ORIGINAL ARTICLE

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Phase I Study of percutaneous 4-hydroxy-tamoxifen with analyses of 4-hydroxy-tamoxifen concentrations in breast cancer and normal breast tissue

Abstract 4-OH-tamoxifen is an active metabolite of tamoxifen that is detectable in the serum and tumour tissue of patients treated by oral tamoxifen. As this metabolite penetrates through the skin, it is possible to compare percutaneous 4-OH-tamoxifen (4-OH-TAM) and oral tamoxifen treatments. We report herein a randomized study of percutaneous 4-OH-TAM versus oral tamoxifen in women with breast cancer. This pharmacology study was designed to compare the 4-OH-TAM concentration in breast cancer and normal breast tissue according to the route and dose used for administration of tamoxifen after a 3-week period prior to surgery and tissue sampling. Women were randomized into one of the five following groups: group I, oral tamoxifen given at 10 mg twice a day; group II, 4-OH-TAM delivered percutaneously at 0.5 mg day to both breast areas; group III, 4-OH-TAM applied percutaneously at 1 mg/day to both breast areas; group IV, 4-OH-TAM delivered percutaneously at 1 mg/day to a large cutaneous area excluding the breasts; and group V, 4-OH-TAM applied percutaneously at 2 mg/day to a large skin area excluding the breasts. 4-OH-TAM plasma and tissue concentrations were significantly higher in the oral tamoxifen group as compared with either the high- or the low-dose percutaneous 4-OH-TAM group. In group II, percutaneous 4-OH-TAM treatment resulted in tissue concentrations of 1,446 and 352 pg/g in tumour tissue and normal breast tissue, respectively. In group I these concentrations were as follows: tumour tissue, 12,453 pg/g; and normal tissue, 10,214 pg/g. 4-OH-TAM concentrations in tumour tissue and normal breast tissue did not significantly differ in any group. In the oral group we observed classic effects on coagulation and lipid metabolism when pre- and post-treatment values of these biological variables were compared, whereas no difference was observed in the percutaneous group. Although precutaneous administration of 4-OH-TAM led to a low plasmatic concentration of this active metabolite, the breast tissue concentration remained lower than those observed after oral tamoxifen treatment. Therefore, at the doses described in this study, percutaneous 4-OH-TAM cannot be proposed as an alternative tamoxifen treatment.

Key words Breast cancer · Chemoprevention · Pharmacology · Tamoxifen · Percutaneous administration

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Introduction

Adjuvant treatment of breast cancer leads to a survival benefit, but the overall mortality in developed countries remains unchanged owing to the increase in breast cancer incidence [1]. Screening for breast cancer can reduce mortality by about 30%–35%; however, it cannot reduce the incidence and, thus, prevention programmes are required. Chemoprevention programmes using oral tamoxifen are under way [20]. These programmes are based on previous studies demonstrating that oral tamoxifen reduces the risk for second breast cancer by 39% [10].

There is controversy about the use of tamoxifen for prevention of breast cancer by the oral route [6, 8].

Tamoxifen can have beneficial side effects such as an improvement in bone density, the regulation of lipid metabolism and a decrease in the risk of cardiovascular disease [11, 12]. On the other hand, some adverse effects of oral tamoxifen have been described (e.g. hot flushes), whereas others, such as an increase in the risk for endometrial cancer, are only suspected [17]. An alternative route of tamoxifen treatment with presumed low systemic effects such as percutaneous 4-hydroxy-tamoxifen (4-OH-TAM) has been proposed [14]. Pharmacology studies to evaluate differences between the percutaneous and oral routes are needed before a clinical applicability for 4-OH-TAM can be proposed.

We report herein a randomized study of percutaneous 4-OH-TAM versus oral tamoxifen in women with breast cancer. This study was designed to compare the 4-OH-TAM concentrations measured in breast cancer and normal breast tissue after treatment with either oral tamoxifen or 4-OH-TAM gel at 3 weeks prior to surgery and tissue sampling.

Patients and methods

Eligibility criteria

Women with histologically proven breast cancer were prospectively entered in the study. Pre-requirements for inclusion consisted of: menopause status lasting for more than 1 year before inclusion, no hormonal substitutive treatment, normal or large breast volume and operable infiltrative breast cancer measuring over 1 cm in diameter. Women with a body weight exceeding 20% of the normal value, women with a previous history of cancer or cancer therapy before surgery and patients with skin diseases were excluded. The study design was approved by the Montpellier University ethics committee, and written informed consent was obtained from each woman before treatment.

