, 2 h, and 4 h of exposure. Toremifene concentration was fixed at 3.3 μ*M*

was thoroughly cleansed and dried prior to all biopsy collections. All biopsied materials were immediately weighed after collection and extracted for toremifene as described above. Biopsy weights averaged 25 mg.

Results

In vitro studies

Growth inhibitory effects and cellular accumulation of toremifene in MCF-7 cells

The MCF-7 growth inhibition curve and the time-dependent toremifene accumulation curve are shown in Fig. 1. The growth inhibition curve showed a clear dose-response relationship between cell growth and increasing toremifene concentrations. The IC_{50} was calculated to be 2.75 μ*M*. The percent survival is expressed as percent of control. The toremifene accumulation curve showed a steady increase in cellular accumulation up to 4 h exposure time to a toremifene concentration of 3.3 μ*M*. Units are nanomoles of toremifene per gram of cells. In a preliminary study, we found that a 4-h exposure to an inhibitory concentration of toremifene (1.0 μ*M*) resulted in a cellular accumulation of 236.45 nmol/g in vitro (data not shown). The number of MCF-7 cells was calculated to be 1.83×10^5/mg.

Mouse studies

Kinetic study

Toremifene concentrations following topical and i.p. treatment are shown in Table 1. Table 1 shows that the topically treated mice had tumors with much greater concentrations of toremifene while having lower serum concentrations at 2.5 times the dose of i.p.-administered toremifene. Mean intratumoral toremifene concentrations were 2678.2 nmol/g in the topically treated group and 17.4 nmol/g in the i.p.-treated group.

Table 1 Toremifene concentrations (mean and range) following i.p. (1 mg/day × 5 days, $n = 3$) and topical (2.5 mg/day × 5 days, $n = 3$) treatment in tumor-bearing nude mice (units are nmol/g except the serum values which are nmol/ml)

Tissue	i.p.	Topical	Topical/i.p.[a]
Tumor	17.4 (8.7–29.2)	2678.2 (57.9–4738.9)	154
Serum	10.8 (0.5–31.5)	2.5 (1.1–4.5)	0.2
Brain	4.6 (2.9–5.4)	3.6 (3.2–4.0)	0.8
Uterus	73.8 (65.2–88.4)	7.2 (5.7–8.7)	0.1
Liver	35.1 (27.6–45.8)	10.4 (8.5–13.8)	0.3

[a] Ratios not corrected for dose

Drug concentrations in blood and various organs achieved by transdermal and i.p. administration were markedly different. Interestingly, the mice receiving topical toremifene had one-tenth the concentrations in the uterus compared to those receiving i.p. toremifene. In the liver, the topically treated mice showed lower toremifene concentrations, suggesting less systemic distribution. The toremifene concentrations in the brain tissue were equivalent.

Topical prevention study

In the prevention study, all four control mice developed tumors (> 48 mm^2). Only one mouse in the 4-OH toremifene group developed a small tumor (< 2 mm^2). A representative photograph of six mice is shown in Fig. 2. Arrows indicate either tumor or inoculation sites. These preliminary mouse experiments demonstrate that adjuvant doses of topically administered toremifene can prevent breast cancer tumor growth in an in vivo mouse model.

