Anti-Cancer Drugs 1998, 9, pp. 181–189

Research paper

Intratumoral toremifene therapy and tissue distribution in the baboon

Gregory T Wurz, Juhani U Mäenpää,[1] Gene B Hubbard,[2] Timothy B Cadman, Robert C Seymour,[3] Lin Soe and Michael W DeGregorio

Department of Internal Medicine, Division of Hematology/Oncology, Cancer Center, University of California, Davis, 4501 X Street Room 3016, Sacramento, CA 95817, USA. Tel: (+1) 916 734-2360; Fax: (+1) 916 734-2374. [1]Department of Obstetrics and Gynecology, University Hospital, PO Box 2000, 33521, Tampere, Finland. [2]Department of Genetics and Laboratory Animal Medicine, Southwest Foundation for Biomedical Research, PO Box 28147, San Antonio, TX 78228-0147, USA. [3]Department of Medicine, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78284, USA.

The purpose of the present study was to evaluate the tissue distribution of toremifene (TOR) in baboons following intratissue injections and to examine the effectiveness of intratumoral TOR therapy of baboons with various spontaneous neoplasms. Five healthy baboons (*Papio* sp.) were used to examine the distribution of TOR following intratissue injections. Twenty-three different tissue specimens were collected for HPLC analysis. In addition, four baboons with various spontaneous neoplasms (myxoma, squamous cell carcinoma, lymphosarcoma and adenocarcinoma) were treated with intratumoral TOR and their responses were evaluated. Tissue TOR distribution was also examined in these animals. In the tissue distribution study, target tissue/serum TOR concentration ratios ranged from 138 to 8873 and the target tissue/other tissue ratios ranged from 1.2 to 2428. The distribution of TOR was very favorable, with the highest concentrations outside the injection sites noted in adjacent organs. A marked response was observed in the myxoma and partial responses were observed in the other three cases. Drug level analysis data from these four animals revealed tissue concentrations similar to those seen in the TOR tissue distribution study. Intratumoral administration of TOR can achieve effective local tumor and tissue concentrations, while systemic distribution via circulation to other organs is limited. [© 1998 Rapid Science Ltd.]

Key words: Antiestrogen, baboon, intratumoral, toremifene.

Introduction

Toremifene (TOR) is a non-steroidal triphenylethylene which has demonstrated antiestrogenic activity in breast and endometrial cancer,[1–3] and it is currently approved for first-line treatment in patients with estrogen receptor (ER)-positive metastatic breast cancer. At lower concentrations (1.0 μM or below), TOR acts as an antiestrogen, much like tamoxifen, by preventing the binding of estrogen to its receptors. At higher concentrations (6 μM or above), TOR has non-specific, hormone-independent cytotoxic antitumor effects which are not associated with its antiestrogenic properties.[4,5] In addition, these high concentrations have been shown to have chemosensitizing activity in multidrug-resistant cells also apparently unrelated to TOR's antiestrogenic effects.[6,7] TOR is well tolerated at relatively high doses in humans with minimal toxicity.[4]

Most side effects associated with the use of triphenylethylenes (e.g. hot flushes) derive from their partial estrogenic effects, especially in organs containing high levels of hormonal receptors such as the uterus. The incidence of endometrial cancer has been shown to increase with the long-term use of tamoxifen, and the cancers which appear can be aggressive and associated with a poor prognosis.[8] The overall effectiveness of antiestrogens depends upon the balance of the given benefits and side effects. A drug's therapeutic advantage may be increased by maximizing its efficacy and/or by reducing its side effects. For example, the topical application of TOR has achieved concentrations *in vivo* far in excess of those found to

This work was partially supported by grants from the Orion Corp. Pharma, Finland, and Tess Diagnostics and Pharmaceuticals, Inc., Blanco, TX.