Study design

Oral tamoxifen consisted of 10-mg tablets purchased from ICI. 4-OH-TAM is an active metabolite of tamoxifen that can be delivered by the percutaneous route [14]. The hydroalcoholic gel used was packaged in a dispenser with a metered valve delivering 250 μg 4-OH-TAM per dose (Besins Iscovesco Laboratories, Paris, France).

At the time of their inclusion in the study, patients underwent the following blood tests: haemogram, bilirubin, serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase, creatinine, estradiol, follicle-stimulating hormone (FSH), luteotrophic hormone (LH), serum hormone-binding globulin (SHBG), cholesterol, high-density lipid (HDL), low-density lipid (LDL), triglycerides and antithrombin III.

Women were randomized into one of the five following groups: group I, oral tamoxifen given at 10 mg twice a day; group II, 4-OH-TAM delivered percutaneously at 0.5 mg/day on both breast areas; group III, 4-OH-TAM applied percutaneously at 1 mg/day on both breast areas; group IV, 4-OH-TAM delivered percutaneously at 1 mg/day on a large cutaneous area excluding the breasts; and group V, 4-OH-TAM applied percutaneously at 2 mg/day on a large

skin area excluding the breasts. In all groups, tamoxifen treatment lasted for 21 days before surgery. Treatment compliance was monitored by measuring the remaining gel in the dispenser. On the day before surgery, all biological variables were tested again. In addition, the plasma 4-OH-TAM concentration was assessed twice (on the day before and the day of surgery).

During surgery, 1 cm^3 of tumour tissue and 1 cm^3 of normal breast tissue were taken as samples and frozen in liquid nitrogen until 4-OH-TAM tissue determination. Estrogen and progesterone receptors were routinely tested in the specimens by means of radioligand (dextran-coated charcoal) analysis and the results of biochemical titration were expressed in fentomoles per milligram of cytosol protein. The cell-growth fraction was evaluated in situ using indirect immunoperoxidase reaction of KI-67 monoclonal antibody as previously described in detail [24]. Ploidy was determined using a computer-assisted image processor following Feulgen staining of cytology prints as previously described [24]. The cathepsin D concentration in breast cancer cytosol was measured using a solid-phase sandwich immunoenzymatic assay [13].

Quantitative measurement of 4-OH-TAM in human plasma and mammary tumors

A new, highly specific assay was recently developed for the quantitative measurement of 4-OH-TAM at the femtomole level in human plasma and breast tissue [9]. The drug and the deuterated internal standard (4-OH-TAM D$_4$) were measured using a negative chemical ionization gas chromatography/mass spectrometry assay. This assay required 0.5 ml of plasma or 0.5 g of mammary tissue, and the quantification limits of the method were 20 pg/ml for the body fluids or 100 pg/g for the tissue samples. The relative standard deviations calculated during the different within-day repeatability assays were always less than 5%, and the mean percentage of error ranged from -8.4% to 1.4% for plasma samples fortified from 20 to 2,000 pg/ml. The results of the tissue repeatability assays were quite similar; relative standard deviations ranged between 1.0% and 6.8% and the mean percentage of error ranged from -0.5% to 1.4% for breast tissue concentrations tested from 100 to 3,000 pg/g.

Statistical analysis

Kruskal-Wallis one-way analysis of variance we used to determine whether any difference existed between the five groups at the time of inclusion (using the following parameters: age, body weight and height) and at the time of surgery for plasma and tissue 4-OH-TAM concentrations. The Mann-Whitney test was used for inter-individual comparisons between two groups and the Wilcoxon rank-sum test, for intra-individual comparisons within each group.

Results

Patients' characteristics

Between January and August 1992, 31 women were entered into the study. Three patients were excluded from the analysis (one refused to continue the preoperative treatment for personal reasons, one had a major treatment schedule violation and one had a metastatic status contraindicating surgery). The remaining 28 women underwent the complete protocol, except in 1 case where the tumour 4-OH-TAM concentration was not due owing to insufficient tumour tissue.

Histology disclosed an invasive adenocarcinoma in all cases. Among them were 10 stage T1, 16 stage T2 and 2 stage T3 tumours according to the International Union Against Cancer tumour mode metastasis (UICC TNM) staging system. After randomization, 6 patients were assigned to groups I, II and V, and 5 patients are assigned to groups III and IV. The distribution of tumour stage did not differ significantly among the different groups. There was no difference between the five groups in terms of age and body weight, but the patients in treatment group V were significantly taller (mean $\pm$ SD, 164 $\pm$ 3.4 cm) than those in the other groups (the lowest value being found in group II: 154 $\pm$ 3 cm;$P < 0.02$).

