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Implications of Tamoxifen Metabolism in the Athymic Mouse for the Study of Antitumor Effects upon Human Breast Cancer Xenografts*

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Abstract—*The metabolism of tamoxifen in the human has been well established and may be important in the antiestrogenic activity of this agent. This study examines whether tamoxifen metabolism in the athymic mouse xenograft model is similar to tamoxifen metabolism in the breast cancer patient.*

Serum taken from athymic mice 24 h *after a single large oral dose* (200 mg/kg) *of tamoxifen contained compounds corresponding to standards of tamoxifen, 4-hydroxytamoxifen,* N*-desmethyltamoxifen, 4-hydroxy-*N*-desmethyltamoxifen and tamoxifen-*N*-oxide when analyzed by high pressure liquid chromatography. The administration of large single doses* (200 mg/kg) *of 4-hydroxytamoxifen and* N*-desmethyltamoxifen either alone or in combination produced the expected peaks for the administered agents and a peak confirming the identity of 4-hydroxy-*N*-desmethyltamoxifen. 4-Hydroxy-*N*-desmethyltamoxifen was detected in serum from six out of* 10 *breast cancer patients receiving* 10 mg *bid of tamoxifen. These patients had tamoxifen, 4-hydroxytamoxifen and* N*-desmethyltamoxifen levels of* 108 ± 23, 2.6 ± 0.5, *and* 238 ± 58 ng/ml, *respectively.*

Repeated large oral doses (200 mg/kg/*day for* 6 *days*) *of tamoxifen to athymic mice produced a similar array of serum metabolites as seen after the single dose and in the breast cancer patient. However, levels of 4-hydroxytamoxifen* (628 ± 192 ng/ml) *were similar to those of tamoxifen* (441 ± 208 ng/ml) *whereas* N*-desmethyltamoxifen* (1343 ± 388 ng/ml) *levels were* 2–3 *times greater. A similar pattern of metabolites was produced with a* 50 mg/kg *dose of tamoxifen although levels were considerably reduced.*

Subcutaneous administration of tamoxifen (200 mg/kg/*day for* 6 *days*) *produced serum levels of the parent compound* (120 ± 19 ng/ml) *in the same range as tamoxifen levels in the breast cancer patient. However, although* N*-desmethyltamoxifen was the major metabolite, levels* (115 ± 18 ng/ml) *were only equivalent to those of tamoxifen itself and 4-hydroxytamoxifen levels* (26 ± 5 ng/ml) *were appreciably higher than the breast cancer patient. Lowering the dose of tamoxifen* (50 mg/kg) *administered s.c. produced not only lower circulating tamoxifen levels* (41 ± 3 ng/ml) *but also changed the metabolite profile. Rather than* N*-desmethyltamoxifen levels equivalent to those of tamoxifen, as seen with the higher dose, they were reduced to the level of 4-hydroxytamoxifen* (7 ± ng/ml). *Low levels of both* N*-desmethyltamoxifen and 4-hydroxytamoxifen compared to the parent compound was characteristic of metabolite profiles produced by* 0.25, 1.25 *or* 2.5 *cm silastic capsules releasing an average* (±*S.E.*) 53 ± 9, 84 ± 8 *and* 192 ± 12 μg *of tamoxifen per day, respectively. These capsules demonstrated their ability to inhibit* 17 β*-estradiol stimulated MCF-7 tumor growth in a dose related manner. The largest capsule* (2.5 cm) *produced serum levels of tamoxifen* (51.8 ± 4.9 ng/ml), *4-hydroxytamoxifen* (3.5 ± 0.4 ng/ml) *and* N*-desmethyltamoxifen* (3.8 ± 0.6 ng/ml) *which were sufficient to produce complete inhibition of tumor growth.*

The athymic mouse hydroxylates oral tamoxifen more readily than the human and therefore produces a different metabolite profile.

