trans-4-Hydroxytamoxifen Concentration and Metabolism after Local Percutaneous Administration to Human Breast

Pierre Mauvais-Jarvis, Nicole Baudot, Damienne Castaigne, Pierre Banzet, and Frederique Kuttenn

Department of Endocrinology and Reproductive Medicine, Hôpital Necker, 149, rue de Sèvres, 75743 Paris Cedex 15 [P. M-J., F. K.], Department of Biochemistry, Hôpital La Pitié, 91, bd de l'Hôpital, 75013 Paris [N. B., F. K.], Institut Gustave Roussy, 39-41, rue Camille Desmoulins, 94800 Villejuif [D. C.], and Service de Chirurgie Plastique et Reconstructive, Hôpital St. Louis, 1, avenue Claude Vellefaux, 75010 Paris [P. B.] France

ABSTRACT

trans-4-Hydroxytamoxifen (4-OHTAM), a very active metabolite of the antiestrogen tamoxifen, was percutaneously administered to the affected breast of nine patients before surgery for breast cancer in order to evaluate 4-OHTAM absorption through the skin and its subcellular localization and metabolism. After percutaneous administration of 80 μCi, 4-[3H]-4-OHTAM was detected in breast tissue. It was especially concentrated in tumor tissue and nuclear and cytosolic fractions, in which it remained unmetabolized except for limited isomerization from the trans to the cis form. In contrast to breast tissue, concentrations of radioactivity remained low in plasma but with a high proportion of metabolites. In another experiment, tamoxifen was percutaneously administered over the breast of 5 patients, resulting in tissue retention weaker and shorter than after 4-[3H]-4-OHTAM. In addition, 4-[3H]-4-OHTAM was administered to either breast or abdominal skin; the appearance of radioactivity in plasma and urine was delayed after administration to the breast in comparison with administration to the abdomen.

It therefore appears that 4-OHTAM passes through the skin and is concentrated in receptor structures of breast tissue, thus avoiding the hepatic metabolism subsequent to p.o. administration. We suggest that local percutaneous administration of this active antiestrogen could be useful in the treatment of hormone-dependent benign breast diseases.

INTRODUCTION

The antiestrogen TAM is a triphenylethylene compound that is widely used in the treatment of human breast cancer (1-3). However, metabolic studies suggest that one of the routes of TAM metabolism in vivo involves conversion to a monohydroxy metabolite, 4-OHTAM, that has a higher affinity for estrogen receptor in its trans isomer form (Z) and is an even more potent estrogen antagonist than trans-tamoxifen (1-4-(2-dimethylaminoethoxy)phenyl]-1,2-diphenyl-but-1(Z)-ene) (4-9). In addition, 4-OHTAM shows greater efficacy in vitro than in vivo (8, 10, 11). This is consistent with the possibility that 4-OHTAM could be readily conjugated and inactivated by the liver (12). Confirming the studies of Jordan et al. (5), Jordan and Allen (6), and Allen et al. (7) recent data on MCF-7 cells (10) have shown that the affinity of 4-OHTAM for the estrogen receptor is 100 times greater than that of TAM. In addition 4-OHTAM is more potent than TAM in suppressing cell growth. Therefore the use of 4-OHTAM would be of interest in human beings but is limited by its inactivation in the liver when administered p.o.

Previous studies have reported that progesterone, when topically applied to the breast in an alcoholic solution, may be retained by the gland before its release into the systemic circulation (13, 14). This percutaneous route of progesterone administration is useful in the treatment of benign breast diseases (15, 16) because of the local antiestrogenic activity of progesterone (17, 18).

In the present study, skin permeability to trans-4-OHTAM was evaluated after topical administration over the breast, and the subsequent distribution of 4-OHTAM in subcellular fractions of breast tissue containing receptors for estrogens and antiestrogens was measured. Results obtained confirm our preliminary data (19).

MATERIALS AND METHODS

Chemicals and Materials

Nonradioactive TAM, 4-OHTAM, desMeTAM, desMeOHTAM, and bisphenol were kindly provided by ICI, Ltd. (Macclesfield, United Kingdom). Pure trans-4-[3H]-4-OHTAM (specific activity, 76 Ci/mmol) was obtained from Amersham and [3H]TAM (specific activity, 75.6 Ci/mmol) from New England Nuclear (Boston, MA). They were purified before use on TLC in 2 solvent systems as described by Robertson et al. (9). These purification steps permitted the pure trans isomers to be isolated.

