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Distribution of Tamoxifen and Its Metabolites in Rat and Human Tissues during Steady-State Treatment[1]

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ABSTRACT

A procedure for the extraction of tamoxifen and metabolites from various rat and human tissues was developed and verified. With this method, we determined the drug and metabolite concentrations during one dosing interval in various tissues (brain, fat, liver, heart, lung, kidney, uterus, and testes) of rats given tamoxifen once daily for 3 or 14 days, and in various normal and malignant tissues obtained during surgery or at autopsy from patients with breast cancer treated with tamoxifen.

In the rat, the concentrations of tamoxifen and metabolites in most tissues were 8- to 70-fold higher than in serum. The highest levels were observed in lung and liver; substantial amounts were also recovered from kidney and fat. Fluctuations of metabolites and tamoxifen content in most tissues were observed during one dosing interval, corresponding to a ratio of 4:8 between C_{max} and C_{min}, except in fat and testicular tissues, where the drug concentrations were relatively stable. In addition to tamoxifen, *N*-desmethyltamoxifen, followed by 4-hydroxytamoxifen, 4-hydroxy-*N*-desmethyltamoxifen, and *N*-desdimethyltamoxifen, were abundant in most tissues. In contrast, adipose tissue contained only small amounts of these metabolites.

The concentrations of tamoxifen and metabolites found in human normal and malignant tissues confirmed and extended the conclusions made in the experiments with rats. In humans, levels were 10- to 60-fold higher in tissues than in serum, and relatively high concentrations were detected in liver and lung. Additionally, pancreas, pancreatic tumor, and brain metastases from breast cancer and primary breast cancer retained large amounts of drug. Again, the amounts of demethylated and hydroxylated metabolites were high in most tissues, except in fat. Tamoxifen and some metabolites were also present in specimens of skin and bone tissue. In one patient, significant amounts of drugs could be detected in lung, heart, ovary, and intestinal wall 14 months after withdrawal of tamoxifen, demonstrating efficient retention and slow washout of these compounds in human tissue.

INTRODUCTION

The nonsteroid antiestrogen tamoxifen [*trans*-1-(4β-dimethylaminoethoxyphenyl)-1,2-diphenylbut-1-ene] is a first-line drug in the treatment of estrogen receptor positive breast cancer (1). Tamoxifen has also been considered a therapeutic agent in other cancers (2, 3), in some nonmalignant endocrine diseases (2), and as prophylactic intervention in women at high risk of developing breast cancer (4).

Tamoxifen is extensively metabolized, and several metabolites of tamoxifen have been detected in human serum. Tamoxifen and metabolites formed by demethylation (metabolites X[3] and Z) are the predominating species in serum, whereas the concentrations of the more hydrophilic, hydroxylated metabolites (Y, B, and BX) are low. Metabolism may play a role in tamoxifen action, especially since the hydroxylated metabolites B and BX have a higher affinity for the estrogen receptor than the parent drug (5). A simplified metabolic scheme is depicted in Fig. 1.

The distribution of tamoxifen into tissues and the availability of the drug and active metabolites to target cells are obvious determinants of tamoxifen effects. In serum, more than 98% of the tamoxifen is bound to albumin. The apparent volume of distribution for tamoxifen is high (50 to 60 liters/kg) (6), suggesting extensive distribution into peripheral tissues and the presence of only a minor portion of drug (0.1%) in the serum. These pharmacokinetic properties of tamoxifen may explain why no correlation between plasma levels and clinical response has been observed (7–9). However, in studies in rats, tamoxifen and metabolite concentrations in tumor cells were related to tumor regression (9), and a dose-response relationship for tamoxifen has been demonstrated *in vitro* (10–13) and in female athymic mice and rats (9, 14). Such experimental data motivate investigations of the amount of tamoxifen and metabolites in tissues and distribution into extravasal compartments.

Tissue distribution of tamoxifen has been reported in animals (15–20) and humans (21–24). To our knowledge, only two investigations deal with tissue levels following repeated p.o. dosing of tamoxifen (18, 25), and the variations in tissue levels during one dosing interval have not been investigated. Two studies reported high levels of tamoxifen and metabolite X in breast tumor (23–25) and muscle (23) from patients treated for a sufficient time to obtain steady-state.

We have previously reported that tamoxifen and its metabolites, including the newly discovered metabolite 4-hydroxy-*N*-desmethyltamoxifen, BX (26), are readily distributed into biological fluids from patients receiving chronic tamoxifen treatment. The conjugated, hydroxylated metabolites were the prevailing species in excretory fluids like urine and bile, suggesting a role in drug elimination (6, 26).

The aim of the present work was to investigate the distribution of tamoxifen and its metabolites, including both the demethylated species and the more hydrophilic metabolites formed via hydroxylation, into various tissues of rat and human. Rat tissues were selected on the basis of diversity of lipid content, blood flow, and drug metabolizing activity. The availability of human specimens was restricted to tissues obtained at surgery or autopsy. We first verified the extraction procedure, using various rat and human tissues. With this method, we studied tamoxifen and metabolite concentrations and the kinetics of drug distribution into various tissues from rats given tamoxifen p.o. for 3 and 14 days. The results were compared with data on drug content in normal and malignant tissues obtained from patients given long-term p.o. treatment with tamoxifen.