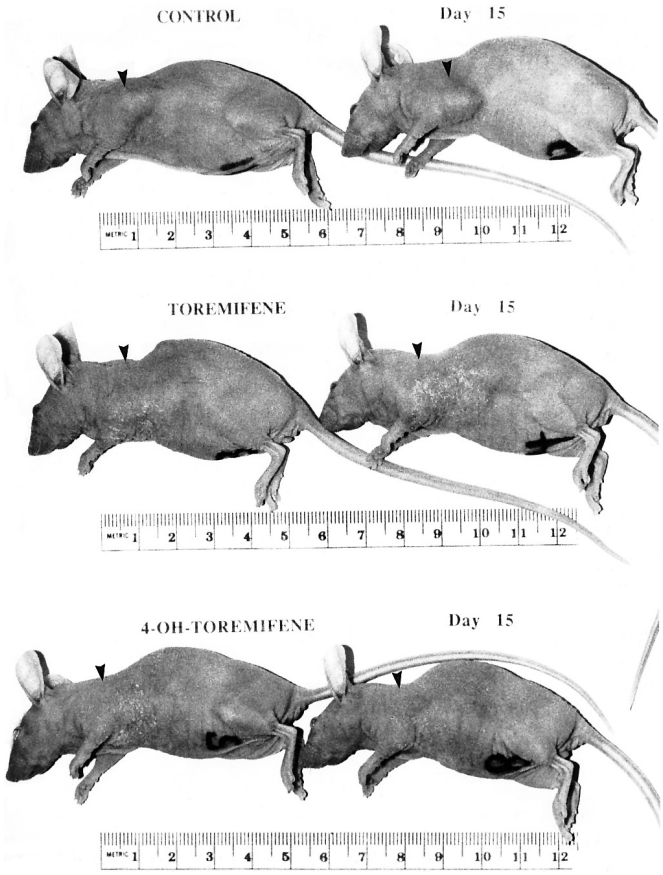

Fig. 2 Photograph of two mice in the control (*top*), toremifene (*center*), and 4-hydroxytoremifene (*bottom*) groups clearly shows that topical administration of toremifene or 4-OH toremifene prevented tumor growth in the presence of estradiol. *Arrows* indicate either tumor or inoculation site following 15 days of therapy

Baboon studies

Baboon pharmacokinetic study

Concentrations of toremifene from breast skin and underlying subcutaneous tissues were much higher than the plasma levels at 30 min, 24 h, and 7 days following topical application (Table 2). In the areas directly underneath the application area, both in skin and fat, toremifene concentrations were significantly and consistently higher than in plasma – well above the known therapeutic level of 5 μM. Drug levels of 20.4 nmol/g in skin and 2.4 nmol/g in fat were still detectable on day 7 after the application, whereas plasma levels were no longer detectable. Similar to the biopsy data from the treated areas, the drug levels in surrounding tissues without direct contact with the drug were maintained at considerably high concentrations. These toremifene levels were 2.3 nmol/g in skin and 1.3 nmol/g in fat on day 7, with no detectable toremifene in plasma. This suggests that toremifene diffuses regionally in dimethylsulfoxide. The difference was persistent from the time immediately after the application through day 7.

Pharmacokinetic study in a baboon with lymphoma

The treatment of a regional lymphoma in a female baboon showed that topical toremifene can produce an in vivo antitumor response. This baboon offered a unique opportunity because of the two distinct tumor sites. Although we fully realize that topical toremifene can diffuse regionally outside the treatment area (see above), we elected to treat primarily one tumor site with topical toremifene and use the second tumor as a control.

The treated lymphoma in the right groin had an initial size of 1462 mm^2. The left inguinal lymphoma had a size of 280 mm^2. The response to therapy is given in Fig. 3. A clear response was observed in the treated right inguinal lymphoma whereas the untreated left inguinal lymphoma (Fig. 4) increased in size during the topical treatment period. The histologic examination of the right inguinal lymph node following topical therapy showed a marked reduction in neoplastic lymphoid cells, and a marked increase in reticuloendothelial cells in the medullary sinuses. Pockets of mature plasma cells were recognized in the medullary region of the lymph node. The lymphoma was negative for both ER and PgR by immunohistochemistry.

Table 2 Toremifene concentrations (mean and range) in skin, subcutaneous fat, and plasma of four baboons following topical administration of 500 mg toremifene in DMSO

Time	Treatment area		Plasma (nmol/ml)	Control area	
	Skin (nmol/g)	Fat (nmol/g)		Skin (nmol/g)	Fat (nmol/g)
30 min	1908.7 (574.4–3290.7)	262.3 (87.1–559.3)	0.03[a]	52.9[a]	39.1[a]
24 h	177.1 (30.8–355.6)	20.6 (1.5–39.3)	0.006 (0–0.01)	68.1 (7.1–184.5)	14.4 (5.8–22.9)
1 week	20.4 (4.4–36.9)	2.4 (1.6–3.2)	0	2.3 (0.3–3.3)	1.3 (0.3–2.2)

[a] Based on one animal

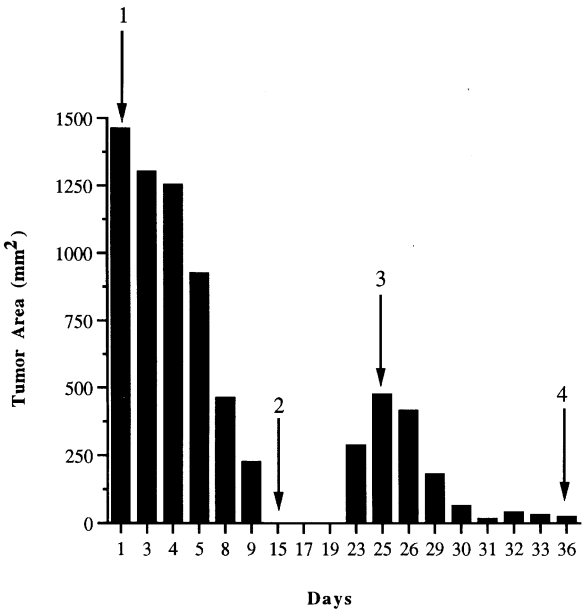

Fig. 3 The response of the right inguinal lymphoma in a baboon to toremifene therapy. *Arrows*: *1* start of topical treatment; *2* start of systemic treatment; *3* start of combined treatment; and *4* end of therapy

