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Tumor and serum tamoxifen concentrations in the athymic nude mouse*

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Summary. The athymic nude mouse has been used as an in vivo model for pharmacologic studies of the antiestrogen, tamoxifen. Serum and tumor tamoxifen and metabolite concentrations were examined during and after 100 and 1000 μg/day doses injected s.c. Tamoxifen and tamoxifen metabolites were quantitated by high-performance liquid chromatography. Tamoxifen was detected in tumors after a dose of 100 μg/day, although serum concentrations were not detected. At a dose of 1000 μg/day, tumor tamoxifen and its active metabolites were detected in high concentrations ranging up to >6 mmol/g tissue. Serum tamoxifen metabolites were not detected at either dose. In summary, high doses of tamoxifen were required in the nude mouse to obtain clinically relevant serum concentrations, and significant tumor levels were achieved at doses that resulted in undetectable serum levels. The relationship between serum tamoxifen concentrations, tumor tamoxifen levels, and the biologic activity of the drug requires further study.

Introduction

Tamoxifen is an antiestrogen commonly used in the treatment of breast cancer. 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 the G1 phase [4, 9]. In these studies, tamoxifen concentrations of ≥10 n*M* were required for a biologic effect, whereas the maximal inhibition of cell proliferation was observed at a concentration of 1 μ*M*. This concentration of tamoxifen is similar to the steady-state serum levels achieved in patients treated with therapeutic doses [3].

Several studies have used cultured human breast cancer cells inoculated into the immune-deficient athymic nude mouse [5–8] as an in vivo model of human breast cancer. Estrogen receptor-positive cell lines are estrogen-dependent in this model. Optimal tumor growth requires estrogen supplementation even in intact female nude mice, because of the low circulating estrogen level, which is equivalent to that seen in postmenopausal women [8]. In this model, estrogen receptor-negative cells lines form tumors that are independent of the estrogen status of the animal [5]. This model system has been used to study the effects and mechanism of action of antiestrogens on the proliferation of human breast cancer cells.

We previously examined the steady-state serum tamoxifen concentrations in female nude mice following three different methods of administration [2]. Mice were treated with various doses of tamoxifen given by daily s.c. or i.p. injection or as slow-release pellets placed s.c. to determine a method of administration that achieves serum steady-state tamoxifen concentrations similar to those observed in patients receiving tamoxifen therapy. In the present study, we examined tumor tamoxifen and active metabolite levels after s.c. tamoxifen administration.

Methods

Tamoxifen administration. Tamoxifen citrate was generously provided by Stuart Pharmaceuticals. Female 4 to 5-week old BALB/c athymic nude mice received daily s.c. injections of tamoxifen suspended in peanut oil at doses of either 100 or 1000 μg/mouse. The mice were sacrificed, and blood was obtained after disruption of the axillary vessels. After clotting, serum was collected by centrifugation in a microfuge to eliminate contaminating red blood cells. Tumors were collected by surgical resection for the 100 and 1000 μg/day time-course experiments. Serum and weighed tumor specimens were then immediately frozen at −20°C.

Assay of tamoxifen and metabolites. Tamoxifen and tamoxifen metabolites were quantified as previously reported [10]. Briefly, serum and minced tumor 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 its fluorescent phenanthrene structure. 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 >0.985 for all compounds. To assure consis-

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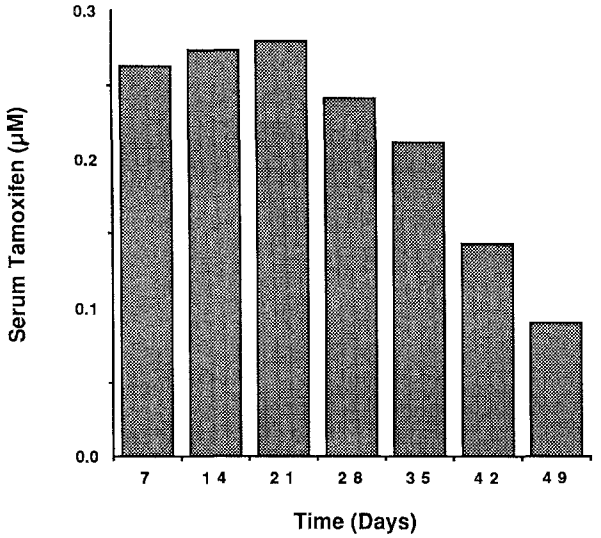

Fig. 1. Serum tamoxifen levels during and after 35 days of 1000 μg/day doses injected s.c. and 7 and 14 days after the final tamoxifen dose. Each point represents the average of at least duplicate samples

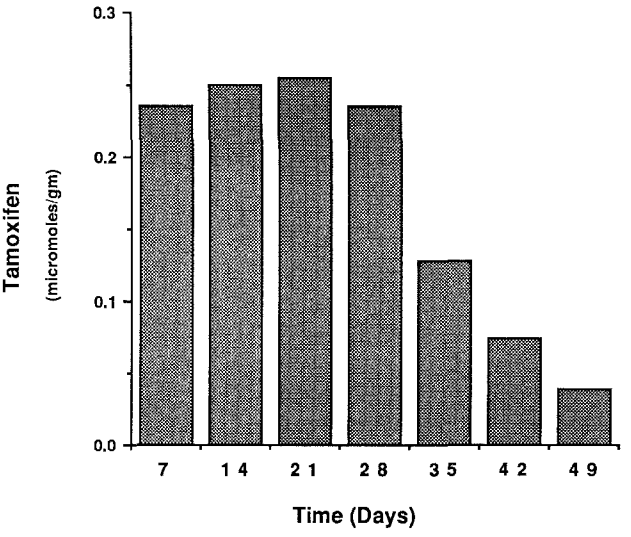

Fig. 3. Tumor tamoxifen during and after 35 days of 100 μg/day doses injected s.c. and 7 and 14 days after the final tamoxifen dose. Each point represents the average of at least duplicate samples

tent linearity throughout the study period, additional standards were analyzed following every second or third 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.