MATERIALS AND METHODS

Chemicals. Tamoxifen, metabolites Y, B, BX, X, and Z were gifts from Imperial Chemical Industries, PLC, Pharmaceuticals Div., Mac-

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[3] The abbreviations used are: metabolite X, *N*-desmethyltamoxifen; metabolite Y, *trans*-1-(4β-hydroxyethoxyphenyl)-1,2-diphenylbut-1-ene; metabolite B, 4-hydroxytamoxifen; metabolite BX, 4-hydroxy-*N*-desmethyltamoxifen; metabolite Z, *N*-desdimethyltamoxifen; HPLC, high-performance liquid chromatography.

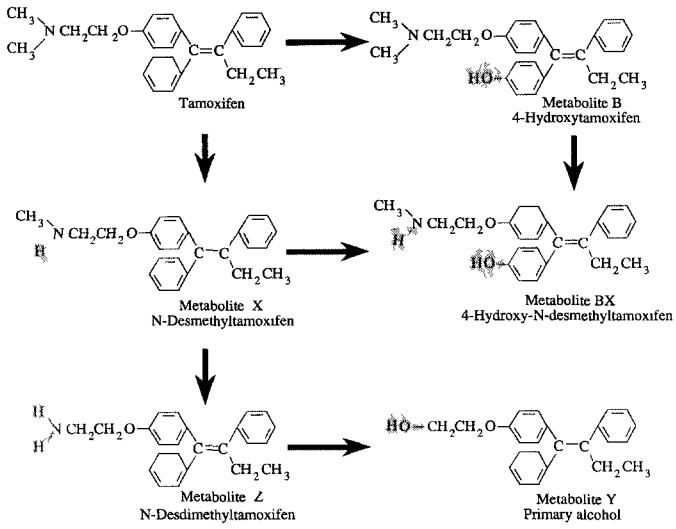

Fig. 1. Proposed metabolic pathways for tamoxifen.

clesfield, UK. "HPLC-grade" acetonitrile was purchased from Rathburn Chemicals, Ltd., Walkerburn, Scotland, UK. Acetic acid and diethylamine were from Merck AG, Darmstadt, Federal Republic of Germany. The reversed-phase analytical column (0.21 x 10 cm) packed with 5-μm particles of octadecylsilane (ODS-Hypersil) was purchased from Shandon, Palo Alto, CA. The precolumn (0.21 x 3 cm) was packed with 5-μm particles of ODS-Hypersil.

Animals. Male and female albino Wistar rats weighing 250 to 350 g, supplied by Møllegård, Ltd., Skensved, Denmark, were used. They were kept in metal wire cages in a room with 12-h light-dark cycles, and at a constant temperature of 20 ± 3°C. The animals were acclimatized for at least 5 days under these conditions before the start of the experiments.

Animal Study. Tamoxifen citrate was dissolved in propylene glycol (0.2 mg/ml). The animals were given doses of 1 mg/kg body weight/day via an esophageal tube for 3 or 14 days. On the third day of treatment, three male and three female rats were sacrificed 0, 2, 4, 6, 12, and 24 h after the last tamoxifen dose. Six female rats received tamoxifen for 14 days, and groups of three animals were killed 6 and 24 h after the last dose. Samples from brain, liver, heart, lung, retroperitoneal fat, kidneys, testes (male rats), and uterus (female rats) were immediately dissected and frozen in liquid N_2 and stored at −80°C until sample processing.

Patients and Collection of Tissue Samples. Samples from normal tissue and tumor were obtained during surgery or at autopsy from patients occasionally succumbing while receiving chronic tamoxifen. Since tamoxifen is used as first-line treatment of breast cancer, the access to such material was limited. The tissue samples from 14 patients, age 28 to 89 years, were collected from four Norwegian hospitals during the period from May 1987 to November 1990.

We determined the amounts of tamoxifen and its metabolites in 37 tissue samples from 14 patients. Nine patients were in steady-state (treatment for more than 35 days) and three patients had received tamoxifen for 7 to 13 days. Patients BiE and AM had received tamoxifen for 3 to 3.5 years at the time of tissue sampling. Patients EB and ED had a tamoxifen-free period of 28 days and 14 months, respectively, prior to tissue sampling. Tissue specimens from all other patients were obtained within one half-life of tamoxifen [4 to 60 h; $T_{1/2} > 7$ days (21)] (Table 1).

Patient EB received alternating tamoxifen or medroxyprogesterone acetate, each drug for 8 weeks. Patient ED was treated consecutively with megestrol, Adriamycin, and 5-fluorouracil in combination with mitomycin in the tamoxifen-free period.

Tissue specimens obtained during surgery were washed in saline and immediately frozen in liquid nitrogen. Blood samples were drawn simultaneously if possible, and the serum was prepared and frozen. Biological materials were stored at −80°C until analysis.