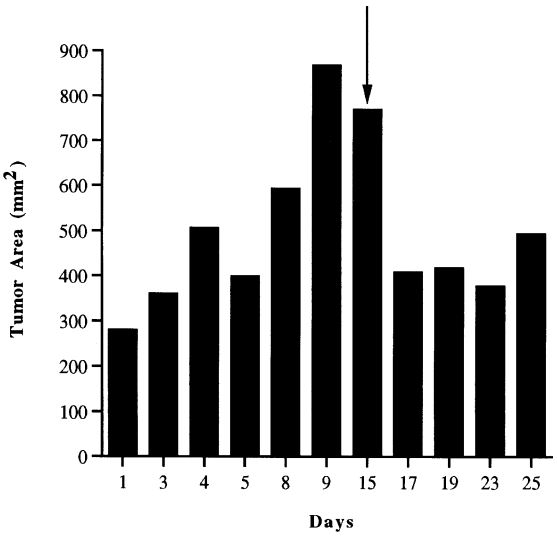

Fig. 4 The response of the left inguinal lymphoma in a baboon to no therapy and to systemic toremifene therapy. *Arrow* indicates the beginning of systemic toremifene therapy

During oral treatment, the right inguinal lymphoma began to regrow (Fig. 3). Consequently, topical toremifene therapy was reinstituted on this tumor. Similarly to the initial response to topical therapy, the lymphoma regressed from a tumor area of 476 mm^2 to 24 mm^2. The residual tumor was removed for histologic examination. Microscopically, the lymph node was atrophic with a markedly thinned cortex, minimal prominence of germinal centers and an apparent reduction in lymphocytes throughout the lymph node.

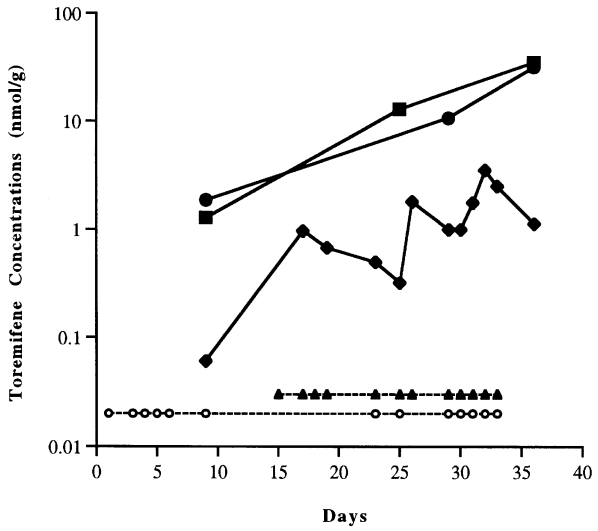

Fig. 5 Toremifene concentrations in tumor, plasma, and fat in a baboon with lymphoma following topical, oral, and combination therapy. *Solid squares* represent tumor concentrations, *solid diamonds* represent plasma concentrations (nmol/ml), *solid circles* represent fat concentrations, *solid triangles* represent oral treatment days, and *open circles* represent topical treatment days

Islands of mature plasma cells were still evident and the reticuloendothelial cell population was disproportionately prominent.

The results of several measurements of toremifene concentrations in the tumors, fat, and plasma are shown in Fig. 5. Although no definite conclusions can be made regarding tumor concentrations and antitumor response, it is clear that topical administration produced an effective antitumor toremifene concentration with minimal systemic distribution.

The baboon's weight, blood chemistry, and hematologic parameters remained stable during therapy, reflecting the atoxicity of toremifene. A slight local rash caused by the topical treatment was the only side effect noted. Physical examination of the animal showed no evidence of disease 5 months after discontinuation of therapy. She was still alive and well 4 years later. Hematologic and blood chemistry parameters were all normal. A control chest radiograph taken 1 week after discontinuation of treatment was negative.