The buffers used were Tris-HCl-EDTA (GTED buffer), GTED-sucrose, and GTED with 0.4 M KCl.

Patients

Twelve women with mammary carcinoma volunteered for this study according to the ethical standard of the Helsinki declaration of 1975 and the local ethical committees (Villejuif and Necker). The patients were carefully selected in order to present a homogeneous group for tumor characteristics. Tumor diameter was 25 to 30 mm. In 10 cases, a drill biopsy was performed before surgery both for pathological diagnosis and cytosolic estradiol receptor measurement (single point assay) to ascertain that the tumor was estradiol receptor positive. Furthermore, an estradiol binding assay was performed on all 12 tumors after surgery.
by exchange method and Scatchard analysis (20). The tumors had a histopredictive grade 1 (21), and cytosolic estradiol receptor levels ranged from 150 to 620 fmoI/mg protein.

**Experiments**

Eighty μCi of trans-[3H]-4-OHTAM in a 60% alcoholic solution were topically applied to the affected breast of 9 patients and mastectomy was carried out 12, 24, 36, 48, 72 and 96 h or 7 days after application. In 3 other patients, 80 Ci of trans-[3H]TAM were applied to the breast and surgery was performed 24, 36, or 72 h later. Moreover, one of the carcinoma patients received the same dose of trans-[3H]-4-OHTAM topically on the abdomen 3 wk after the topical administration of the same compound to the breast.

Blood samples were taken at different times following [3H]-4-OHTAM or [3H]TAM percutaneous administration, 12, 24, and 36 h and every day from the third to the seventh day, depending on the day of surgery, or [3H]TAM percutaneous administration, 12, 24, and 36 h or 7 days after application. Two thousand dpm of 14C tracers were added to all the samples before the extraction step in order to monitor recovery. In all experiments, recovery was more than 75% for the extraction steps and more than 95% for TLC.

No isomerization of trans-[3H] to cis-[3H]-4-OHTAM occurred through the extraction procedure.

Final identification of the compounds was made after addition of nonradioactive compounds to the radioactive extracted ones and co-crystallization to constant specific activity.

**RESULTS**

Concentration of [3H]-4-OHTAM in Human Tumor Breast. The amounts of radioactivity recovered from mammary tumor tissue after trans-[3H]-4-OHTAM percutaneous administration at different times before surgery was given on Table 1. The maximal radioactive concentration occurred 24 h after percutaneous administration of radioactive 4-OHTAM and then plateaued for 2 days before decreasing until day 7; 35% radioactivity was recovered in the microsomal fraction, 28% in the cytosol, 28% in the KCI nuclear extract, and 11% in the nuclear pellet (Fig. 1). In the "normal" mammary tissue surrounding the tumor 50% radioactivity was found in the cytosolic fraction and 20% in the microsomes. After thin layer chromatography, radioactivity detected in both cytosolic and nuclear fractions was essentially formed by 4-OHTAM (Fig. 2); 80% was the unchanged trans isomer and 20% the cis-isomerized compound.

**Plasma and Urine Studies**

Plasma and urine samples were divided into 3 fractions for separated extraction and counting of the free and conjugated forms of [3H]-4-OHTAM and its metabolites. Free compounds were directly extracted with cyclohexane-ethyl acetate (v/v). Solvolysis was performed on the second fraction by acid hydrolysis at pH 1 with H2SO4. On the third fraction, glucuronides were hydrolyzed with a β-glucuronidase (Pasteur Prod., Paris) at pH 6.0 and 37°C for 2 h before extraction with ethyl acetate. The extract was then washed by 1 N NaOH (twice) and H2O (once).

**Identification of TAM, 4-OHTAM and Their Metabolites**

Unlabeled carrier compounds were added to the extracts, the extracts were evaporated, redissolved in ethanol, and analyzed on TLC (Merck F 254 silica gel plates) in 2 different solvent systems, benzene-piperidine (9:1) which provides optimum resolution of 4-OHTAM isomers and metabolites, and benzene-triethylamine (9:1). Unlabeled markers were localized in UV absorbance at 254 nm. The TLC plate was then divided into 30 sections, scraped, and radioactivity was extracted with acetone and ethanol. After evaporation to dryness, radioactivity was counted in 10 ml of liquid scintillator (Amer sham Corp., Arlington, IL) in a Packard 300C Tri-Carb liquid scintillation spectrometer (Packard, Downers Grove, IL) with 40% efficiency for 3H and 60% efficiency for 14C.