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INTRODUCTION

LABORATORY RESEARCH on the pharmacology of new drugs relies heavily on animal models of human disease. Since the first report of the successful use of the athymic mouse as a host for supporting the growth of human tumor xenografts [1], this system

has been increasingly applied to study potential anticancer agents. The support and growth of a large number of different tumor types has been achieved either by direct implantation of tumor pieces of primary tumor [2, 3] or inoculation of cultured cancer cell lines [4, 5]. This model has, therefore, provided the potential to examine the action of a particular antitumor agent on a specific type of human tumor *in vivo*. Recently, hormone dependent breast [5–10] and endometrial tumors [11–14] have been grown in the athymic mouse by hormone supplementation. From preliminary reports these models may be useful to study the antitumor action of antiestrogens such as tamoxifen (TAM) [8–13] which has been used so successfully for the treatment of breast cancer [15].

Sensitive methods are available to measure TAM and certain metabolites in human plasma using HPLC separation, UV conversion to phenanthrene derivatives and detection of fluorescence [15, 16]. Recently these methods have been used to determine TAM levels in athymic mice following different routes of administration. However, the methodology was not sufficiently sensitive to detect and identify metabolites [17]. In a preliminary study [18] we found that if large oral doses of TAM are used to raise circulating TAM and metabolite levels in rats and mice into the range seen in breast cancer patients, then this allows the metabolism and pharmacokinetics to be studied. In the present study, a similar approach was adopted using large oral doses to establish potential metabolite pathways in the athymic mouse and compare metabolite profiles with the human. Subsequently, lower doses and different routes of administration were examined to determine the characteristics of the handling of TAM in the athymic mouse and document circulating levels that produce antiestrogenic effects on estradiol-stimulated MCF-7 tumor and uterine growth.

MATERIALS AND METHODS

TAM free base was obtained from Sigma Chemical Company (St. Louis, MO). *Trans* isomers of 4-hydroxytamoxifen, *N*-desmethyltamoxifen, *N*-didesmethyltamoxifen, and *N*-desmethyl-4-hydroxytamoxifen were all gifts from Imperial Chemical Industry, plc (Macclesfield, Cheshire, U.K.). Tamoxifen-*N*-oxide was synthesized from TAM free base by the method of Foster *et al.* [19].

Animals and MCF-7 tumors

Ovariectomized female athymic mice (4–5 week old BALB/c strain) were purchased from Harlan Sprague Dawley (Indianapolis, IN), housed in laminar flow hoods with sterile cages and bedding, and fed autoclaved LM-485 chow (Taklad, Madison, WI) and sterile water *ad libitum*.

MCF-7 cells (ATCC passage 144) were grown into solid tumors in athymic mice in the manner outlined previously [10]. A tumor was transplanted from a 17β-estradiol-treated animal by sterile dissection, mincing into 2 mm^3 pieces and s.c. implantation of tumor pieces into the axillary fat pads of athymic mice. Tumor growth was followed by weekly caliper measurements and cross sectional area was calculated using the formula length/2 × width/2 × π.

Drug administration and sampling

TAM, 4-hydroxytamoxifen and *N*-desmethyltamoxifen doses were prepared by dissolving in peanut oil before 0.1 ml was administered either p.o. or s.c. Large doses were given as a crystalline suspension following rigorous mixing.

Estradiol and TAM administration to mice implanted with MCF-7 tumors was by silastic capsules implanted s.c. on the back of the animal using a trochar.

Silastic capsules were formed by plugging one end of lengths of medical grade silastic tubing (0.078 inches ID by 0.125 inches OD; Dow Corning, Midland, MI) with silastic 382 medical grade elastomer (Dow Corning) and then filling with either crystalline TAM or 17β-estradiol (mixed 1:3 w/w with elastomer without catalyst). Capsules were completed by filling the open end with elastomer and sterilizing with radiation (20,000 rads). TAM release was calculated as capsule preimplantation weight minus dry capsule weight after removal.

Blood samples were taken from mice 24 h after the final dose by light ether anesthesia and bleeding from the eye orbit.

TAM analysis

TAM and metabolite analyses in serum were as previously described [20]. Briefly, serum (300 μl) was spiked with enclomiphene (Merrel Dow, Cincinnati, OH) (internal standard) and extracted three times with 1 ml hexane:amyl alcohol (98:2). The three extracts were combined and evaporated to dryness before being resuspended in 100 μl mobile phase (iso-octane:ethanol:isopropanol: diethylamine:acetic acid; 75:22.0:3.0:0.05:0.05). Extracts were injected (Gilson Model 231 autosampler; Gilson Medical Electronics Inc., Middleton, WI) into a Gilson Series 4000 chromatograph and metabolites separated on a silica 5 μm particle size column (100 × 4 mm; Scientific Glass Engineering, Austin, TX). TAM and metabolites underwent post-column on-line UV activation from the triphenylethylene to the fluorescent phenanthrene derivatives [20]. Compounds were detected with either a Gilson 121 or Shimadzu RF 535 fluorometer. Identification and quantitation were perfor-

med using pure metabolite standards spiked into control serum. Concentrations were calculated from standard curves using the enclomiphene internal standard to correct for extraction losses.