Correspondence to M DeGregorio

inhibit melanocyte cell lines *in vitro*, with minimal systemic distribution.[9]

The basis for the development of a regional (intratumoral) therapy is the achievement of effective target tissue concentrations while minimizing systemic distribution and therefore toxicity. Recently, we reported on the distribution of TOR in the tissues of baboons following transdermal application,[10] another type of regional therapy. Examples of existing, clinically used, regional chemotherapies include intra-arterial infusion for liver and kidney neoplasms, limb perfusions for melanoma and sarcomas, intrathecal administration for CNS neoplasms, and i.p. administration for intra-abdominal neoplasms.[11–15] More recently, a direct intratumoral injection of pure ethanol for primary hepatomas has been developed.[16–19] The reported response rates of primary hepatomas have been 60–90%.[17–19] In addition, solitary liver metastases seem to respond well.[16] In general, this mode of treatment has been reasonably well tolerated with, to our knowledge, only one treatment-related death.[20]

One major drawback associated with many cytotoxic chemotherapeutic agents is the fact that they are strong vesicants[21] and thus they are not ideal candidates for intratumoral administration. However, the cytostatic agents such as the triphenylethylenes (e.g. TOR) lack significant vesicant properties and hence are good candidates for this type of therapy. Intratumoral administration of TOR is another potential method of achieving high local concentrations of drug with minimal distribution to other tissues, thereby limiting the systemic side effects.

Materials and methods

Animals

All baboons were purchased from the Southwest Foundation for Biomedical Research (San Antonio, TX). 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.

Drugs

TOR was supplied by Orion Corp. Pharma (Turku, Finland) in the form of TOR citrate (molecular weight 598.14).

Toremifene tissue distribution study

Five healthy adult baboons (*Papio* sp.), three males (nos 8839, 9042 and 8677) and two females (nos 8835 and 6760), were used in this study. The subject animals were of various ages and weights. Toremifene citrate was dissolved in pure dimethylsulfoxide (DMSO; Fisher Scientific, Pittsburgh, PA) at an equivalent concentration of 150 mg TOR free base per milliliter prior to each injection. Three animals were injected in the right kidney (nos 8839, 8677 and 6760) and two intra-hepatically (nos 8835 and 9042) at a dose of 43 mg/kg using a 10 ml syringe fitted with a 1.5 inch 20-gauge needle. All injections were performed under anesthesia and were ultrasound-directed. One 10 ml blood specimen was collected for baseline drug level analysis from each animal in a heparinized tube prior to injection. Twenty-four hours after treatment, the animals were anesthetized again, 10 ml blood specimens were drawn and the baboons were then euthanized by i.v. administration of a lethal dose of phenobarbital. Immediately after euthanasia, the animals were necropsied, and 23 different tissue specimens were collected for analysis of TOR and its major metabolites by HPLC as described below. All tissue samples were collected in pre-weighed polypropylene extraction tubes and immediately extracted for TOR as described below. The following tissues were collected: left inguinal node; ovaries; axillary node; adrenal gland; liver; left kidney; right kidney; pancreas; spleen; muscle; sciatic nerve; lung; heart; mesentary node; paraaortic; fat; nipple; bone marrow; uterus; cerebellum; cerebrum; and eye.

HPLC analysis of TOR

TOR concentrations in the above specimens were quantitated by a HPLC system as previously described.[22] Briefly, tissue samples were placed in pre-weighed extraction tubes (16 × 100 mm) and internal standard (nafoxidine HCl, 200 ng; Sigma, St Louis, MO) was added. Samples were homogenized, extracted with 6 ml of 2% butanol in hexane, vortexed for 1 min, centrifuged for 10 min at 1000 *g* and the organic layer was evaporated to dryness at 37 C under a gentle stream of nitrogen. Samples were then reconstituted in 200 µl methanol, transferred to an Infrasil quartz cuvette and irradiated for 1 min with high-intensity UV 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 Programmable Fluorescence Detector

set at an excitation wavelength of 266 nm. Retention times and peak heights were recorded with a Spectraphysics 4100 integrator. Standard curves were prepared for TOR, 4-hydroxytoremifene and *N*-desmethyltoremifene. The correlation coefficient for each curve was above 0.985.

The case reports

Four adult baboons (*Papio* sp.), consisting of three females (nos 1C0071, 1X0229 and 9587) and one male (no. 7349), with spontaneous neoplasms were administered intratumoral TOR, and tumor responses and toxicity were evaluated. The treated tumors included a myxoma (no. 1C0071), a squamous cell carcinoma (no. 9587), a lymphosarcoma of the liver (no. 7349), and an adenocarcinoma of the parotid salivary gland (no. 1X0229). All TOR doses described below represent the equivalent amount of TOR free base delivered. All procedures described below were performed under anesthesia.