Two thousand dpm of 14C tracers were added to all the samples before the extraction step in order to monitor recovery. In all experiments, recovery was more than 75% for the extraction steps and more than 95% for TLC.

No isomerization of trans-[3H] to cis-[3H]-4-OHTAM occurred through the extraction procedure.

**Final identification of the compounds was made after addition of nonradioactive compounds to the radioactive extracted ones and co-crystallization to constant specific activity.**

**Table 1**

<table>
<thead>
<tr>
<th>Time after administration of trans-[3H]-4-OHTAM</th>
<th>Concentration of radioactivity (% of dpm/g tumor)</th>
<th>% metabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 h</td>
<td>4.2</td>
<td>&lt;1</td>
</tr>
<tr>
<td>24 h</td>
<td>12.8</td>
<td>&lt;1</td>
</tr>
<tr>
<td>36 h</td>
<td>12.1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Day 4</td>
<td>8.1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Day 7</td>
<td>0.4</td>
<td>2</td>
</tr>
</tbody>
</table>

**CONCLUSIONS**

1. The amounts of radioactivity recovered from mammary tumor tissue after trans-[3H]-4-OHTAM percutaneous administration at different times before surgery were given on Table 1. The maximal radioactive concentration occurred 24 h after percutaneous administration of radioactive 4-OHTAM and then plateaued for 2 days before decreasing until day 7; 35% radioactivity was recovered in the microsomal fraction, 28% in the cytosol, 28% in the KCI nuclear extract, and 11% in the nuclear pellet (Fig. 1).

2. In the "normal" mammary tissue surrounding the tumor 50% radioactivity was found in the cytosolic fraction and 20% in the microsomes. After thin layer chromatography, radioactivity detected in both cytosolic and nuclear fractions was essentially formed by 4-OHTAM (Fig. 2); 80% was the unchanged trans isomer and 20% the cis-isomerized compound.

**RESULTS**

At surgery, specimens of the tumor were reserved for pathological study and steroid receptor determination. The remaining tissue was then separated into 3 parts: tumor; tissue immediately surrounding the tumor; and normal tissue; and immediately frozen in liquid nitrogen.

All tissue samples were processed for biochemical investigations less than 2 wk after surgery. All procedures were carried out at 0-4°C. The amounts of radioactivity recovered from mammary tumor tissue after trans-[3H]-4-OHTAM percutaneous administration at different times before surgery was given on Table 1. The maximal radioactive concentration occurred 24 h after percutaneous administration of radioactive 4-OHTAM and then plateaued for 2 days before decreasing until day 7; 35% radioactivity was recovered in the microsomal fraction, 28% in the cytosol, 28% in the KCI nuclear extract, and 11% in the nuclear pellet (Fig. 1). In the "normal" mammary tissue surrounding the tumor 50% radioactivity was found in the cytosolic fraction and 20% in the microsomes. After thin layer chromatography, radioactivity detected in both cytosolic and nuclear fractions was essentially formed by 4-OHTAM (Fig. 2); 80% was the unchanged trans isomer and 20% the cis-isomerized compound.

**Plasma and Urine Studies**

Plasma and urine samples were divided into 3 fractions for separated extraction and counting of the free and conjugated forms of [3H]-4-OHTAM and its metabolites. Free compounds were directly extracted with cyclohexane-ethyl acetate (v/v). Solvolysis was performed on the second fraction by acid hydrolysis at pH 1 with H2SO4. On the third fraction, glucuronides were hydrolyzed with a β-glucuronidase (Pasteur Prod., Paris) at pH 6.0 and 37°C for 2 h before extraction with ethyl acetate. The extract was then washed by 1 N NaOH (twice) and H2O (once).

**Identification of TAM, 4-OHTAM and Their Metabolites**

Unlabeled carrier compounds were added to the extracts, the extracts were evaporated, redissolved in ethanol, and analyzed on TLC (Merck F 254 silica gel plates) in 2 different solvent systems, benzene-piperidine (9:1) which provides optimum resolution of 4-OHTAM isomers and}

CANCER RESEARCH VOL. 46 MARCH 1986
1522
and even TAM and desMeTAM after day 4.