4-OH-TAM concentration

On the day before surgery the plasma 4-OH-TAM concentration was significantly higher in group I (oral tamoxifen; 2,326 $\pm$ 585 pg/ml) as compared with the other groups (plasma tamoxifen was undetectable in group II, $P < 0.002$; Table 1). On the day of surgery a similar difference was observed (Fig. 1).

In tumour tissue the 4-OH-TAM concentration varied significantly according to the route and dose of administration ($P < 0.001$; Fig. 2), being higher in group I as compared with the four other groups. The 4-OH-TAM concentration did not differ significantly when the two breast-area percutaneous treatment groups were compared (group II, 1,446 $\pm$ 2,673 pg/g; group III, 1,867 $\pm$ 2,472 pg/g). The tumour tissue concentration was slightly higher in groups II and III as compared with groups IV and V, suggesting direct transcutaneous delivery of 4-OH-TAM when the drug was applied to the breast area. In normal breast tissue, a similar distribution of 4-OH-TAM concentration was observed, i.e. the highest level was seen in group I and the lowest value was observed in the low-dose percutaneous treatment groups (II and IV). Within each group the 4-OH-TAM concentration was higher in tumour tissue as compared with normal breast tissue, although this difference did not reach statistical significance.

Clinical toxicity

There was no cutaneous intolerance in the percutaneous treatment groups.

Modifications of blood tests

No biological effect was observed when pre- and post-treatment values were compared in the percutaneous 4-OH-TAM groups (II–V). In the oral tamoxifen group (I) we observed a significant decrease in anti-thrombin

Table 1 4-OH-TAM plasma concentrations

Treatment group	Day before surgery: Mean $\pm$ SD (pg/ml)	Day of surgery: Mean $\pm$ SD (pg/ml)
I	2,326 $\pm$ 585	2,317 $\pm$ 1,098
II	0	17 $\pm$ 27
III	164 $\pm$ 131	62 $\pm$ 71
IV	93 $\pm$ 75	13 $\pm$ 29
V	78 $\pm$ 137	73 $\pm$ 114
P value[a]	0.002	0.002

[a]According to Kruskall-Wallis one-way analysis of variance
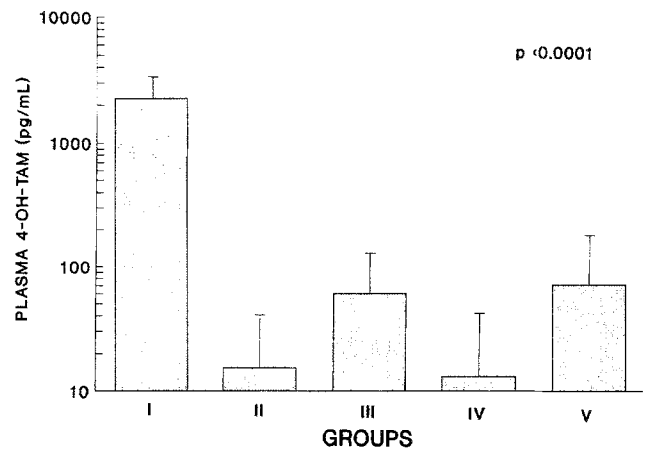

Fig. 1. 4-OH-TAM plasma concentrations as assessed on the day of surgery according to treatment group. Statistical analysis was carried out using one-way analysis of variance
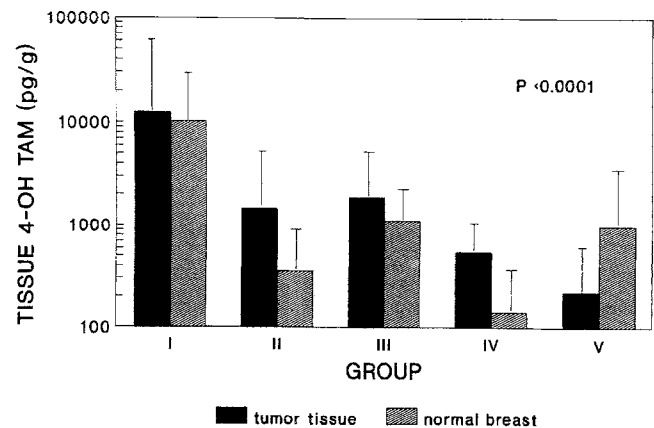

Fig. 2. 4-OH-TAM tissue concentrations according to treatment group. Statistical analysis was separately in each tumour tissue and normal breast tissue series using Kruskall-Wallis one-way analysis of variance

III and fibrinogen and a significant increase in both platelet and lymphocyte counts (Fig. 3). Pre- and post-treatment levels of Estradiol (E^2), FSH, LH and SHBG did not differ significantly.

