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Serum tamoxifen concentrations in the athymic nude mouse after three methods of administration*

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Summary. The athymic nude mouse has been used as an in vivo model for pharmacologic studies of the antiestrogen, tamoxifen. We have examined the steady-state serum tamoxifen concentrations achieved in mice with s.c. slow-release pellets, s.c. injections, and i.p. injections, in an attempt to identify a method that would yield serum levels similar to those observed in patients receiving tamoxifen therapy. Tamoxifen and tamoxifen metabolites were examined by a high-performance liquid chromatography assay which has a sensitivity of 8 ng/ml. Tamoxifen metabolites were not observed with any dose or schedule. After slow-release pellets containing 5 or 25 mg tamoxifen no tamoxifen was detectable, even after 2 weeks of treatment. Very low levels (0.07 μM) were found with 50-mg pellets. Tamoxifen was also not detected either with daily s.c. injections of 500 μg/mouse or with i. p. injections of 2.5 mg/kg. However, daily s. c. injections of 1000 μg or i. p. injections of 25, 50, or 100 mg/kg resulted in tamoxifen concentrations ranging from 0.21 to 0.51 μM which are similar to those observed in patients. Thus, clinically relevant tamoxifen concentrations can be achieved in the nude mouse with either of these methods of administration.

Introduction

Tamoxifen is an antiestrogen that is commonly used in the treatment of breast cancer. Its mechanism of action is not fully understood at the present time, but most investigators believe that tamoxifen exerts its antiestrogen effects by competitive blockade of the estrogen receptor. In vitro studies of human breast cancer have shown that the antiestrogens inhibit important metabolic pathways, resulting in the eventual slowing of cell proliferation and the accumulation of cells in G_1 phase [3, 8]. In these studies, using cells growing in charcoal-stripped serum, tamoxifen concentrations of at least 10 nM were required for a biological effect. Maximal inhibition of cell proliferation was observed with a tamoxifen concentration of 1 μM, which is similar to the steady-state serum concentrations achieved in patients treated with therapeutic doses of the drug [2].

In an effort to develop an in vivo model of human breast cancer, in several studies cultured human breast cancer cells have been inoculated into the immune-deficient athymic nude mouse [4–7]. This model system has been used to study the effects and mechanism of action of antiestrogens on proliferation of human breast cancer cells. Tamoxifen treatment has been shown to antagonize the effect of estrogen and to inhibit tumor growth [4]. However, there is little information on the pharmacokinetics or steady-state serum levels of tamoxifen in the athymic nude mouse. In the present study, we examined the steady-state serum tamoxifen concentrations in female nude mice following three different methods of administration. Mice were treated with various doses of tamoxifen given by daily s.c. or i.p. injection, or in the form or slow-release pellets placed s.c. The purpose of this study was to determine a method of administration that achieves serum steady-state tamoxifen concentrations similar to those observed in patients receiving tamoxifen therapy.

Methods

Animals. Female Balb/c athymic nude mice 4–5 weeks old implanted with MCF-7 human breast cancer cells were used. Mice were maintained in laminar air-flow cabinets with sterile bedding, food, and water as previously described [4].

Tamoxifen administration. Tamoxifen citrate was generously provided by Stuart Pharmaceuticals. Mice were treated with tamoxifen by three different modes of administration. One group received slow-release pellets containing 5 mg, 25 mg, or 50 mg tamoxifen citrate (Innovative Research, Rockville, Md). Pellets were fused and compressed individually with filler material, including cholesterol, microcrystalline cellulose, alpha lactose, di- and tricalcium phosphate, calcium and magnesium stearate, and stearic acid. The pellet was placed s.c. in the interscapular region using a 14-gauge needle to puncture the skin. A second group received daily s.c. injections of tamoxifen suspended in peanut oil at doses of either 500 or 1000 μg/mouse. A third group was treated with daily i.p. injections of tamoxifen citrate dissolved in ethanol and diluted 1:5 in sterile phosphate-buffered saline prior to administration. Doses of 2.5, 25, 50, or 100 mg/kg per day were used.

Mice were sacrificed 24 h after treatment, and blood was obtained after disruption of the axillary vessels. After clotting, serum was obtained by centrifugation in a microfuge to eliminate contaminating red blood cells. Serum was frozen at −20° C prior to analysis.

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Assay of tamoxifen and metabolites. Tamoxifen and tamoxifen metabolites were quantified as previously reported [9]. In brief, serum samples were spiked with an internal standard (nafoxidine), extracted with 10 ml hexane/2% butanol solution, vigorously vortexed, centrifuged for 10 min at 1000 *g*, dried under nitrogen, and frozen until analysis. Each sample was reconstituted with methanol and activated to the fluorescent phenanthrene structures. Fluorescent compounds were injected onto a high-performance liquid chromatography system and detected at 266 nm.