Myxoma

Intratumoral therapy of an 18-year-old, 17 kg female baboon (1C0071) with a spontaneous myxoma was evaluated. This animal was presented with a large, white, highly vascular glistening mass at the base of its tail. The mass was biopsied for histology. The surgical biopsy was white with minimal lobulation and was attached to the skin. Microscopically, the mass was composed of anaplastic spindle to stellate-shaped cells with a massive fibrillar and non-staining parenchyma. These neoplastic cells were morphologically consistent with myxoid cells. Collections of mixed inflammatory cells and neovascularization were evident in the tumor. Unstained sections were evaluated immunohistochemically using the avidin-biotin-peroxidase complex method.[23] The neoplasm was diagnosed as a myxoma. All tissues for light microscopic examination were preserved in 10% neutral buffered formalin, processed conventionally, embedded in paraffin, cut at 5 μm and stained with hematoxylin & eosin.

ER and progesterone receptors (PgR) were measured in tumor tissue with an immunoperoxidase staining technique using the H226 antibody for ER and the B39 antibody for PgR (both provided by Dr C Green, University of Chicago, Chicago, IL), and a standard streptavidin-biotin-peroxidase detection system.[24-26]

The myxoma baboon 1C0071 was administered TOR by intratumoral injection in three separate treatment periods. The tumor was measured using a vernier caliper prior to each treatment. During period 1, she was injected with 1000 mg TOR suspended in approximately 20 ml peanut oil over a period of 1 h, followed by an additional 1000 mg dissolved in 4 ml pure DMSO 19 days later. Peanut oil is a commonly used vehicle in the delivery of drugs in rat and mouse models. A 10 ml blood specimen was collected 24 h after the second dose. Injections were performed as described in the distribution study. The initial injection of TOR in peanut oil resulted in excessive irritation of the treatment area. At this point it was decided that DMSO, in which TOR is highly soluble, and which would allow the injection of a smaller volume, should be used. The tumor was resected 44 days later and analyzed by HPLC as described above.

One month later, following a recurrence of the tumor, treatment period 2 was begun. The baboon was administered a total of 6 g TOR (1000 mg/week) by intratumoral injection over a 5 week interval. A 10 ml blood specimen for HPLC drug level analysis was collected in a heparinized tube prior to each injection. Each dose was dissolved in 4 ml pure DMSO and injected as described in the tissue distribution study.

Treatment period 3 covered a 5 month interval after the end of period 2. During this period we decided to institute oral therapy. Twenty-one days following the end of treatment period 2, the animal was administered four 1000 mg doses of TOR suspended in normal saline via a nasal-gastric tube over a 3 week period. She received no treatment for the following month. A 10 ml blood specimen was collected 24 h after the first oral dose. Seventy days after the end of period 2, 1 month following the last oral dose, the tumor recurred. She was then administered four 500 mg doses of TOR in DMSO by intratumoral injection over a 3 week period. Two months later, following another recurrence, the tumor was again resected.

Squamous cell carcinoma

A 3-year-old, 9.4 kg female baboon (9587) was found to have abnormal gingival growth. Radiographs revealed bony proliferation under soft tissue swelling. The tumor was massive and extended from an ulcerated area on the gingiva into subepithelial collagenous connective tissue and may have penetrated into the maxilla. A biopsy was taken for histologic examination. Microscopically, the neoplasm was comprised of variable shaped eosinophilic epithelial cells with intercellular bridges. The nuclei were often multiple or multilobulated. Mitotic figures were

common. Occasional large single nucleoli were seen. The neoplasm formed keratin rings. The animal was diagnosed with squamous cell carcinoma of the gingiva.

This animal was administered five doses of TOR by intratumoral injection over a 35 day period. The drug was prepared in pure DMSO at 150 mg/ml. It was administered once a week at the following doses (in order of administration): 1000, 330, 500, 500 and 200 mg. Due to necrosis of the treatment area following the initial dose, consistent subsequent doses of drug could not be delivered. The doses were divided as necessary in order to deliver the entire volume. One 10 ml blood specimen was collected for HPLC drug level analysis prior to each dosing. Because the tumor was diffuse and difficult to measure, magnetic resonance imaging (MRI) was utilized to assess tumor response. One MRI was performed midway through the treatment period. Additional biopsies were collected for histological examination following the third and final doses. The animal died 24 h following the last dose and was necropsied. The tissues listed above were collected for drug level analysis by HPLC.