Patient characteristics and tissue sampling (at operation or autopsy) are listed in Table 1.

Extraction and Recovery of Tamoxifen and Metabolites. Recovery studies were performed in brain, lung, liver, kidney, heart, and adipose tissue from rat and human. The human tissues were obtained from autopsy material.

Tissue samples weighing about 2 g were homogenized (1:5, w:v) in 50 mM Tris-HCl, pH 7.4, at 20,000 rev/min, using an Ultra Turrax homogenizer. The volume of the homogenization buffer was increased for the skin samples (1:10, w:v) and for tissue specimens weighing less than 2 g, *i.e.*, uterus from rats (1:40, w:v), tumor and breast glandular tissue from patient SR (1:10, w:v), and tumor tissue from patient RT (1:20, w:v). The homogenate was mixed with an equal volume of 100% acetonitrile, precipitated protein was removed by centrifugation, and supernatants were transferred to sample vials, capped, and analyzed.

Bone tissue was obtained from a patient who received a femoral head prosthesis. Cortical bone was obtained by tangential sawing in the cortex of the femoral head. The sawdust was homogenized as described above.

The recovery of tamoxifen and its metabolites was evaluated as

Table 1 *Patient characteristics and drug treatment*

Patient	Age (yr)	Tamoxifen treatment: Duration (days)	Tamoxifen treatment: Daily dose (mg)	Interval between last dose and tissue sampling	Other drugs
RA	46	7	30[a]	28 h	Dexamethasone
EB	68	56	20[b]	28 days	Medroxyprogesterone acetate Estriol (intravaginal)
BiE	89	1263	20	24 h	None
BoE	73	249			None
ED[c]	28	210	30	418 days	Diflunisal Furosemide Amphotericin Atenolol Bromhexine
BH	76	371	20	27 h	Furosemide Triazolam Methenamine Nitrofurantoin
AM	75	935	30	26 h	None
AN	46	7	30	28 h	Dexamethasone
BO	71	180	50[d]	4 h	Furosemide
SR	71	227	20	27 h	Furosemide Triazolam Methenamine Nitrofurantoin Dienestrol (intravaginal)
MS[c]	67	188	30	49 h	Morphine Haloperidol Triazolam Trimethoprim Diazepam Hydrocodone
TS	49	120	20	26 h	None
RT	72	592	40	25 h	Warfarin
OW	61	13	80[e]	61 h	Cephalexin Metoclopramide

[a] Sixty mg daily for the initial 3 days.
[b] Alternating 8 weeks tamoxifen and 8 weeks medroxyprogesterone acetate.
[c] Samples taken during autopsy. The samples from the other patients were obtained during operations or diagnostic procedures.
[d] Twenty mg at the day of operation.
[e] OW received only two doses of tamoxifen: 90 mg 13 days before operation and 80 mg 61 h before operation.

follows. A solution was prepared containing 100 μg/ml of tamoxifen and metabolites B, BX, X, and Z in methanol. Aliquots of 10 μl of this solution were added to 10 ml of tissue homogenate (tube A), of 100% acetonitrile (tube B), and to 100% acetonitrile (tube C), giving final concentrations of 100 ng/ml for each compound. Homogenate spiked with drug (in tube A) was incubated at 37°C for 15 min in a shaking water bath. Then the homogenate was mixed with an equal volume of acetonitrile. The acetonitrile containing drug (tubes B and C) was mixed with an equal volume of either homogenate (tube B) or water (tube C). Precipitated protein was removed by centrifugation, and the supernatants were analyzed by HPLC. The concentration of drugs in the acetonitrile diluted with an equal volume of water (tube C) is taken as 100%.

High-Performance Liquid Chromatography. We used a liquid chromatography system which was developed for the determination of tamoxifen and metabolites in serum (27). The assay was modified to improve the separation and isolation of the early eluting, hydrophilic metabolites. The method (27) and the modification are briefly described below.

Large samples of 250 μl were injected into a small precolumn with an internal diameter of 0.21 cm, packed with 5 μm octadecylsilane material. The length of this column was increased from 2 cm to 3 cm. The samples were on-column concentrated by equilibrating the precolumn with 50% acetonitrile in water, containing 6 mM acetic acid and 4 mM diethylamine. The analytes were then directed into an analytical ODS-Hypersil column (0.21 x 10 cm) by changing the mobile phase followed by column switching. The composition of the mobile phase was 91% acetonitrile containing 1 mM acetic acid and 0.67 mM diethylamine, and the flow rate was 0.7 ml/min. Tamoxifen and its metabolites were eluted in the following order: metabolite Y, metabolite B, metabolite BX, tamoxifen, metabolite Z, and metabolite X. These compounds were post-column converted to fluorophors by ultraviolet illumination while passing through a 10-m transparent knitted PTFE reaction coil inside the model Beam Boost, manufactured by ict Handels GmbH, Frankfurt, Federal Republic of Germany. The fluorescence was monitored using an HPLC detector, model RF-535, from Shimadzu, Kyoto, Japan. The excitation wavelength was 251 nm, and the emission wavelength was 360 nm.