Human serum from breast cancer patients was obtained from the University of Wisconsin Clinical Cancer Center serum bank and analyzed as previously described [20].

RESULTS

Serum taken from athymic mice 24 h after a single large oral administration (200 mg/kg) of TAM contained compound corresponding to standards of TAM, 4-hydroxytamoxifen, *N*-desmethyltamoxifen, 4-hydroxy-*N*-desmethyltamoxifen and tamoxifen-*N*-oxide (Fig. 1) when analyzed by HPLC separation, post column UV activation and detection of fluorescence (Fig. 2). The oral administration of large single doses (200 mg/kg) of either 4-hydroxytamoxifen or *N*-desmethyltamoxifen alone produced the expected peak for the administered agent and a peak corresponding to 4-hydroxy-*N*-desmethyltamoxifen. When 4-hydroxytamoxifen and *N*-desmethyltamoxifen were administered together, peaks corresponding to the administered agents were again observed together with a single metabolite peak corresponding to 4-hydroxy-*N*-desmethyltamoxifen (Fig. 2). This demonstrates that the formation of 4-hydroxy-*N*-desmethyltamoxifen from TAM can occur either by 4-hydroxylation and subsequent *N*-desmethylation or vice versa.

The compound with a retention time corresponding to tamoxifen-*N*-oxide, observed following large doses of TAM, was not seen following large doses of the 4-hydroxy or *N*-desmethyl metabolites. This is consistent with this compound being formed from TAM directly and being the *N*-oxide metabolite. However, a peak corresponding to tamoxifen-*N*-oxide was also detected in spiked serum standards containing large amounts of TAM (≥250 ng) whereas analyte standards containing similar amounts of TAM did not. This suggests that either serum can metabolize TAM to *N*-oxide or the extraction procedure converts a small amount of TAM to this compound.

Examination of TAM and metabolite levels in human breast cancer patients receiving 10 mg bid indicated higher circulating *N*-desmethyltamoxifen levels than TAM with low levels of 4-hydroxytamoxifen (Table 1). Very small peaks corresponding to 4-hydroxy-*N*-desmethyltamoxifen and tamoxifen-*N*-oxide were also (six out of 10 samples) seen (Fig. 3); however, these could not be quantitated accurately.

Using standard curves of mouse serum spiked with TAM, 4-hydroxytamoxifen and *N*-desmethyltamoxifen, it was possible to quantitate the circulating levels of these metabolites in the athymic mouse. Additional metabolites could not be quantitated because insufficient amounts of pure metabolite standards were available. The repeated administration of large daily oral doses (200 mg/kg/day for 6 days) of TAM to athymic mice produced a similar array of metabolites as seen after the single dose (Fig. 4). Levels of 4-hydroxytamoxifen were in a range similar to those of TAM whereas *N*-desmethyltamoxifen levels were 2–3 times greater (Table 1). A similar pattern of metabolites was produced with lower oral doses (50 mg/kg) of TAM although levels were considerably reduced (Table 1). There were non-detectable circulating levels of either the parent compound or metabolites after repeated oral doses of 12.5 mg TAM/kg (approximately 300 μg/mouse).

