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First-in-human pharmacokinetics of tamoxifen and its metabolites in the milk of a lactating mother: a case study

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ABSTRACT

Background

Breast cancer represents the most frequent neoplasm diagnosed in women of childbearing age. When the tumour is oestrogen receptor-positive, tamoxifen is among the recommended endocrine treatments. Lactating women are advised not to breastfeed while receiving tamoxifen. However, information about tamoxifen transfer into breast milk is lacking.

Methods

We measured the concentration of tamoxifen and its metabolites by liquid chromatography-tandem mass spectrometry in the milk of a nursing mother that was treated for pregnancy-associated breast cancer diagnosed a few months after delivery. She was advised not to breastfeed her child and she collected milk samples for 23 days while the baby was fed with formula.

Results

Tamoxifen concentrations in milk increased reaching a maximum of 214 nM. The two active metabolitesZ-4-hydroxy-tamoxifen and Z-endoxifen, could not be quantified in milk the first days after tamoxifen intake, but increased over time and reached clinically significant levels after day 18.

Conclusion

This study demonstrates for the first time in human that tamoxifen and its metabolites transfer into milk. Since tamoxifen has a complete oral bioavailability, a long half-life (>7 days) and may interfere with the normal development of the infant, mothers should not breastfeed during tamoxifen treatment.

Key questions

What is already known about this subject?

- Women treated with tamoxifen are advised not tonurse their babies since the drug may interfere with child development. This recommendation was made on a cautionary basis, as no data on tamoxifen transfer nor on the transfer its metabolites into breast milk has been available.

What does this study add?

- We measured tamoxifen and its main metabolites concentration in a patient's breast milk during tamoxifen treatment. Tamoxifen and its metabolites were found to be present at clinically significant levels which might therefore exert an effect on the child.

How might this impact on clinical practice?

- All aspects of oncofertility, including breastfeeding, are becoming more and more important since the number of cancer cases in women of reproductive age are increasing. Providing pharmacological data helps physicians in providing evidence-based indications on this delicate aspect.

INTRODUCTION

Breast cancer (BC) is by far the most common cancer diagnosed in women during their childbearing years.[1] As the average age for starting families has risen, so has the number of pregnancy-associated breast cancers (PABC).[2] PABC is defined as BC diagnosed during pregnancy or within 1 year after delivery and complicates around 1 out of 3000 pregnancies. Because of the increased incidence of this condition, patients are enquiring about the possibility of breastfeeding when diagnosed with BC during pregnancy or during puerperium.

Tamoxifen is the adjuvant treatment of choice for premenopausal women with low-risk oestrogen receptor (ER)-positive BC.[3–5] Its activity, efficacy and side effects are well described in older adults,[6 7] but data on the effects of tamoxifen during pregnancy and lactation is insufficient.[8] In agreement with previously published data,[9] a recent review reported a malformation rate of 20.5% in infants exposed to tamoxifen prenatally, thus supporting a contraindication of its use during pregnancy.[10] It has been shown that tamoxifen might suppress spontaneous lactation,[11] but tamoxifen transfer into milk has only been hypothesised due to its low molecular weight[12] without any experimental evidence. Thus, recommendations that breastfeeding

Table 1 Quality control and lower limit of quantification (LLQ) for tamoxifen and its metabolites with the chromatography-tandem mass spectrometry assay

	Quality control			LLQ
Compound	**C (nM)**	**CV, %**	**Accuracy, %**	**C, nM**
Tamoxifen	29.6	10.5	111	6.2
Z-endoxifen	4.2	4.8	105	1.8
Z-4'-endoxifen	9.7	6.4	100	2.1
NDtam	59.3	4.1	108	6.3
TamNoX	7.3	6.4	100	1.5
Z-4OHtam	0.7	10.3	101	0.6
Z-4'-OHtam	0.6	4.2	99	0.5
NNDDtam	14.7	14.0	111	6.2

C, concentration; CV, coefficient of variation; NDtam, N-desmethyl-tamoxifen ; nM, nanomolar; NNDDtam, N,N-D-desmethyl tamoxifen; TamNoX, tamoxifen N-oxide; Z-4'-endoxifen, Z-4'-endoxifen; Z-endoxifen, Z (cis) stereoisomer of endoxifen; Z-4OHtam, Z-4-hydroxy-tamoxifen; Z-4'-OHtam, Z-4'-hydroxytamoxifen.

should be avoided in mothers who are using tamoxifen are based on an acceptable safety principle, without available data. The aim of this case-study is to determine if tamoxifen and its metabolites transfer into milk during tamoxifen treatment.