The chromatograms of the tissue extracts contained a broad solvent front which prevented the determination of metabolite Y.

Liquid Chromatography/Mass Spectrometry. Homogenates were extracted with 10 volumes of hexane:butanol (98:2, v:v). The supernatant was evaporated in plastic beakers at 55°C under nitrogen, redissolved in 1 ml 50% acetonitrile, and centrifuged. The supernatant was transferred to sample vials, capped, and analyzed. The analytical column was connected to a liquid chromatography/mass spectrometry thermospray system (model 201; Vestec, Houston, TX). Before entering the thermospray, the effluent from the column was mixed with 0.1 M ammonium acetate, delivered at a rate of 0.3 ml/min via a zero dead volume T-connector.

RESULTS

Recovery of Tamoxifen and Metabolites from Tissue Extract. Aqueous homogenates from various human and rat tissues were spiked with tamoxifen and its metabolites and incubated at 37°C for 15 min. The amounts recovered were determined and compared with those determined in a solution of 50% acetonitrile spiked with the same amount of drugs. These values were taken as 100%.

For most tissues, the recoveries were between 70 and 100%, and there was a trend toward a slightly higher recovery for hydroxylated metabolites B and BX than for tamoxifen itself and metabolites X and Z. Notably, the variability of the recovery from adipose tissue was higher (≥8%) than for other tissues (usually <5%). Similar results were obtained for human and rat tissues (Table 2).

Recovery below 100% (Table 2) may be due to trapping of drugs in the protein pellet or incomplete extraction from tissue particles. We tried to differentiate between these causes by comparison of recovery when either the homogenate or the acetonitrile was spiked with drugs. Essentially, no difference was noted (data not shown).

Homogenates of liver and brain of rats treated with tamoxifen for 3 days were kept for 5 h at 4 or 37°C. No degradation or interconversion of tamoxifen and metabolites was observed (data not shown).

Distribution of Tamoxifen and Metabolites into Tissues of Rats. Rats were given tamoxifen p.o. for 3 days (female and male rats) and for 14 days (male rats). In all tissues except fat, essentially the same amounts of drugs were found after 3 days and 14 days of treatment, suggesting that steady-state is obtained within 3 days (Fig. 2). The content of tamoxifen and its metabolites in tissues was orders of magnitude higher than in serum. Assuming that 1 ml of serum is equivalent to 1 g of

Table 2 *Recovery of tamoxifen and metabolites from rat and human tissue*[a]

Tissue	Recovery (%)				
	TAM[b]	B	BX	X	Z
Brain					
Human	103 ± 1	102 ± 3	110 ± 5	107 ± 2	99 ± 2
Rat	85 ± 3	100 ± 8	103 ± 2	86 ± 1	76 ± 2
Adipose tissue					
Human	73 ± 10	94 ± 9	102 ± 5	83 ± 11	86 ± 13
Rat	85 ± 9	69 ± 12	108 ± 5	102 ± 9	95 ± 7
Lung					
Human	88 ± 3	89 ± 4	87 ± 3	89 ± 3	89 ± 4
Rat	96 ± 8	103 ± 3	106 ± 3	94 ± 3	87 ± 4
Liver					
Human	89 ± 4	99 ± 3	81 ± 5	86 ± 4	79 ± 2
Rat	89 ± 4	81 ± 7	109 ± 5	87 ± 4	80 ± 7
Kidney					
Human	87 ± 1	89 ± 1	83 ± 2	99 ± 2	98 ± 2
Rat	85 ± 7	71 ± 5	103 ± 1	88 ± 3	78 ± 3
Testes					
Human					
Rat	86 ± 3	69 ± 3	92 ± 1	84 ± 4	77 ± 4
Heart					
Human	80 ± 2	88 ± 6	102 ± 4	93 ± 3	82 ± 1
Rat	89 ± 2	102 ± 3	98 ± 4	87 ± 2	73 ± 3

[a] Values are given as mean ± SD of 6 replicates.
[b] TAM, tamoxifen.

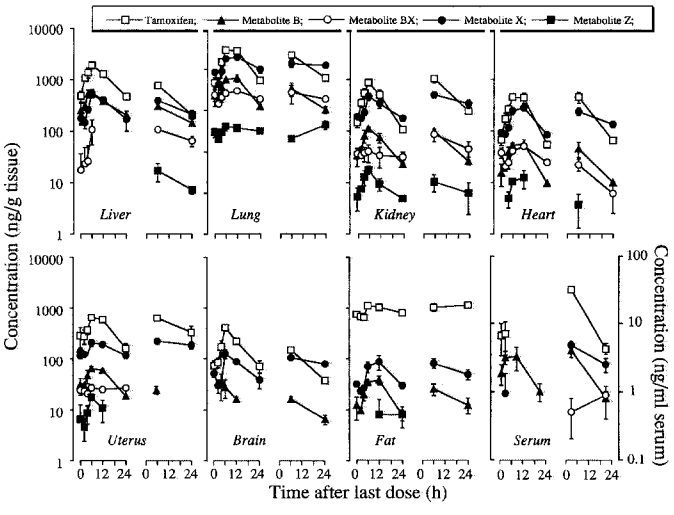

Fig. 2. Concentrations of tamoxifen and its metabolites during two dosing intervals in various tissues and serum of rats given p.o. doses of tamoxifen for 3 or 14 days. The tamoxifen dose was 1 mg/day/kg body weight. For each tissue (serum), two sets of data are shown: at *left*, levels after treatment during the preceding 3 days; at *right*, levels after 14 days of treatment. Data are given as mean ± SEM.