Repeated subcutaneous administration of TAM (200 mg/kg/day for 6 days) produced substantially lower circulating levels of TAM and metabolites

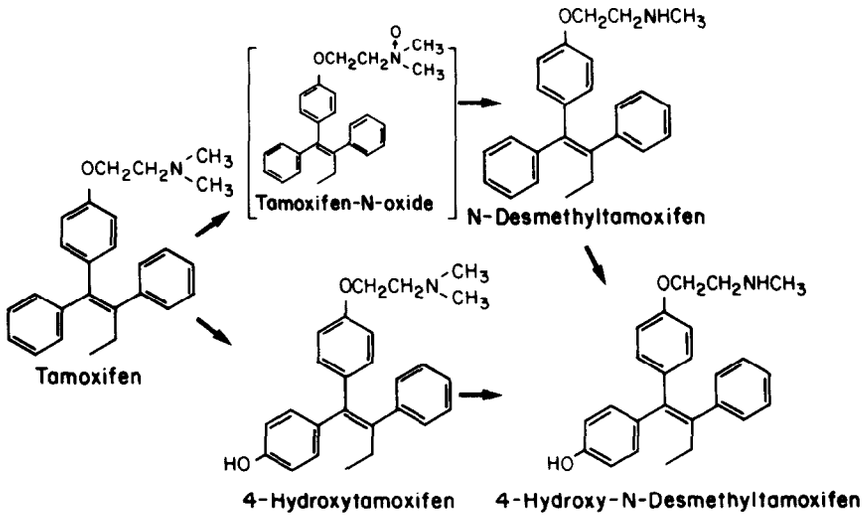

Fig. 1. Structure of TAM and metabolites described in the text.

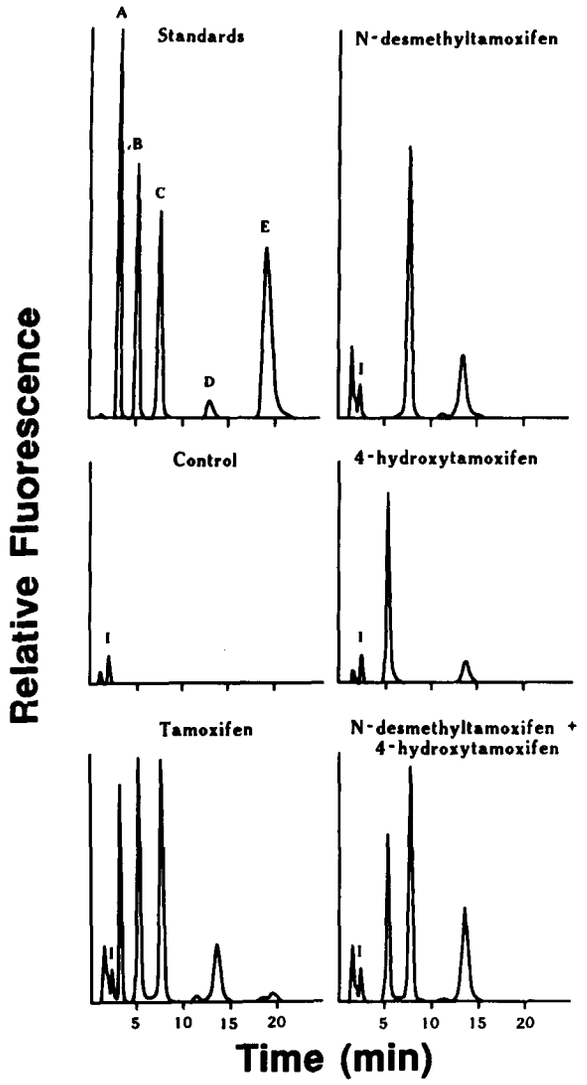

Fig. 2. Chromatography of TAM and metabolites separated by HPLC on a silica column, converted to the phenanthrene derivatives by on-line UV activation and subsequent fluorescence detection. The first panel shows the resolution of an analyte standard mixture of TAM (100 ng; *A*), *4-hydroxytamoxifen* (100 ng; *B*), *N-desmethyltamoxifen* (100 ng; *C*), *4-hydroxy-N-desmethyltamoxifen* (10 ng; *D*) *and tamoxifen-N-oxide* (200 ng; *E*). *The subsequent panels show the chromatograms of extracts of serum* (0.3 ml) *spiked with* 100 ng *of enclomiphene* (*I*) *from control, TAM* (200 mg/kg), *N-desmethyltamoxifen* (200 mg/kg), *4-hydroxytamoxifen* (200 mg/kg) *and N-desmethyltamoxifen* (200 mg/kg) *plus 4-hydroxytamoxifen* (200 mg/kg) *treated* (*p.o. in* 0.1 ml *peanut oil* 24 h *prior to sampling*) *athymic mice. The relative fluorescence between panels is comparable. A peak of fluorescence corresponding to the 4-hydroxy-N-desmethyltamoxifen standard was present in serum from mice treated with 4-hydroxytamoxifen and N-desmethyltamoxifen either alone or in combination whereas a peak corresponding to tamoxifen-N-oxide is present in TAM treated animals.*