Fig. 3A,B. Comparison of pre- and post-treatment serum variables. **A** Treatment group I. **B** Treatment group II. Statistical analysis was carried out using the Wilcoxon test. $*P < 0.05$. Units: fibrinogen, g/l; anti-thrombin III, %; FSH, mU/ml; cholesterol, triglyceride and HDL, mmol/l
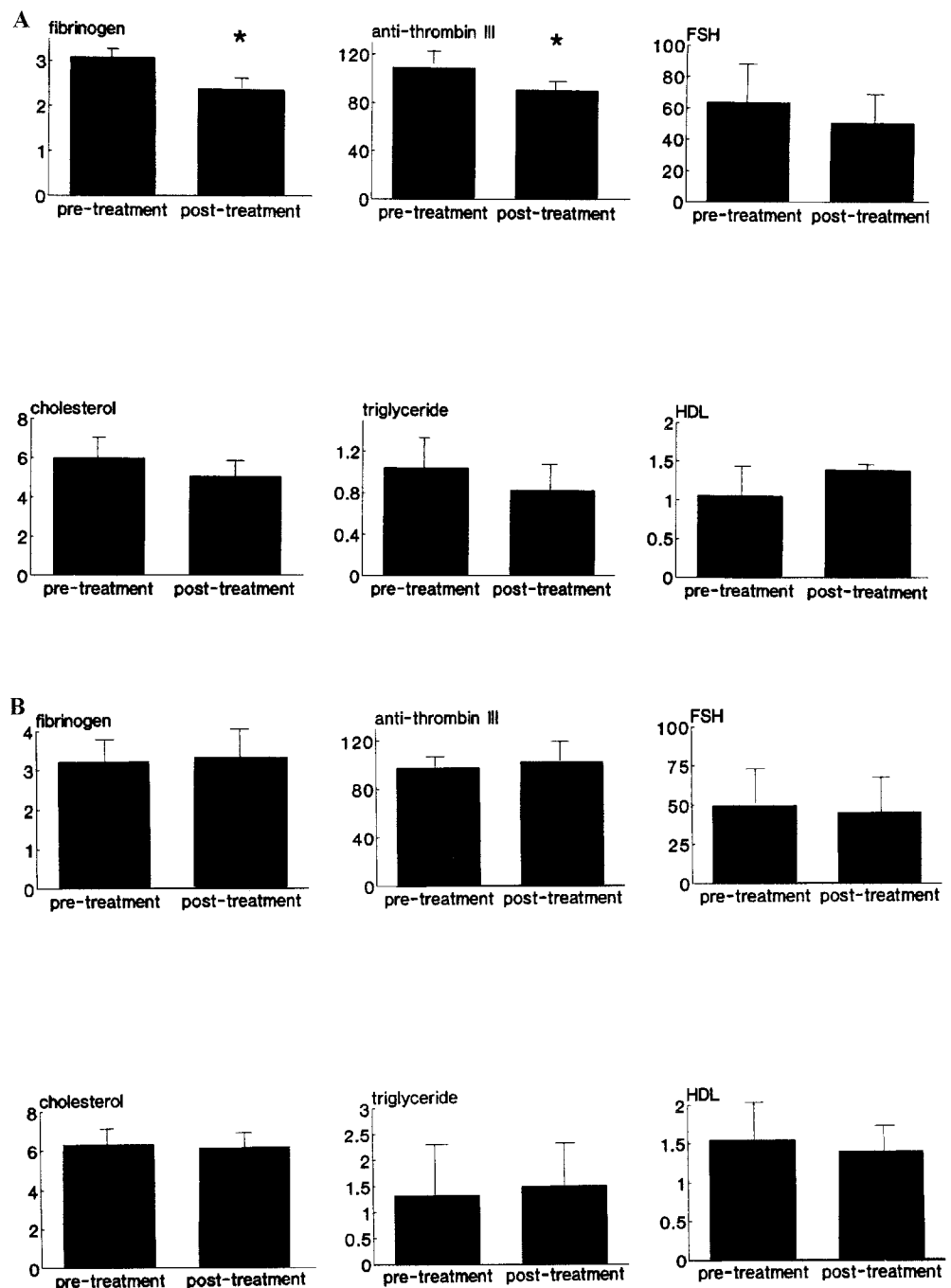
In situ tumour markers

There was no significant difference in tumour tissue between the five treatment groups when the intratumoural concentration of estrogen receptors, progesterone receptors and cathepsin D were considered (Table 2). A similar observation was made when these marker concentrations were analysed in normal breast tissue. No difference in growth fraction or ploidy status was observed when oral and percutaneous treatment groups were compared.