Horse Study

Kinetic data from the horse melanoma showed that the tumor toremifene concentrations reached 16.50 nmol/g 24 h following the first dose, and 45.07 nmol/g 24 h after the sixth and final topically administered dose. The tissue elimination half-life was calculated to be 38.9 h. Interestingly, the peak concentration was higher than the concentration which caused a response in the baboon lymphoma (35.34 nmol/g). This concentration

is 40 times higher than those seen following adjuvant oral tamoxifen therapy in humans. Tamoxifen has been reported to result in concentrations in the order of 1.35 nmol/g in breast tumors [15]. A slight reduction in tumor volume was noted, although no histologic verification of response was made. This clearly shows that transdermal delivery of toremifene achieves significant local concentrations of drug that are more than adequate for antitumor activity.

Discussion

Transdermal administration of a variety of agents has proven to be an effective and safe mode of delivery [6, 9, 20, 21]. In the case of transdermal estradiol, clonidine, and nitroglycerin, the goal of transdermal administration is an even systemic distribution, while that of scopolamine is a more regional effect on the vestibular system. The basic idea in the transdermal application of toremifene is to achieve local concentrations of drug sufficient to inhibit tumor cell growth, with limited systemic exposure. The target concentrations in breast tissues and breast tumors during transdermal toremifene therapy should be comparable to those seen following effective oral antiestrogen dosing. Ideally, these local tumor toremifene concentrations should far exceed those seen systemically. The potential indications of transdermal toremifene include the prevention of contralateral breast cancer postmastectomy, adjuvant therapy following conservative breast cancer surgery in ER + -disease, prevention of breast cancer in dysplastic breast lesions, premenstrual mastalgia, and adjuvant treatment after local excision of malignant melanoma in high-risk patients (Clark, ≥ 2, lesions in the so-called BANS-areas).

Johnston et al. conducted a study investigating the intratumoral concentrations of tamoxifen following oral therapy of 20 mg/day in patients with *de novo* and acquired tamoxifen resistance. They reported concentrations of 1.78 nmol/g and 1.16 nmol/g in the *de novo* and acquired resistance patients, respectively. Mean serum concentrations were the same, with tumor/serum ratios of 4.3 in *de novo* resistance patients and 2.0 in acquired resistance patients [15]. In our studies, the peak toremifene concentrations in tumors following short-course transdermal therapy in the horse and baboon were significantly higher, with minimal distribution to plasma. Clearly, transdermal administration of toremifene has a distinct pharmacologic advantage over systemic therapy because it can achieve locally elevated concentrations with relatively low systemic distribution.

Pharmacokinetic data of transdermal *trans*-4-OH tamoxifen have been presented by Mauvais-Jarvis et al. [18]. In their study, radioactive tamoxifen and active metabolites of tamoxifen were administered percutaneously to the breasts of nine patients before resection. Drug concentrations were examined in the breast tissue and compared to those in blood and urine after surgery performed at different intervals – at 12, 24, 48, 72 and 96 h (or on day 7). *Trans*-4-OH tamoxifen is found predominantly concentrated in breast tissue and remains unmetabolized. It remains in breast tissue for a longer period than tamoxifen, and appears in blood and urine very slowly. This pattern changes when the compounds are administered to the abdominal wall where there are no hormone receptors. *Trans*-4-OH tamoxifen appears in blood and urine quickly. Mauvais-Jarvis et al. did not report the concentration of *trans*-4-OH tamoxifen in fat.

In our study with transdermal toremifene, the highest concentrations of drug were in the tumor and peritumoral adipose tissue. A rapid clearance of *trans*-4-OH tamoxifen noted in local tissue following administration to the abdominal wall suggests that 4-OH tamoxifen or toremifene may reside in adipose tissue, acting as a depot form of the drug. Once the parent drug or its 4-OH metabolite is made available to the systemic circulation, it is cleared rapidly.

From the data obtained from our studies, it is apparent that by administering toremifene transdermally, the distribution of drug to local tissues such as breast and tumor is much higher than the distribution to other organs via the circulation. When toremifene is administered locally, it is bound to fat nearby, then diffuses slowly, and its level is almost undetectable in blood after a few days when it is still present at high concentrations in local tissue. It can be assumed from our studies that transdermal application of toremifene vastly improves the therapeutic index of the drug. High local concentrations of drug may improve efficacy and could potentially avoid acquired resistance and high systemic exposure to the drug. Reduced systemic exposure may be particularly beneficial in light of the mounting evidence that antiestrogens have adverse effects on endometrial tissues. Endometrial cancer is especially known to be an increased risk with long-term use of tamoxifen [10]. Combining local administration of toremifene with systemic therapy in cases of melanoma and non-Hodgkin's lymphoma may improve the local effectiveness and control without causing increased risk of side effects. Topical toremifene may also be a preferred alternative to oral antiestrogens in prevention of breast cancer, with minimal toxic and carcinogenic effects, should the use of antiestrogens be found beneficial in the prevention of breast cancer.

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