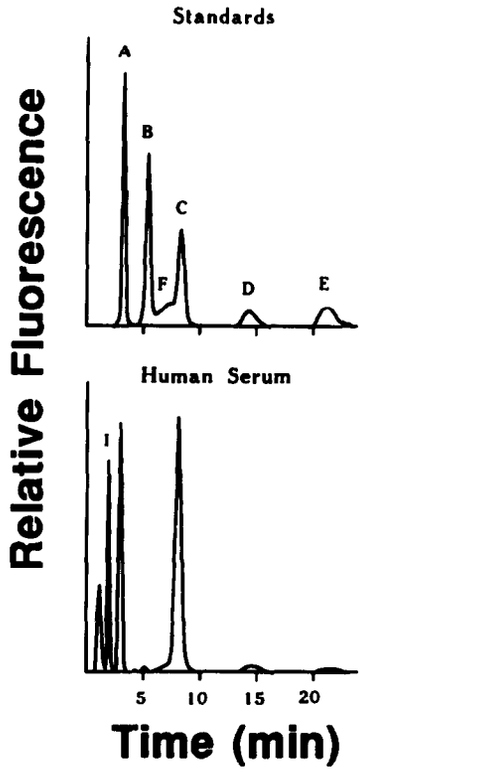

Fig. 3. Chromatography of TAM and metabolites separated as described in Fig. 2 and Materials and Methods. The first panel shows the resolution of an analyte standard mixture of TAM (50 ng; *A*), *4-hydroxytamoxifen* (50 ng; *B*), *N-desmethyltamoxifen* (50 ng; *C*), *4-hydroxy-N-desmethyltamoxifen* (10 ng; *D*), *tamoxifen-N-oxide* (25 ng; *E*) *and N-didesmethyltamoxifen* (50 ng; *F*). *The second panel shows the chromatogram of an extract of human serum* (0.25 ml) *from a patient receiving* 10 mg *TAM bid and spiked with enclomiphene* (100 ng; *I*). *Peaks of fluorescence were quantitatable from standard curves made in serum from TAM* (101 ng/ml), *4-hydroxytamoxifen* (2 ng/ml) *and N-desmethyltamoxifen* (256 ng/ml). *Peaks of fluorescence corresponding to 4-hydroxytamoxifen and tamoxifen-N-oxide can also be seen.*

compared to the same dose given orally (Table 1). However, compounds corresponding to the same metabolites including 4-hydroxy-*N*-desmethyltamoxifen and *N*-oxide were detected (Fig. 4). In contrast to oral administration, less 4-hydroxytamoxifen than the parent compound is present following s.c. administration. Interestingly, similar levels of *N*-desmethyltamoxifen and TAM were seen with a 200 mg/kg dose of TAM. However, with the lower 50 mg/kg dose, not only is the TAM reduced but the amount of *N*-desmethyltamoxifen is considerably lower and in the same range as 4-hydroxytamoxifen (Table 1). The circulating levels of TAM and metabolites were non-detectable at 24 h after repeated s.c. administration of 12.5 mg/kg TAM for 6 days.

MCF-7 tumor growth

Pieces of MCF-7 tumor implanted in athymic mice were stimulated to grow by 17β-estradiol supplementation (1 cm silastic capsule). In contrast, no growth of tumors occured in either control mice or mice treated with TAM (2.5 cm silastic capsule) alone. Tamoxifen administered from 0.25, 1.25 or 2.5 cm silastic capsules was capable of inhibiting 17β-estradiol (1 cm silastic capsule) stimulated MCF-7 tumor growth in a dose related manner (Fig. 5). The uterine wet weight was markedly stimulated in the group of athymic mice receiving

Table 1. Serum levels of tamoxifen and metabolites in the athymic mouse and human breast cancer patients after different routes of administration