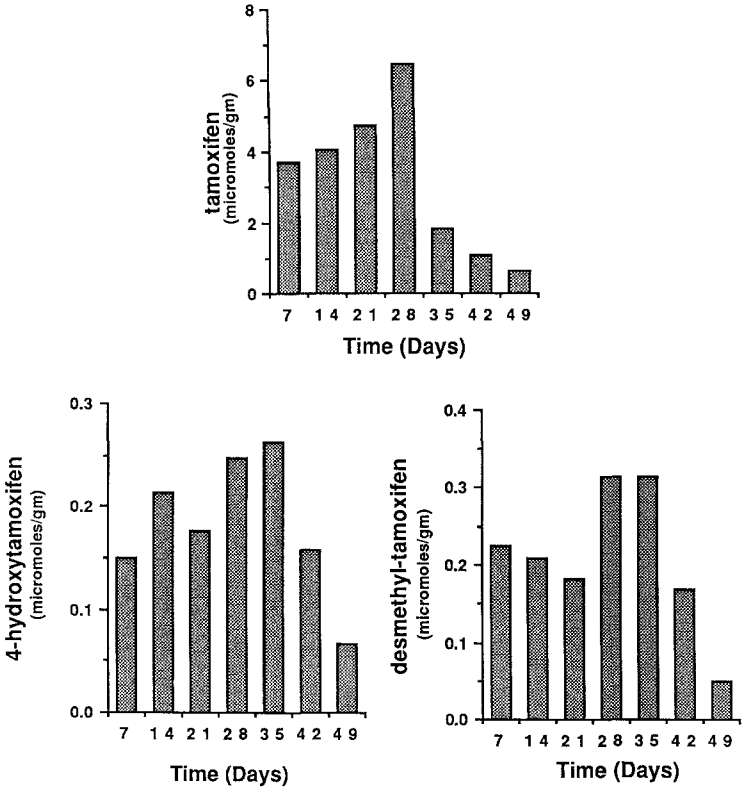

Fig. 2. Tumor tamoxifen, 4-hydroxytamoxifen, and N-desmethyltamoxifen durig and after 35 days of 1000 μg/day doses injected s.c. and 7 and 14 days after the final tamoxifen dose. Each point represents the average of at least duplicate samples

Results

Figure 1 shows the serum tamoxifen concentrations from a time-course experiment during 35 days of 1000 μg/day s.c. injections and for 14 days after the final tamoxifen dose. Steady-state serum tamoxifen concentrations of >0.25 μ*M* were achieved within 3 weeks after the start of therapy. The tamoxifen level fell below 0.1 μ*M* at 14 days following the last injection. No detectable serum concentrations were seen during the 100 μg/day s.c. time-course experiment. The terminal half-life of tamoxifen was approximately 8 days.

Figure 2 shows tumor tamoxifen, *N*-desmethyltamoxifen and 4-hydroxytamoxifen levels during and after the 35 days at s.c. doses of 1000 μg/day. Cellular tamoxifen concentrations were 10–20 times greater than those of 4-hydroxytamoxifen and *N*-desmethyltamoxifen throughout the study period. Interestingly, detectable tumor levels were seen during the 100 μg/day s.c. time-course experiment as shown in Fig. 3, although no serum concentrations were detected during this study. Both serum and tumor concentrations declined after the final tamoxifen dose (Figs. 1–3).

Discussion

In the present study, tumor tamoxifen levels were detected at s.c. doses of both 100 and 1000 μg/day, despite undetectable serum tamoxifen or metabolite levels at the lower dose. The effect of tamoxifen on the growth of human breast cancer in the nude mouse has previously been reported [5, 7]. In one study, tamoxifen was injected s.c. in peanut oil at a dose of 5 μg/mouse per day [7]. This dose, which is 200-fold lower than that resulting in clinically relevant tamoxifen serum concentrations in our previous study [2], was nevertheless associated with significant regression of MCF-7 human breast cancer cells.

Biologic effects of tamoxifen, including the saturation of tumor Estrogen Receptors (ER), induction of Progesterone Receptors (PgR), and growth inhibition, have been

observed with doses producing undetectable serum levels [5]. These data raise questions about the serum tamoxifen level necessary for tumor growth inhibition in postmenopausal patients with low endogenous estrogen concentrations. A very low dose may provide a tumor tamoxifen concentration sufficient to antagonize the effects of residual estrogen. Another study measured tamoxifen concentrations in castrated male nude mice after a single i.m. injection in peanut oil [1]. Concentrations of 0.1 and 0.13 μM 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.

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 [2]. Daily s.c. injections of 1 mg or i.p. injections of 25–100 mg/kg achieved clinically relevant serum tamoxifen levels and, therefore, can be used to mimic the clinical situation. However, since significant tumor levels of the drug as well as biologic effects are obtained at markedly lower doses resulting in undetectable blood levels, questions are raised about the necessity of achieving high blood levels of tamoxifen in patients. The relationship between serum tamoxifen and estrogen concentrations, tumor tamoxifen levels, and antiestrogenic effects requires further study.

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