Hepatic lymphosarcoma

Baboon 7349 was an 8-year-old, 22.5 kg male which was found to have an elevated white blood cell count after a routine health check. The animal did not respond to antibiotic therapy, but was eating well and was alert and active. An ultrasound scan of his abdomen revealed hypoechoic areas throughout the liver. A biopsy was taken. Microscopically, there was hypercellularity in portal triads in occasionally scattered areas of the parenchyma. These areas were comprised primarily of neoplastic lymphocytes and occasional macrophages. Numerous eosinophils were scattered throughout these foci. Mitotic figures were rare. A preliminary diagnosis of lymphosarcoma (malignant lymphoma) was made and later confirmed by immunohistochemical staining. Another biopsy taken from a lymph node also revealed changes morphologically consistent with lymphosarcoma.

This baboon was administered TOR by intratumoral injection, with the aid of ultrasound, four times over an interval of 67 days. The drug was again prepared in pure DMSO at 150 mg/ml. The animal was given doses of 1000, 500, 500 and 1000 mg, respectively. One 10 ml blood specimen was collected in a heparinized tube for drug level analysis prior to each treatment. During therapy, another biopsy of the liver was collected for histologic examination. The final dose represented 43 mg/kg and was administered after a 5 week washout period so that this animal could be used for distribution studies. Tumor measurements were made by ultrasound. This baboon was euthanized 24 h following the final dose. A necropsy was performed, and the tissues listed above were collected for histologic examination and for drug level analysis by HPLC.

Parotid salivary gland adenocarcinoma

Baboon 1X0229 was an 18-year-old, 14.5 kg adult female. A nodular, well-circumscribed, firm mass extending below the ear was found on her left cheek. A biopsy was taken for histologic examination. Microscopically, the tissue was morphologically consistent with an adenocarcinoma of the parotid salivary gland. The cells were formed into small, epithelial-lined ducts and glands that were often double-layered and contained mucus. Some of the glandular areas were completely filled by irregular vacuolated glandular epithelium. The small, more basophilic cells that formed the ducts were invasive of local connective tissue. There was essentially no inflammation or necrosis associated with this tumor.

Intratumoral TOR therapy was instituted 3 days after the diagnosis. This animal received nine doses over a 76-day interval. The drug was again dissolved in pure DMSO at 150 mg/ml. The first eight doses (1000, 1000 and six 500 mg doses) were administered once a week over 7 weeks. The final dose of 500 mg (43 mg/kg) was administered after a 1 month delay so that this baboon could be used for distribution studies. The tumor was measured periodically by vernier caliper. Blood specimens (10 ml) were collected for HPLC analysis prior to each drug treatment. This animal was euthanized 2 weeks following the final dose. During necropsy, the tissues listed above were collected for drug level determination and histologic examination.

Results

TOR tissue distribution study

Table 1 shows the distribution of TOR in six of the 23 different tissues collected from the five baboons, 24 h following a single intra-tissue injection of 43 mg/kg TOR in DMSO. Male and female animals were included in this study because TOR's cytotoxic effects are hormone independent. Generally, the distribution of the drug was found to be very favorable. The highest concentrations outside the target area were noted in

Table 1. Tissue distribution of toremifene in selected tissues from baboons 24 h following a single intra-tissue injection of 43 mg/kg toremifene in DMSO

Animal	Dose (mg)	Injection site	TOR tissue concentrations (μg/g)					
			Liver	Right kidney	Spleen	Brain	Uterus	Serum[a]
8835 (female)	576	liver	266.2	3.4	13.9	3.6	2.3	0.03
8839 (male)	434	right kidney[b]	7.7	291.3	130.1	7.9	–	0.28
9042 (male)	512	liver	71.9	9.1	9.1	12.9	–	0.52
8677 (male)[c]	735	right kidney	74.7	200.9	453.2	59.0	–	22.3
6760 (female)	813	adrenal[d]	10.6	18.6	8.1	6.0	1.1	0.39

[a]Concentration in μg/ml.
[b]Adrenal concentration was 769.0 μg/g.
[c]Animal died 5 h after dosing.
[d]Kidney was original target; adrenal concentration was 969.9 μg/g.

the adjacent tissues. The target tissue/serum concentration ratios ranged from 138 to 8873 and the target tissue/other tissue concentration ratios ranged from 1.2 to 2428 (excluding baboon 8677, which died 5 h after injection). The distribution of TOR in the tissues not included in Tables 1 and 3 was similar to the distribution in the included non-treated tissues. The histopathology reports showed that if the injection was technically successful, the area of tissue destruction was limited to the surroundings of the injection site, while preserving the remainder of the organ as well as the other organs.