Route and dose	Sample time	Number	Tamoxifen (ng/ml)	4-Hydroxytamoxifen (ng/ml)	*N*-Desmethyltamoxifen (ng/ml)
Athymic mice					
oral 200 mg/kg/day	6 days + 24 h	2	441 ± 208	628 ± 129	1343 ± 288
oral 50 mg/kg/day	6 days + 24 h	3	20 ± 9	18 ± 6	106 ± 8
oral 12.5 mg/kg/day	6 days + 24 h	3	0	0	0
sc 200 mg/kg/day	6 days + 24 h	3	120 ± 19	26 ± 5	115 ± 18
sc 50 mg/kg/day	6 days + 24 h	3	41 ± 3	3 ± 0	7 ± 4
sc 12.5 mg/kg/day	6 days + 24 h	3	0	0	0
2.5 cm capsule 192 ± 12 μg/day	8 weeks	6	51.8 ± 4.9	3.5 ± 0.4	3.8 ± 0.6
1.25 cm capsule 84 ± 8 μg/day	8 weeks	4	14.5 ± 2	1.2 ± 0.2	0.8 ± 0.2
0.25 cm capsule 53 ± 9 μg/day	8 weeks	3	8.4 ± 1.1	0	0
Breast cancer patients					
oral 10 mg bid	465 ± 59 days	10	108 ± 23	2.6 ± 0.5	238 ± 58

Tamoxifen and metabolite levels were determined as described in Fig. 2 and Materials and Methods in serum from athymic mice treated with tamoxifen by the different routes of administration and varying doses shown. The release rate of tamoxifen from silastic capsules was estimated from preimplantation weight minus dry capsule weight after removal divided by time implanted. Tamoxifen levels in breast cancer patients were determined in serum from patients receiving the standard 10 mg bid dose for at least 6 months.

17β-estradiol alone for 8 weeks whereas TAM alone (2.5 cm capsule) produced only a small stimulation above control weights. TAM administered from the 0.25, 1.25 or 2.5 cm silastic capsules inhibited 17β-estradiol stimulated uterine weight to that of TAM alone (Fig. 6).

The circulating levels of TAM and metabolites produced by the silastic capsules are shown in Table 1. Administration of TAM from the 2.5 cm silastic capsule produced low 4-hydroxytamoxifen and *N*-desmethyltamoxifen levels compared to TAM. These levels were similar to those seen 24 h after chronic dosing (6 days) with 50 mg/kg TAM administered s.c. in peanut oil. Shorter silastic capsules released smaller amounts of TAM, however a similar pattern of metabolites was produced. Additional small peaks corresponding to 4-hydroxy-*N*-desmethyltamoxifen and *N*-oxide were also detected together with an unidentified peak (Fig. 4).

DISCUSSION

The athymic mouse implanted with human breast cancer cells allows examination of the action of TAM on human tumor growth *in vivo*. Consistent with previous reports [8–10], TAM markedly inhibited estradiol stimulated breast tumor growth. In the present study, this inhibition was seen to be dose related with less inhibition of tumor growth occurring when lower amounts of TAM were administered and lower circulating levels of TAM were produced. Interestingly, estradiol stimulated MCF-7 tumor growth appeared to be more senstive to TAM inhibition than mouse uterine growth although the mouse uterus was weakly stimulated by the prolonged TAM administration whereas the MCF-7 tumor was not.

The 1.0 cm 17β-estradiol silastic capsules used in the present study have previously been shown to produce a constant circulating level of 17β-estradiol of approximately 350 pg/ml [21]. The circulating TAM level produced by the largest silastic capsule (2.5 cm) was therefore approximately 150 times greater than that of the 17β-estradiol which, on the basis of affinities for the estrogen receptor, would be sufficient to prevent 17β-estradiol binding [22]. However, both *N*-desmethyltamoxifen and 4-hydroxytamoxifen were detected in the serum and have been shown *in vivo* to inhibit estrogen stimulated MCF-7 tumor growth [10]. Furthermore, 4-hydroxytamoxifen has a high affinity for the estrogen receptor [23] and is about 100 times more potent than TAM at inhibiting MCF-7 cell growth in culture [24]. 4-Hydroxytamoxifen has previously been suggested to be important in the antiestrogenic action of TAM *in vivo* [25].