Discussion

Trials of tamoxifen chemoprevention of breast cancer are under way in the United States and in Europe [19–21]. Healthy women with a high risk of developing breast cancer (i.e. familial risk) are eligible. The observation that there is a 39% of odds-ratio decrease in contralateral cancer in tamoxifen-treated breast cancer is the rationale of these studies [5, 7, 10]. In addition, it has been suggested that tamoxifen might have some

Table. 2. Tumour markers

Treatment group	Estrogen receptors: Mean ± SD (fmol/mg)	Progesterone receptor: Mean ± SD (fmol/mg)	Cathepsin D: Mean ± SD (pmol/mg)
I	17.0 ± 17.1	199.1 ± 294.1	64.6 ± 50.9
II	48.0 ± 36.7	233.6 ± 208.2	32.1 ± 7.4
III	21.8 ± 14.6	36.8 ± 27.4	32.8 ± 13.9
IV	99.8 ± 80.1	53.6 ± 48.5	65.6 ± 69.5
V	81.0 ± 94.0	89.4 ± 112.2	54.4 ± 62.2
P value	0.1	0.28	0.61

positive side effects on lipid metabolism and bone density [12] and might also decrease the cardiovascular risk [4, 15].

However, when tamoxifen was prescribed for healthy women, controversy arose about both methodologic difficulties and possible side effects, which include thromboembolic events, endometrial cancer, retinopathies and hepatitis [3, 5, 17, 18]. The clomiphene-like effect of oral tamoxifen induces an overstimulation of the ovaries that limits its use in young woman [23]. Moreover, a contralateral tumour in a breast cancer patient and a primary breast cancer in a healthy woman might be seen as different diseases. Thus, proof of the hypothesis that the odds-ratio reduction obtained using oral tamoxifen in the former case might also be possible in the latter is pending the results of the above-mentioned trials [16].

4-OH-TAM is an active metabolite of tamoxifen (TAM) that is present in the serum of patients treated with TAM [2]. The affinity of this compound to estrogen receptors is 100- to 1,000-fold stronger than that of TAM. As the proliferation inhibition induced by anti-estrogens is correlated with the estrogen-receptor affinity, it can be hypothesized that 4-OH-TAM is one of the most potent anti-estrogens [22]. Oral administration of 4-OH-TAM is inefficient owing to inactivation of the metabolite by the liver. On the other hand, this compound penetrates through the skin. In a previous feasability study it was demonstrated that the percutaneous breast administration of a radiolabeled 4-OH-TAM alcoholic solution resulted in a significant uptake of radioactivity in subsequently resected tumour tissue, whereas there was no detectable 4-OH-TAM in the plasma [14].

In our prospective, controlled study we analyzed tissue and plasma concentrations of 4-OH-TAM and compared the concentrations induced by oral TAM with those induced by different doses and modalities of percutaneous 4-OH-TAM administration. oral TAM given at 20 mg/day induced a higher 4-OH-TAM concentration in both breast cancer and normal breast tissues as compared with the levels observed in the percutaneous 4-OH-TAM groups. In the oral TAM group a high plasma concentration of this active metabolite together with well-known coagulation and lipid-metabolism modifications were also observed. In contrast, the plasma concentration induced transcutaneously by the 0.5-mg/day 4-OH-TAM dose was almost undetectable, whereas at the 1-mg/day dose it remained significantly lower than that induced by oral TAM. No modification of coagulation or lipid metabolism was seen at either dose. This might be regarded as the lack of effect of a low 4-OH-TAM concentration in the serum. However, it cannot be ruled out that some very small modifications remained undetectable. When similar doses of 4-OH-TAM were applied to areas other than the breast, plasma concentrations were in the same range, whereas tissue concentrations were lower in comparison with those observed following gel administration on the breast area.

Thus, this study suggests that 4-OH-TAM gel administration might be associated with low systemic effects yet induce moderate breast tissue concentrations. In the setting of chemoprevention, the optimal breast tissue concentration of 4-OH-TAM is not yet known. The tissue concentration needed to achieve chemoprevention might possibly be lower than that needed to obtain anti-proliferative activity. Moreover, a lower inhibitory effect might result in a better estrogen-progesterone imbalance.

In conclusion, this pharmacology study demonstrates that percutaneous 4-OH-TAM gel administration on the breast leads to a very low plasma concentration of this metabolite together with a breast tissue concentration significantly lower that induced by oral TAM, the only route known to reduce the contralateral breast cancer risk in women with breast cancer. Therefore, at the doses described in this report, 4-OH-TAM is not an alternative method of chemoprevention and new studies must thus be done.

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