**Comparative Retention of [3H]-4-OHTAM and [3H]TAM in Breast Tissue.** A striking difference was noted between the amount of radioactivity retained in breast tissue after percutaneous administration of [3H]TAM and [3H]-4-OHTAM. Whereas [3H]-4-OHTAM was retained at a high level for 4 days, [3H]TAM retention after percutaneous administration was far weaker and shorter (Fig. 4).

**Plasma Studies after Percutaneous Administration of trans-[3H]-4-OHTAM or trans-[3H]TAM.** In contrast to the near absence of [3H]-4-OHTAM metabolites in breast tissue, there was evidence of marked metabolism in blood. For instance, only 24 h after its administration 68% radioactivity was found to be 4-OHTAM (66% trans isomer and 34% cis isomer), 18% radioactivity was identified as desMeOHTAM, and 11% as bisphenol (Fig. 2). The amount of radioactivity observed in blood during the 36 h following percutaneous administration of [3H]-4-OHTAM was approximately 6.8 ± 1.5·10³ (SD) dpm/dl plasma. This corresponds to 0.5% of the administered radioactivity for the total blood compartment (4.5 liters). Regarding the circulating radioactivity, 52% were directly extracted by ethyl acetate and could be assimilated to free compounds, 38% were glucuron conjugated, and only 10% sulfoconjugated.

When plasma kinetics for [3H]-4-OHTAM and [3H]TAM were compared, striking differences were observed. Radioactivity due to [3H]TAM appeared more rapidly, peaked on day 2, and decreased more rapidly than after percutaneous administration of [3H]-4-OHTAM (Fig. 4). Appearance of radioactivity due to [3H]-4-OHTAM was indeed delayed and plateaued from days 4 to 6.

In addition, if we compare the appearance of radioactivity in the plasma according to the site of percutaneous administration of [3H]-4-OHTAM (Fig. 5), we can observe a profile similar to that observed in the preceding experiment, namely, a more rapid appearance of radioactivity in plasma after abdominal administration as compared with administration to the breast followed by a more rapid decrease in blood radioactivity after abdominal administration than after administration to the breast.

**Urinary Studies.** The metabolism of [3H]-4-OHTAM can be best followed in the urine. Quantitatively, radioactivity appeared in the urine 6 h after topical application of the radioactive precursor to the breast and peaked at 48 h (Fig. 6). Thereafter, radioactivity decreased rapidly after 72 h, then more slowly from days 4 to 7. The total radioactivity collected in one case during 22 days was equal to 0.3% of the labeled precursor administered; 66% were glucuroconjugated and 32% sulfoconjugated compounds. Free compounds represented no more than 2 to 3% of the total radioactivity. With time, there was a progressive decrease in 4-OHTAM and a progressive increase in the percentage of different metabolites, particularly desMeOHTAM which was the predominant metabolite at day 4 (Fig. 6, insert).

As in plasma, the appearance of radioactivity in the urine was delayed when [3H]-4-OHTAM was percutaneously administered to the breast as compared with the abdominal application of [3H]-4-OHTAM (Fig. 5) and also as compared with percutaneous administration of [3H]TAM to the breast (Fig. 4).

**DISCUSSION**

The antiestrogen tamoxifen is a well-established drug for treatment of endocrine responsive breast cancers. However, its use has not yet been extended to nonmalignant breast diseases and particularly those considered to be linked to estrogen hyperresponsiveness such as fibrocystic disease (18, 22). Most benign breast diseases occur in premenopausal women with functional ovaries. Since the clomiphene-like effect of tamoxifen...
induces an overstimulation of the ovaries (23), the use of this antiestrogen is not possible in these diseases. However, when administered with a progestin with strong antigonadotropic activity, tamoxifen seems to be a very efficient therapeutic agent in some benign breast diseases (24).

The present experiment was undertaken to determine if 4-OHTAM could be concentrated directly in the breast. 4-OHTAM is a very active antiestrogen which is produced in the liver after p.o. administration of TAM (12). Results obtained in this study largely confirm this hypothesis. 4-OHTAM when topically applied in an alcoholic solution over the human breast is absorbed through the skin. This compound is predominantly concentrated in the subcellular fractions of breast tissue where estrogen receptors are present, namely, the cytosolic and nuclear fractions. In these cellular sites, 4-OHTAM is not only retained in situ but it remains unmetabolized, in contrast to the extensive metabolism observed after the p.o. administration of TAM or 4-OHTAM (25).