The circulating levels of TAM, produced by the largest sustained release preparation (2.5 cm capsule), was only about half the TAM steady-state level achieved in breast cancer patients receiving the standard 10 mg dose of TAM bid whereas 4-hydroxytamoxifen levels were similar. The level of *N*-desmethyltamoxifen on the other hand was more than 60 times higher in breast cancer patients than

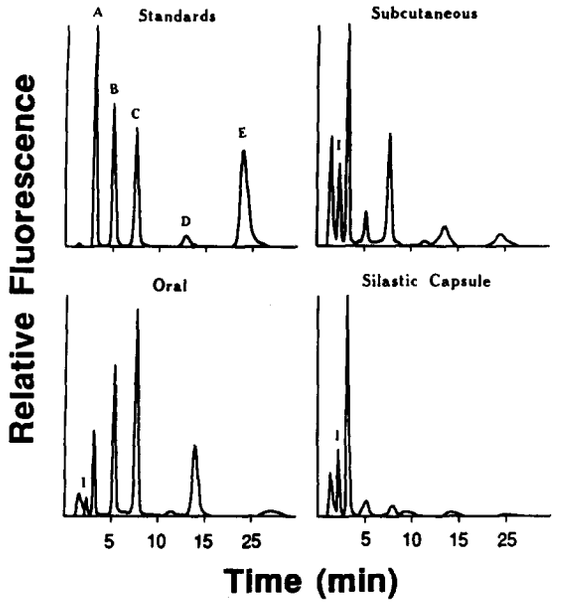

Fig. 4. Chromatography of TAM and metabolites separated as described in Fig. 2 and Materials and Methods. The standards panel shows the resolution of an analyte standard mixture of TAM (100 ng; A), 4-hydroxytamoxifen (100 ng; B), N-desmethyltamoxifen (100 ng; C), 4-hydroxy-N-desmethyltamoxifen (10 ng; D), and tamoxifen-N-oxide (200 ng; E). The oral panel shows the chromatogram of an extract of serum (0.3 ml) spiked with 100 ng of enclomiphene (I) from an athymic mouse treated orally with TAM (200 mg/kg/day for 6 days). TAM, 4-hydroxytamoxifen, and N-desmethyltamoxifen peaks represent 234, 453 and 945 ng/ml, respectively. The subcutaneous panel shows the chromatogram of an extract of serum (0.3 ml) spiked with 100 ng of enclomiphene (I) from an athymic mouse treated s.c. with TAM (200 mg/kg/day for 6 days). TAM, 4-hydroxytamoxifen and N-desmethyltamoxifen peaks represent 112, 72 and 100 ng/ml, respectively. The silastic capsule panel shows the chromatogram of an extract of serum (0.3 ml) spiked with 30 ng enclomiphene (I) from an athymic mouse treated for 8 weeks with a 2.5 cm TAM silastic capsule. TAM, 4-hydroxytamoxifen and N-desmethyltamoxifen peaks represent 47.4, 4 and 6.1 ng/ml, respectively. Relative fluorescence between panels is not comparable. Peaks corresponding to 4-hydroxy-N-desmethyltamoxifen and tamoxifen-N-oxide are seen in samples from each route of administration.

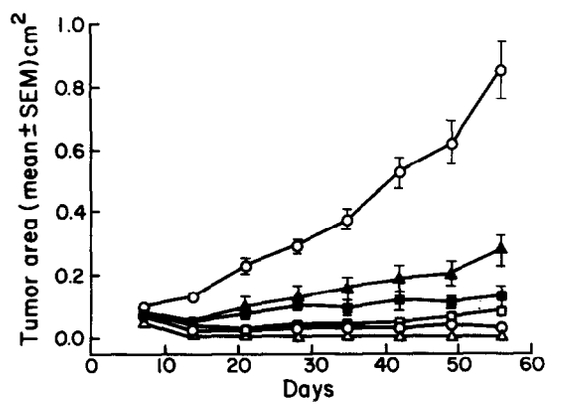

Fig. 5. Growth rates of MCF-7 tumors (n = 12) transplanted in athymic mice and treated by 17β-estradiol silastic capsules (1.0 cm) alone (○—○), TAM (2.5 cm silastic capsule) alone (●—●) or 17β-estradiol silastic capsule in combination with 2.5 cm (□—□), 1.25 cm (■—■) or as 0.25 cm (▲—▲) TAM silastic capsule. Control animals (△—△) received no treatment. TAM release from the silastic capsules is shown in Table 1.