Despite the aid of ultrasound, the renal injections were technically difficult. The injection of baboon 6760 was mistargeted to the adrenal gland, as reflected in the adrenal TOR concentration of 969.9 μg/g. A higher adrenal gland concentration in baboon 8839 (769.0 μg/g) as opposed to a right kidney concentration of 291.3 μg/g (Table 1) also indicates trouble with the drug delivery. Baboon 8677 died 5 h after injection and was found to have severe hemolysis. It could not be determined directly whether this was caused by the drug, the DMSO or both. The pathology report indicated edema and hemorrhage of the lungs, colon and heart. The concentration of TOR in the spleen of this animal was 453.2 g/g (Table 1), more than twice that of the intended target (right kidney), indicating a mistargeted injection.

Case reports

Table 2 summarizes the local responses in each of the four tumor-bearing baboons following intratumoral TOR therapy. Table 3 summarizes the kinetic tissue distribution of TOR in each of these animals. The detailed results from each of the cases are described below.

Table 2. Summary of the treatment results obtained with intratumoral TOR (500–1000 mg in DMSO) in baboons

Tumor type	Initial tumor size	Final tumor size	Percent of initial size
Myxoma			
period 1	140 cm^3	72 cm^3	51
period 2	339 cm^3	0 cm^3	0
period 3	13.5 cm^2	0 cm^2	0
Liver lymphosarcoma	7.7 cm^3	2.7 cm^3	35
Squamous cell carcinoma	see text		
Parotid adenocarcinoma	347 cm^3	116 cm^3	33

Myxoma

The initial tumor biopsy revealed that the myxoma was ER-negative but PgR-positive (173 fm/mg). A partial response (51% reduction) was achieved in the myxoma following two treatments in period 1, after which the tumor was surgically removed. The tumor recurred 1 month later and then completely responded to TOR in period 2. The tumor biopsy taken after treatment period 1 had a TOR concentration of 3.3 μg/g, 44 days after the last dose, compared to undetectable levels in plasma (Table 3). One week post-treatment plasma levels throughout treatment period 2 averaged 23 ng/ml, while the two 24 h post-treatment plasma specimens collected contained 211 and 304 ng/ml following intratumoral and oral therapies, respectively.

Twenty-one days after the end of period 2, we decided to administer TOR orally as a prophylactic in order to delay or possibly prevent another recurrence. However, the tumor did recur during treatment period 3 after having remained undetectable for 70 days. The

Table 3. Tissue distribution of TOR in selected tissues from four case study baboons following intratumoral injection of toremifene in DMSO

Animal	Dose (mg)[a]	TOR tissue concentrations (μg/g)						
		Tumor	Liver	Right kidney	Spleen	Brain	Uterus	Serum[b]
1C0071 (myxoma)[c]	1000	3.3	–	–	–	–	–	<0.01
7349 (lymphosarcoma)	1000[d]	430.2	178.8	14.2	13.9	16.5	–	1.7
9587 (squamous cell carcinoma)	200	365.2	4.6	4.7	7.2	5.4	1.0	0.77
1X0229 (adenocarcinoma)[e]	500[d]	11.4	0.79	0.27	0.15	0.09	<0.01	<0.01

[a]Doses listed are those given just prior to tissue collection.
[b]Concentration in μg/ml.
[c]This animal was not euthanized; tumor biopsy taken 44 days post-treatment.
[d]Doses represent 43 mg/kg.
[e]Tissue collected 2 weeks after final injection.

tumor initially measured 13.5 cm^2 (Table 2). Another complete response was observed following the four 500 mg intratumoral injections. The tumor recurred yet again 2 months later and was resected for histological examination. The biopsy tissues were comprised of connective tissue, a diffuse pyogranulomatous surface exudate and neoplastic tissue morphologically consistent with the previously diagnosed myxoma. The neoplasm looked essentially the same as it did at the beginning of the study. Although the myxoma continued to relapse, tumor growth was controlled throughout the study period. Therapy was discontinued following each of the responses. This animal was euthanized 2 years following the completion of this study due to tumor progression.