tissue, the ratios between tissue and serum levels for tamoxifen were in the range 8 to 70 for most tissues. Especially high concentrations were detected in lung and liver, intermediary levels in kidney and fat tissue, and lower amounts in brain (Fig. 2).

Within one dosing interval, marked fluctuations in tissue concentrations of tamoxifen and metabolites were observed in rats receiving steady-state treatment. For example, the C_{max}:C_{min} ratios for tamoxifen in female lung and liver were 6.3 and 4.1, respectively. In contrast, minor fluctuations were observed for the tamoxifen levels in fat tissue and testes within one dosing interval (Fig. 2). In these tissues, C_{max}:C_{min} ratios for tamoxifen were 1.7 and 2.0, respectively (data not shown).

The demethylated and hydroxylated metabolites were abundant in most tissues, except in fat tissue, where tamoxifen was the predominating species. The concentrations of the hydroxylated metabolites (B and BX) and the demethylated metabolite X were especially high in lung and liver and kidney (Fig. 2), whereas the hydroxylated metabolite BX was not detected in brain and adipose tissue.

Human Liver, Lung, Pancreas, Brain, and Adipose Tissue. Liver and pancreas were obtained from two patients (MS and OW), brain from four patients (RA, AN, BO, MS), and adipose tissue from seven patients. Lung tissue was obtained from only one patient (MS). She had a distal obstruction of the small intestine, which might have influenced the enterohepatic circulation of drug, and thereby tissue levels (Table 1).

The concentrations of tamoxifen and its metabolites were particularly high in liver, lung, and pancreas, and somewhat lower in brain. In those patients from whom serum was obtained, the tissue concentrations were 10- to 60-fold higher than the serum concentrations. These tissues contained substantial amounts of the demethylated metabolite X, the concentration of which often exceeded that of the parent drug, but also contained significant amounts of the hydroxylated species B and BX. A similar metabolite profile was observed in brain. The quantitative relations between drug and metabolites in these tissues, given in terms of the ratio between concentrations, were often 1:1.2:0.2:0.05:0.01 for tamoxifen:X:Z:BX:B. Notably, adipose tissue accumulated tamoxifen, and to a lesser degree metabolite X, whereas only minor amounts of the hydroxylated metabolites B and BX could be detected. In patient AM (treated for 935 days), the metabolite X concentration in fat tissue was relatively high (Table 3). Thus, the tissue distribution of tamoxifen and metabolites shows similarities with that observed in the rat. One difference was noted: metabolite X seemed to be more abundant in human compared with rat tissues.

Various Nonmalignant Human Tissues. Skeletal muscle, postmenopausal ovary, and breast glandular tissue also contained significant amounts of tamoxifen and its metabolites. We found low concentrations, equaling those in serum, in cortical bone from one patient (BoE), and in epidermal tissue from two patients (AM and BoE), but higher concentrations in skin from patient BiE, who had been treated with tamoxifen for 3.5 years (Table 3).

The low content in skin and cortical bone may be related to inefficient extraction of drugs from these tissues, whereas the high levels of tamoxifen in trabecular bone may suggest the presence of fat tissue in this specimen.

The quantitative relation between drug and metabolites resembles that reported above for human liver, pancreas, and brain (Table 3).

Human Malignant Tumors, Including Breast Cancer. Tissue samples from primary breast cancer were obtained from patients BH and SR. Tamoxifen (12,173 ng/g) and metabolite X (23,656 ng/g) were extremely abundant in the specimen from patient BH, *i.e.*, 100 times the concentrations found in serum. The other serum metabolites were present in lower but significant amounts (Table 3).

The hydroxylated metabolites B and BX eluted in front of tamoxifen, and their identities were confirmed by liquid chromatography/mass spectrometry by comparing retention times and the $(M+1)^+$ ions (388 and 374 m/z, respectively), with those of authentic standards. One additional peak eluted in this region of the chromatogram of breast cancer tissue. The material had an $(M+1)^+$ ion of 388 and may represent another hydroxylated metabolite of tamoxifen, yet to be identified (data not shown).

Three metastatic breast tumors investigated contained high concentrations of tamoxifen and its metabolites, and so did additional three metastases from breast cancer localized within brain and a metastasis from a clear cell carcinoma (patient MS). One primary pancreatic tumor (from patient OW) was analyzed and contained amounts of drug and metabolites similar to the levels found in normal pancreas from the same individual (Table 3).