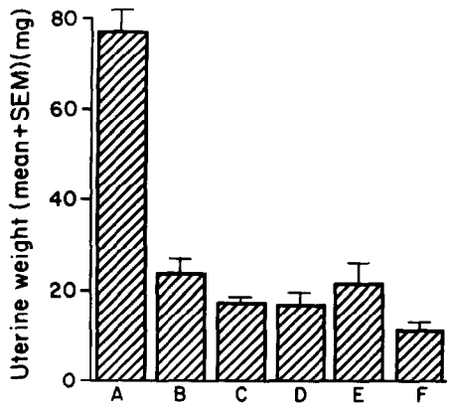

Fig. 6. Effect of long term (8 weeks) 17β-estradiol and TAM treatment either alone or in combination on athymic mouse uterine wet weight. Mice were treated with (A) 1.0 cm 17β-estradiol silastic capsule, alone or in combination with (B) 0.25 cm, (C) 1.25 cm or (D) 2.5 cm TAM silastic capsule or (E) 2.5 cm TAM silastic capsule alone. Control animals (F) received no treatment. The bars represent mean ± S.E. of groups of at least six mice.

the athymic mouse implanted with a 2.5 cm TAM silastic capsule.

Different routes of administration were examined to ascertain the most appropriate method of TAM administration to represent the human breast cancer patient. Repeated large oral doses of TAM produced *N*-desmethyltamoxifen levels greater than those of TAM, a finding consistent with the metabolite profile in the human breast cancer patient. However, unlike the breast cancer patient, 4-hydroxytamoxifen levels were similar to those of TAM and correspond to our findings in the normal mouse [18].

Repeated large s.c. doses (200 mg/kg) of TAM produced circulating levels of the parent compound in the same range as TAM levels in the human breast cancer patient receiving 10 mg TAM bid. However, although *N*-desmethyltamoxifen was the major metabolite, levels were only equivalent to those of TAM itself, and 4-hydroxytamoxifen levels were appreciably higher than in the breast cancer patient. Interestingly, decreasing the dose of TAM (50 mg/kg) administered s.c. produced not only lower circulating levels of TAM but also changed the metabolite profile. Rather than *N*-desmethyltamoxifen levels equivalent to those of TAM as seen with the larger dose, they were reduced to the levels of 4-hydroxytamoxifen. Low levels of both *N*-desmethyltamoxifen and 4-hydroxytamoxifen are characteristic of the metabolite profile produced by the sustained release preparations and may explain why *N*-desmethyltamoxifen has not previously been identified as a major metabolite in the mouse.

Using a long administration time, metabolites corresponding to 4-hydroxy-*N*-desmethyltamoxifen and tamoxifen-*N*-oxide standards were identified in both athymic mouse and human serum. In the

athymic mouse, compounds corresponding to these standards were formed regardless of the route of administration. Recently, 4-hydroxy-*N*-desmethyltamoxifen has been identified in the bile of breast cancer patients receiving TAM [26]. The biological activity of this compound has not yet been examined, however, a high binding affinity for the estrogen receptor may be expected because of the 4-hydroxyl group and the *N*-desmethylated side chain will probably impart antiestrogenic activity [22].

Tamoxifen-*N*-oxide has previously been reported to be formed by rat liver microsomes *in vitro* and may be an intermediate in the formation of *N*-desmethyltamoxifen [19]. However, the presence of this compound in spiked serum standards containing large amounts of TAM suggests either serum can metabolize TAM to *N*-oxide or the extraction procedure converts a small amount of TAM to this compound.

The observation that 4-hydroxytamoxifen is formed from TAM (po) more readily in the mouse [18] and athymic mouse than in the human, may be an important difference between the species, as this metabolite is a considerably more active antiestrogen in culture than TAM [24]. The use of sustained release preparations can produce TAM and 4-hydroxytamoxifen profiles in the athymic mouse that mimic those seen in the human breast cancer patient. However, the *N*-desmethyltamoxifen levels are not representative. Our preliminary report that the profile of TAM metabolites in the rat is similar to the human [18] suggests that the athymic rat implanted with human breast cancer cells could be used as a more accurate model for future studies of the clinical pharmacology of TAM.

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