Squamous cell carcinoma

This tumor was growing inward and was therefore difficult to measure. However, based on MRI, an objective response was achieved. The tumor became necrotic after the initial dose and was no longer visible 1 week following the third dose. Twenty-four hours following the fifth and final dose of TOR (21 mg/kg), the tumor concentration was 365.2 μg/g, while the concentration in plasma was 0.77 μg/ml (Table 3). Table 3 shows the TOR concentrations in other selected tissues.

This baboon died 24 h following the last dose due to progressive metastatic disease. The pathology report revealed that the maxillary mass that had been well documented previously by MRI and histopathology had extended into the maxilla approximately 3–4 cm with a distinct boundary between the expanding tumor and surrounding bone and soft tissue. The adjacent lymph nodes (submandibular) were minimally enlarged and firm. Foci 1 mm in diameter were scattered throughout the lung. Microscopically, squamous cell carcinoma was evident in all areas of the maxillary mass viewed. Islands of this tissue could be seen extending into the surrounding medullary cavity of the maxilla, and cells were evident within the vessels and peripherally. Multifocal collections of lymphocytes with associated myocardial degeneration were seen in the heart. Occasional vessels in the lung contained fibrin, and areas of the lungs had alveolar edema, inflammation and vascular congestion. The lymph nodes that were firm and enlarged grossly in the submandibular region had subcapsular collections of foamy macrophages with necrosis. Although a local response was achieved, this animal had massive systemic disease.

Hepatic lymphosarcoma

Based on ultrasound, the liver lymphosarcoma was reduced in size by approximately 65% (Table 2). Twenty-four hours after the final injection of TOR (43 mg/kg), the tumor concentration was 430.2 μg/g, compared to a plasma concentration of 1.65 μg/ml (Table 3). A follow-up tumor biopsy taken during therapy revealed no change in the morphologic appearance of the tumor. There may have been some increase in spindling and loss of cytoplasmic eosinophilia.

The pathology report after necropsy showed that the regional lymph nodes were enlarged, and multiple, variably sized, white foci were scattered throughout the liver and lung. Microscopically, the treated areas of the liver were often necrotic/infarcted with a fibrous periphery and inflammatory cell infiltrates. In these areas, the malignant lymphocytes were generally

undetectable or remarkably reduced in number. In other areas of the liver, focal collections of lymphocytes were quite evident, especially in portal areas. Additionally, some vessels were necrotic and may or may not have been an extension from the treated areas. Other than that, typical changes associated with lymphosarcoma were evident in this case.

Parotid salivary gland adenocarcinoma

This neoplasm reduced in size by 67% after nine intratumoral injections of TOR (Table 2). Two weeks following the final injection, the tumor concentration was 11.4 μg/g (Table 3). A plasma specimen collected upon euthanasia contained undetectable levels of TOR. Throughout treatment, plasma levels of drug averaged 15 ng/ml 1 week post-injection and one 24 h post-treatment specimen contained 968 ng/ml. The initial biopsy revealed that the tumor was ER- and PgR-negative.

A necropsy revealed that the baboon was emaciated. The adenocarcinoma was firm and approximately 116 cm^3 in size. The core of the tumor was yellow and soft with surrounding tumor tissue resembling a normal to neoplastic salivary gland. The consistency of the tumor was firm to gelatinous. The tumor was attached to the subjacent bone, but when defleshed no remarkable bone changes were noted. Submandibular lymph nodes on the left side had a possible metastatic lesion. There were scattered, variably sized metastatic foci approximately 10 mm in diameter throughout the lung lobes. Microscopically, the tumor was morphologically consistent with an adenocarcinoma as previously diagnosed in the surgical biopsy. Other lesions found insignificant to the neoplasm were interstitial collections of lymphocytes in the kidney, amyloid in scattered islets of Langerhans and thyroid papillary adenomas.