The quantitative relations between the compounds in these tumors were similar to those observed in most other human tissues. Metabolite X was the most abundant species, followed by tamoxifen, Z, BX, and B, in that order (Table 3). The pancreatic tumor of patient OW was an exception to this rule, since it contained more tamoxifen than metabolite. This patient, however, had received only two doses of tamoxifen, and steady-state had not been obtained.

Tissue Levels after Tamoxifen Withdrawal. We obtained a sample from endometrium from patient EB 28 days after cessation of tamoxifen dosing. Metabolite X, followed by tamoxifen, metabolite Z, and BX, were present in significant amounts, and the tissue:serum ratios were high (>40) (Table 3).

Tissues specimens (from lung, brain, pancreas, uterus, duodenum, ovary, and heart) from patient ED were obtained 14 months after stopping tamoxifen treatment. Trace amounts of tamoxifen metabolites were detected with the routine extraction procedure. When the samples were extracted with hexane:butanol (as described in "Materials and Methods") to enrich tamoxifen and its metabolites, concentrations of tamoxifen and metabolites B, BX, X, and Z up to 70 ng/g tissue could be detected. The limited amounts of tissue available did not allow the optimalization and verification of the extraction procedure, and more quantitative estimates of drug concentrations were not obtained.

DISCUSSION

Method. Extraction of aqueous tissue homogenate with an equal volume of acetonitrile gave 70 to 100% recovery of tamoxifen and derivatives added to the homogenate (Table 2). These compounds were stable in the extracts, and the possibility of low recovery of drugs, present in the intact tissue *in vivo*, seems unlikely. Thus, the simple procedure originally developed for serum determination (27) could be adopted for the determination of the drug in tissues.

We could not determine metabolite Y in tissue extracts because of interference from the solvent front. Metabolite Y is demonstrated in plasma from rat and human (28) (Fig. 2; Table

Table 3 *Distribution of tamoxifen and its metabolites in tissues and serum*

Patient	Days of treatment	Sample	Concentration (ng/g)					
			TAM[a]	Y[b]	B	BX	X	Z
RA	7	Brain metastasis[c]	1,332		46	152	2,250	126
		Brain	882		26	0	1,242	64
		Serum	78	7	0	0	142	11
EB	56	Endometrium	464		12	83	2,139	175
		Serum	7	0	0	0	52	5
BiE	1,263	Metastasis[c]	1,458		29	342	1,824	474
		Subcutaneous fat	1,674		5	0	173	5
		Skin	1,082		22	178	1,153	244
		Serum	122	5	2	14	153	29
BoE	249	Subcutaneous fat	1,920		4	6	456	126
		Muscle	1,338		5	48	702	192
		Skin	164		0	13	117	22
		Cortical bone	386		0	11	385	70
		Red bone marrow[d]	2,474		0	8	819	197
		Serum	116	2	1	12	180	39
BH	371	Primary breast cancer	12,173		168	1,134	23,656	7,322
		Subcutaneous fat	2,376		8	18	893	201
		Serum	94	1	3	0	210	53
AM	935	Metastasis[c]	4,536		14	63	2,532	465
		Subcutaneous fat	2,718		10	33	2,016	390
		Skin	300		0	13	283	30
		Serum	154	4	1	2	280	42
AN	7	Brain metastasis[c]	1,457		32	67	2,189	86
		Brain	854		16	43	1,382	68
		Serum	40	1	6	0	48	4
BO	180	Brain metastasis[c]	1,283		67	82	1,910	336
		Brain	1,491		46	143	2,898	552
		Serum	118	30	3	24	216	42
SR	227	Primary breast cancer	1,611		16	164	2,519	606
		Breast glandular tissue	3,390		0	0	625	114
		Subcutaneous fat	1,777		0	0	372	73
		Serum	115	6	4	6	823	55
MS	188	Brain	3,462		41	115	7,080	1,326
		Lung	4,662		38	116	11,484	2,322
		Ovary	1,656		24	91	2,958	432
		Liver	6,834		89	155	14,988	3,126
		Pancreas	5,628		106	261	10,320	1,788
		Omental metastasis[e]	4,980		37	123	8,436	1,206
TS	120	Subcutaneous fat	426		0	0	102	4
		Serum	21	0	0	0	53	5
RT	592	Metastasis[c]	2,104		36	226	3,600	872
OW	13	Omental fat	918		0	0	20	0
		Subcutaneous fat	392		0	0	21	0
		Liver	1,286		27	61	1,235	109
		Pancreas	571		11	0	340	14
		Pancreatic cancer tissue	768		10	6	557	26
		Duodenal mucosa	208		0	13	154	0
		Serum	20	0	0	0	21	0

[a] Assuming that 1 ml serum is equivalent to 1 g of tissue. TAM, tamoxifen.
[b] The chromatograms of the tissue extracts contained a broad solvent front which prevented the determination of metabolite Y.
[c] Metastasis from breast cancer.
[d] A mixture of fat, hemopoetic cells, and bone tissue including osteoblasts and osteoclasts.
[e] Clear cell carcinoma.