Standard curves ($n = 6$) for tamoxifen, 4-hydroxytamoxifen, and *N*-desmethyltamoxifen yielded correlation coefficients of 0.995, 0.993, and 0.989, respectively. To assure consistent linearity throughout the study period, additional standards were analyzed in every second or third serum sample. Each of the additional standards was within 5% of the predicted concentrations based on our standard curves throughout the study. The sensitivity of the assay is 8 ng/ml.

Results

The major metabolites of tamoxifen, 4-hydroxytamoxifen and *N*-desmethyltamoxifen, were not detected in any of the mice, even those receiving the highest doses: serum concentrations of the parent drug, tamoxifen, are shown in Table 1. Tamoxifen serum levels were below the limits of detectability in all mice treated with the 5- or 25-mg slow-release pellets. Very low levels were also observed with the 50-mg pellets. Similar results were seen in two other similar blind studies using this mode of administration (data not shown).

Appreciably higher serum concentrations were achieved with s.c. or i.p. injections. At doses of 100 μg/day injected s.c. in peanut oil or of 25, 50, or 100 mg/kg per day injected i.p., serum tamoxifen concentrations reached levels similar to those seen in breast cancer patients treated therapeutically, and ranged from a mean of 0.24 to 0.51 μ*M*.

Table 2 shows the serum tamoxifen concentrations of a separate time course experiment of 1000 μg s.c. injections. The mean serum tamoxifen concentrations were approximately equal to those achieved within 2 weeks after the start of therapy, as shown in Table 1. This would suggest that the 2-week time point in the athymic nude mouse model can be used to mimic the in vivo serum pharmacokinetic conditions achieved in patients receiving tamoxifen therapy.

Table 1. Serum tamoxifen concentration by mode of administration

Method of administration	Dose	Serum concentrations (μ*M*)
Slow-release pellets s.c.	5 mg	ND[a]
	25 mg	ND
	50 mg	0.07 (0–0.22)
Injections s.c.	500 μg/day	ND
	1000 μg/day	0.35 (0.26–0.43)
Injections i.p.	2.5 mg/kg/day	ND
	25 mg/kg/day	0.24 (0–0.36)
	50 mg/kg/day	0.29 (0.25–0.32)
	100 mg/kg/day	0.51 (0.20–0.72)

[a] ND, not detectable

Table 2. Serum tamoxifen after s.c. administration

Time (days)	Serum concentrations (μ*M*)
7	0.263 (0.20–0.33)
14	0.274 (0.19–0.32)
21	0.280 (0.27–0.28)
28	0.242 (0.20–0.29)
35	0.212 (0.16–0.25)

Discussion

In the present study, only daily s.c. injections of 1 mg/day or i.p. injections of 25 mg/kg per day (about 625 μg/mouse per day) or greater produced clinically relevant serum tamoxifen concentrations in the athymic nude mouse model. These concentrations are similar to those required for optimal inhibition of proliferation of cultured breast cancer cells [3]. Subcutaneous slow-release pellets, even those containing 50 mg tamoxifen, resulted in very low levels of the drug.

The effect of tamoxifen on the growth of human breast cancer in the nude mouse has been previously reported [4, 6]. In one study tamoxifen was administered as an s.c. injection in peanut oil at a dose of 5 μg/mouse per day [6]. This dose, which is 200 fold lower than that resulting in clinically relevant tamoxifen serum concentrations in the present study, nevertheless was associated with significant regression of MCF-7 human breast cancer cells.

In another study, tamoxifen was administered either as a 5-mg s.c. slow-release pellet or as s.c. injections in peanut oil at doses of up to 100 μg/mouse per day [4]. This study indicated that serum tamoxifen levels approached 1 μ*M* under these conditions. Our present studies do not agree with these findings. We would predict much lower serum levels with these doses. Nevertheless, biological effects of tamoxifen, including saturation of tumor ER, induction PgR, and growth inhibition, were observed despite the low serum concentrations. This raises questions about the serum tamoxifen level necessary for tumor growth inhibition in postmenopausal women with low endogenous estrogen concentrations. In another study, tamoxifen concentrations were measured in castrated male nude mice after a single i.m. injection in peanut oil [1]. Concentrations of 0.1 and 0.13 μ*M* were achieved with tamoxifen doses of 5 and 10 mg, respectively. Growth inhibition was observed in an ER-positive, but not in an ER-negative, cell line.

We did not detect serum levels of 4-hydroxytamoxifen or *N*-desmethyltamoxifen, the major tamoxifen metabolites in humans, in athymic nude mice treated with tamoxifen for up to 2 weeks. It is possible that detectable levels would eventually be achieved after longer treatment periods, or that these metabolic pathways are not operative in the nude mouse.

In summary, serum tamoxifen concentrations similar to those seen in patients receiving tamoxifen therapy can be achieved in the nude mouse model, but relatively large

doses are required. Daily s.c. injections of 1 mg or i.p. injections of 25–100 mg/kg achieved clinically relevant serum tamoxifen levels and can therefore be used to study the effects of tamoxifen on human breast cancer.

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