Discussion

The basic principle behind the regional (intratumoral) delivery of TOR is to increase its therapeutic advantage by raising local drug levels while reducing systemic exposure and thus the risk of side effects. We previously showed that transdermal delivery of TOR can achieve local concentrations capable of producing anti-tumor effects *in vivo*, with minimal systemic distribution.[10] Intratumoral administration is another potential method of regional toremifene therapy which can result in locally elevated drug concentrations. TOR's lack of significant vesicant properties makes it a more ideal candidate for intratumoral administration than most other chemotherapeutic agents.

One of the problems with the intratumoral administration of TOR is its solubility. Although direct injection of pure ethanol has been used successfully in the past,[16-19] TOR is only moderately soluble in ethanol (2.7 mg/ml). Therefore a 10 mg dose would require at least 3.7 ml of ethanol. While a volume of 4 ml would not be a major concern for hepatic injections, this volume would be a concern for other organs such as the kidney. TOR is also practically insoluble in water. Therefore, we chose DMSO, a solvent in which the drug is highly soluble (more than 100 mg/ml) and which has been studied extensively in humans.[27-33] Toxicological data on DMSO in humans do show that it can be used without serious side effects.[27-33] However, the volume of pure DMSO used in our studies was remarkably high (up to 7 ml in some cases). Although this large volume makes it extremely difficult to keep the injected drug within the tumor, in this preclinical study we sought to determine whether this mode of TOR therapy is useful in the treatment of spontaneously occurring neoplasms. The doses we selected for this study are considered high, exceeding the highest oral doses in humans by a factor of 3–6. These doses were selected deliberately to show that cytotoxic drug levels (above 6 μM) could be delivered locally, while limiting systemic exposure. The doses and most appropriate diluent must be determined in future studies.

In our studies with intratumoral TOR in baboons, we examined the drug's distribution and the responses in four baboons with spontaneous neoplasms. We found the distribution of the drug to be generally favorable in the healthy baboons used in the tissue distribution study, with the highest drug concentrations outside the delivery found in adjacent tissues. The concentrations achieved at the injection site were extraordinarily high compared to other tissues, suggesting that the distribution of TOR to local tissues was far greater than the distribution to distal organs via circulation. *In situ*/serum concentration ratios in the distribution study ranged from 138 to 8873 and *in situ*/other tissue ratios ranged from 1.2 to 2428. It is especially remarkable that uterine concentrations of TOR were low, taking into account that triphenylethylenes (at least tamoxifen) have carcinogenic properties in the endometrium. Responses were achieved in all four case studies, including two complete responses in the myxoma. These four animals remained otherwise healthy during the course of the study and no evidence of toxicity was observed. Although the myxoma baboon was euthanized 2 years after the study due

to a recurrence of the tumor, the animal nevertheless remained disease-free for 2 years.

As in our previous study with transdermal TOR, results from our intratumoral case studies show that high concentrations of drug were retained locally several days or weeks after discontinuation of therapy, when TOR could no longer be detected in plasma. Dose intensification of chemotherapy, which attempts to increase concentration over time at the target tissue, usually results in unacceptable toxicity following systemic therapy. By delivering TOR intratumorally, we achieved very high local concentrations which were maintained after the drug could no longer be detected in plasma. This had the effect of increasing concentration over time while limiting systemic exposure to the drug. From this data we can conclude that intratumoral delivery of TOR markedly increases the therapeutic index of the drug and that high local concentrations may potentially improve efficacy while avoiding high systemic exposure to the drug.

Conclusion

By examining the distribution of TOR in healthy baboons following intra-tissue injections and by evaluating the responses achieved from the intratumoral therapy of four baboons with spontaneously occurring neoplasms, we have shown that intratumoral administration of TOR can achieve effective local tumor and tissue concentrations while limiting the systemic distribution to other organs via circulation. In the four case study animals, high local drug concentrations were retained several days or weeks following discontinuation of therapy, when TOR could no longer be detected in plasma. This is important in that the concentration of drug over time was increased locally while minimizing systemic exposure. We can therefore conclude that the intratumoral delivery of TOR markedly increases the therapeutic index of the drug and that high local drug concentrations may potentially improve efficacy while avoiding high systemic exposure to the drug. Further study of this mode of TOR therapy is warranted.

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(Received 28 October 1997; accepted 20 November 1997)