3). In rat, it has less affinity for the estrogen receptor than tamoxifen and expresses estrogen antagonistic as well as agonistic properties (28).

Metabolite E, which is a tamoxifen metabolite cleaved at the ether linkage, is not detected by our method. It is resolved by the HPLC method of Langan-Fahey *et al.* (29), but they could not detect this compound in serum from their patients. By use of gas chromatography/mass spectrometry, trace amounts have been demonstrated in dog bile (16) and tentatively identified in human plasma (30). The occurrence of metabolite E may be of some importance, since it has been reported to act as a pure estrogen agonist in rat and mouse (31).

Tissue Distribution in Rat and Human. The metabolic disposition of tamoxifen in rat resembles that in human, whereas in mouse, metabolite B is a major serum metabolite. Therefore, rats seem to be preferable to mice in studies on tamoxifen metabolism (25) and were chosen as an experimental animal in this study.

In rats, the highest levels of tamoxifen and its metabolites were observed in lung and liver, followed by fat, kidney, and

uterus. The tissue levels were 8- to 70-fold higher than the corresponding serum concentrations, assuming that 1 ml of serum is equivalent to 1 g of tissue (Fig. 2). A similar tissue distribution was found in patients (Table 3) and has previously been reported for radioactive tamoxifen given i.v. in mice (17). In tissues and serum from both rats and humans, tamoxifen or metabolite X was the prevailing species, whereas the amounts of metabolite Z and the hydroxylated metabolites (B and BX) were lower. Adipose tissue contained high concentration of the parent drug and low concentrations of metabolites (Fig. 2; Table 3).

There were differences in the metabolite profile between rat and human. In rat tissues, the amount of the hydroxylated metabolite B equaled or often exceeded that of hydroxylated metabolite BX (Fig. 2), whereas metabolite BX was the most abundant hydroxylated species found in human tissues (Table 3). In brain and fat tissue, metabolite BX was occasionally detected in humans but not in rats (Fig. 2; Table 3). The demethylated metabolite X was abundant in human tissues, where the concentration often exceeded that of tamoxifen itself. In contrast, in rat tissues, tamoxifen was the most abundant species. *Our results are in agreement with earlier reports on the* abundance of metabolite X and tamoxifen in human plasma and tissues (23, 24). They also establish a different metabolic fate of tamoxifen in tissues from rat and human, which in turn may cause different pharmacodynamics.

Tissue Distribution and Pharmacokinetics. The apparent distribution volume (V_d) for tamoxifen in humans is about 50 to 60 liters/kg (6), which indicates that most drug (99.9%) is present in peripheral compartments, suggesting extensive tissue binding. This estimate is supported by the finding in the present paper demonstrating a ratio ranging from 8:1 to 70:1 between the concentrations in tissues and serum. A distribution volume of 50 liters/kg corresponds to a mean (overall) tissue:serum ratio of about 50. Such pharmacokinetic features are typical for highly lipophilic, basic drugs (32) like tamoxifen and may also be related to the long half-life of tamoxifen in man (21) and experimental animals (25).

Robinson *et al.* (25) found that the concentration of tamoxifen in tissues was more than 100 times higher than in plasma. They gave rats tamoxifen doses which were 200-fold higher than those used in the present study. A dose dependent pharmacokinetics should be considered.

The serum levels of tamoxifen were often below the detection limit of the assay. This agrees with previous reports, where higher doses were given than those used by us, and tamoxifen was not detected in plasma from mice and rats (*14, 18*).

Tamoxifen and its metabolites are extensively bound to various tissues (Fig. 2; Table 3), and this may account for large peripheral pharmacokinetic compartments. These compartments, or a major portion thereof, are probably in equilibrium with plasma, because in most rat tissues, the concentrations of tamoxifen and its metabolites fluctuate in parallel with the plasma levels during one dose interval. Furthermore, in humans, the metabolite profile in serum resembles that observed in tissues (Table 3), and this seems to be the case in rats (Fig. 2)

In rat adipose tissue, the fluctuations in the tamoxifen and metabolite concentrations during one dosing interval were less than those observed in other tissues of the rat. Tamoxifen was the predominating species; only small amounts of the demethylated metabolites (X and Z) and the hydroxylated metabolite B were observed. The hydroxylated metabolite BX was not detected (Fig. 2). Similar findings were made in human adipose tissue, except that metabolite X was relatively more abundant (Table 3). These findings may be explained by slow distribution of tamoxifen into fat tissue, where this lipophilic drug partitions into lipid droplets and is preserved due to low activity of drug metabolizing enzymes. Thus, adipose tissue may represent a "deep" peripheral compartment (33), which may sequester drug during treatment and after drug withdrawal. In postmenopausal women, fat tissue may represent 25 to 50% of total body mass (34), indicating the quantitative importance of such a drug depot.

In patient ED, we could demonstrate significant amounts of tamoxifen and metabolites B, BX, X, and Z in lung, postmenopausal ovary, heart, and duodenal wall tissue 14 months after tamoxifen withdrawal. Such residual drug levels in tissues may partly be related to replenishment from a "deep" compartment.