The possibility that 4-OHTAM, when percutaneously administered to the breast, is retained in breast tissue before reaching the peripheral circulation is emphasized by the following 2 observations: (a) a greater amount of radioactive 4-OHTAM is retained for a longer period in breast tissue than occurs with TAM. As a result, TAM appears in the plasma more rapidly than does 4-OHTAM. This correlates well with the lower affinity of TAM for the estrogen receptor; (b) percutaneous administration of radioactive 4-OHTAM through an anatomical site containing receptors, i.e., the breast, is followed by delayed appearance of this compound both in plasma and urine when compared to the same experiment performed through abdominal tissue which does not contain estradiol binding sites.

Delayed appearance of radioactivity in biological fluids when a labeled compound has to pass through receptor structures suggests that it is retained in these structures. Furthermore, the presence of radioactivity in the microsomal fraction of breast tissue might be explained by the fact that 4-OHTAM is also bound to selective binding proteins located in this fraction (26, 27) and distinct from the estradiol receptor. Finally, it should be pointed out that in spite of the lower affinity of 4-OHTAM for the...
antiestrogen binding sites as compared to TAM (26), the higher tissue retention of 4-OHTAM results from and confirms the higher affinity of 4-OHTAM for estradiol receptor.

In contrast to the absence of metabolism of 4-OHTAM in the breast, there is a progressive increase in plasma and urine metabolites following percutaneous administration of [3H]-4-OHTAM. This is an additional proof of the hepatic bypass that results from the percutaneous administration of 4-OHTAM. In addition, one can observe both in plasma and urine a variable and increasing concentration of conjugated 4-OHTAM and metabolites. This is mainly due to the hepatic metabolism of the compound once it has passed into the peripheral circulation.

A progressive isomerization of 4-OHTAM is observed in the breast samples collected from 12 h to day 7 after percutaneous administration of the labeled trans isomer. This contrasts with trans-TAM which does not seem to be isomerized into cis-TAM at least in vitro (10, 25). Recently, Katzenellenbogen et al. (10) have reported that labeled trans- and cis-4-OHTAM are extensively isomerized when incubated with MCF-7 cells in culture. However, in these experiments (10) trans-4-OHTAM was the only isomer to accumulate at the nuclear receptor level, cis-TAM which cannot be transformed into its trans isomer exerts a potent estrogenic agonist effect. In contrast cis-4-OHTAM due to its extensive conversion into trans-4-OHTAM has a strong antiestrogenic activity in vitro (12). One can suppose that the same is true in vivo and particularly after percutaneous administration of 4-OHTAM. The prolonged retention of this compound which we observed in the breast is in favor of such a hypothesis. These findings are important from a pharmacological point of view and one can advance the argument that the percutaneous administration of trans-4-OHTAM could produce a strong antiestrogenic effect at the molecular level.

The therapeutic implications of the present data are evident. Since 4-OHTAM cannot be used p.o. because it is destroyed during its hepatic passage, the possibility of obtaining an effective concentration of this compound by means of topical application over the breast might be useful in several pathological conditions affecting the breast. 4-OHTAM could be used either alone or in combination with a progestin. Thus, the association of the two affecting the breast. 4-OHTAM could be used either alone or in combination with a progestin. This therapeutic approach might be proposed in hormone-dependent benign breast disease (16, 22). A small dose of 29). This therapeutic approach might be proposed in hormone-adrenalin combination with a progestin. Thus, the association of the two affecting the breast. 4-OHTAM could be used either alone or in combination with a progestin. This therapeutic approach might be proposed in hormone-adrenalin combination with a progestin. Thus, the association of the two affecting the breast. 4-OHTAM could be used either alone or in combination with a progestin. This therapeutic approach might be proposed in hormone-adrenalin combination with a progestin. Thus, the association of the two affecting the breast. 4-OHTAM could be used either alone or in combination with a progestin. This therapeutic approach might be proposed in hormone-adrenalin combination with a progestin. Thus, the association of the two affecting the breast. 4-OHTAM could be used either alone or in combination with a progestin. This therapeutic approach might be proposed in hormone-adrenalin combination with a progestin. Thus, the association of the two affecting the breast. 4-OHTAM could be used either alone or in combination with a progestin. This therapeutic approach might be proposed in hormone-adrenalin combination with a progestin. Thus, the association of the two affect...
trans-4-Hydroxytamoxifen Concentration and Metabolism after Local Percutaneous Administration to Human Breast

Pierre Mauvais-Jarvis, Nicole Baudot, Damienne Castaigne, et al.


Updated version Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/46/3/1521

E-mail alerts Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.