Determinants of Drug Retention in Tissues. In general, blood flow and lipid content are important determinants of tissue distribution of lipophilic compounds (35). Tamoxifen and its derivatives would also be expected to partition into the hydrophobic domains of the cell membrane.

Lung contained the highest amounts of tamoxifen and its metabolites (Fig. 2), and these compounds add to certain lipophilic basic amines which have been shown to accumulate in lung tissues (36). Binding sites for basic drugs in lung have not been identified, but the retention may be related to interaction with phospholipids that are constituents of the pulmonary surfactant (37).

Tamoxifen and, to a greater extent, some hydroxylated metabolites bind with high affinity to estrogen receptor present in target tissues, like breast tissue, uterus, and endometrium (5). The low capacity of the receptor sites (38) makes it unlikely that such binding may totally account for the large amounts of tamoxifen and metabolites detected in breast tumor of patient BH and other patients and in mammary tissue of patient SR (Table 3).

Large amounts of tamoxifen were also recovered from drug metabolizing tissues like liver and kidney (Fig. 2; Table 3), which also were rich in the hydroxylated metabolites B and BX. Tamoxifen binds to so-called "antiestrogen binding sites" (39) which may be associated with the drug metabolizing enzyme system in the liver (40). Other acceptors for antiestrogens are protein kinase C, calmodulin and calcium channels (41, 42), muscarinic receptors and receptors for histamine and neurotransmitters (42), and membrane lipids (43).

Tissue Levels in Relation to Clinical Use. We detected high *concentrations of tamoxifen and metabolites in primary and* metastatic breast cancer. This is of obvious relevance to the clinical response, and in agreement with the finding of Daniel *et al.* (23). The therapeutic effect in breast cancer may not be mediated solely through interaction with estrogen receptor, since 10% of estrogen receptor negative tumors responded to tamoxifen (44, 45). However, estrogen receptor independent cytotoxic effects of tamoxifen have been demonstrated in *in vitro* studies. These may be operating at the high concentrations of tamoxifen detected in tissues.

The observation in the present study that tamoxifen metabolites may be retained for several months after drug withdrawal may have consequences for subsequent treatment regimens. Cross-over data from randomized trials of breast cancer treatment indicate that the highest overall response rate is obtained if tamoxifen is used as a first-line drug (46), and patients who relapse after adjuvant tamoxifen treatment have a lower re-

sponse rate to tamoxifen and a shorter median time to disease progression than a control group that has not received adjuvant endocrine treatment (47). It is conceivable that prolonged exposure to low levels of tamoxifen may sustain or produce tumor cells resistant to tamoxifen. Finally, antiestrogens may interfere with the radioligand binding assay for estrogen receptor activity, the result of which may be of importance for the selection of subsequent treatment regimens.

Tamoxifen and most metabolites were present in brain from rat and human (Fig. 2; Table 3). In rat brain, fluctuations were observed during one dosing interval, and the variations paralleled the serum concentration curve (Fig. 2), suggesting that tamoxifen readily crosses the blood-brain barrier and distributes readily into normal brain tissue. In human, tamoxifen and its metabolites were present in equal or even higher amounts in cerebral metastasis compared with normal brain tissue (Table 3) (48). There are only sporadic reports on the effectiveness of tamoxifen against brain metastasis from breast cancer (49–51), and our findings should encourage further studies to evaluate the response to tamoxifen.

A tissue sample from pancreas tumor was obtained from one patient (OW), who had received only two doses of tamoxifen before tissue sampling. The concentrations of tamoxifen and metabolites were higher than in normal pancreas and approached those observed in liver (Table 3). Because estrogen receptor activity has been found in human pancreatic adenocarcinoma (52, 53), tamoxifen treatment was tried in pancreatic carcinoma, but conflicting results were obtained (3). High tissue level may produce both an estrogen receptor dependent and an independent kill of cancer cells (48), and different dosing regimens should be tested.

The observation that tamoxifen and metabolites are present in bone from patients deserves attention, since estrogens may play a role in the maintenance of bone density, possibly through the interaction with estrogen receptor in osteoblasts (54). Tamoxifen has estrogen-like properties in bone tissues (55) and seems to reduce rather than promote postmenopausal bone loss (56).

Summary and Conclusion. Tamoxifen and its metabolites are extensively distributed into rat and human tissues, corresponding to a tissue:serum concentration ratio in the range 8 to 70, and consistent with a high distribution volume of the drug. High concentrations were detected in lung and liver, but substantial amounts of drug were also found in brain and fat tissue and in several other normal and malignant tissues. Tissue kinetics in rat and similar metabolite profiles in most tissues suggest an exchange of tamoxifen and metabolites between most tissues, and between serum and tissues, including brain. In contrast, fat tissue contains low levels of metabolites and seems to sequester tamoxifen; it may function as a "deep" compartment. Data on distribution of tamoxifen and metabolites should influence the design of chemotherapeutic regimens, for example for the treatment of brain metastases.

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