CASE STUDY

A 31-year-old woman with a pathogenic BRCA2 mutation, discovered a breast nodule while breastfeeding. After histological confirmation she underwent nipple-sparing mastectomy and sentinel lymph node biopsy, followed by immediate breast reconstruction. Histology confirmed a moderately differentiated infiltrating ductal carcinoma pT1a (0.45 cm) pN0, ER 80%, PgR 10%, Ki-67 16%, HER2 negative, associated with several areas of intermediate grade ductal intraepithelial neoplasia. Hence, tamoxifen at the dosage of 20 mg/day was prescribed and assumed in the morning at 08:00, before breakfast: the patient was instructed not to breastfeed her child. Being herself a pharmacologist with a special interest in drug distribution, she gave informed consent to collect breast milk samples using a breast pump while the infant was fed with formula. The ethical committee of the treating hospital was informed and the patient agreed in publishing her clinical history and results from the study, provided her identity was concealed.

METHODS

Thirty-nine samples were collected over 23 days. For the first 15 days, two samples were collected each day, one in the afternoon (12:00 to 14:30) and one in the evening (23:00 to 00:30). For the remaining 8 days, only one sample was collected each day. Milk samples from two unexposed volunteers and a baseline sample from the case were used as blank.

Samples were immediately frozen at −20°C, before being transferred for long-term storage at −80°C in the biobank of the European Institute of Oncology, Milan, Italy. Tamoxifen and seven of its metabolites were determined by a modification of a previously published method at University of Bergen, Bergen, Norway.[13] Briefly, 20 µL of milk was protein precipitated in 500 µL of acetonitrile containing deuterated internal standards, followed by evaporation of 80 µL of supernatant to dryness under nitrogen flow. The samples were thereafter reconstituted in 500 µL water:methanol (20:80, v:v) and injected into the column (BEH Phenyl column) for chromatographic separation using the ACQUITY UPLC system from Waters (Milford, Massachusetts, USA). A 0.01% aqueous solution of formic acid was used as the weak mobile phase and 0.01% formic acid in methanol as the strong mobile phase. The compounds were subjected to atmospheric pressure photoionisation and detected in positive mode using a Xevo tandem quadrupole mass spectrometer from Waters. Calibrators and quality controls were created by adding different concentrations of tamoxifen and its metabolites to breast milk from a donor. The modified liquid chromatography-tandem mass spectrometry method was well within Food and Drugs Administration acceptance criteria in terms of precision and accuracy. The total coefficient of variation for the compounds were in

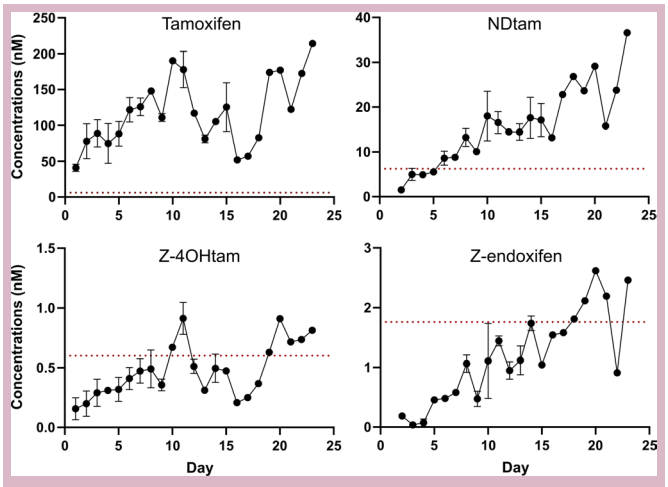

Figure 1 Time curve concentrations of tamoxifen and its major metabolites N-desmethyl tamoxifen (NDtam), 4-hydroxy tamoxifen (Z-4OH-tam) and Z-endoxifen in milk. Longitudinal concentrations of tamoxifen and its main metabolites in breast milk. Concentrations were determined by chromatography-tandem mass spectrometry. Samples were obtained twice a day for the first 15 days and are expressed as mean±SD. After day 15 one sample was obtained each day. Red dashed horizontal line indicates the lower limit of quantification (LOQ), that is, the lowest analyte concentration that can be quantitatively detected with good accuracy and precision. Measures below LOQ are reported anyway as they were above the lower limit of detection, that is, the smallest amount of an analyte that can reliably be detected in the test.

Table 2 Concentrations of tamoxifen and its metabolites at different time points

Day	Time	Tamoxifen	Z-endoxifen	Z-4'- endoxifen	NDtam	TamNox	Z-4OHtam	Z-4'-OH tam	NNDD tam
0	Baseline	–	–	–	–	–	–	–	–
1	12:00	44.487	–	0.001	0.000	0.182	0.091	0.058	–
1	23:00	37.308	–	0.304	0.000	0.006	0.222	0.072	–
2	13:00	95.094	0.186	0.173	1.422	0.255	0.124	0.020	–
2	23:00	60.757	0.000	0.010	1.629	0.122	0.275	0.178	–
3	13:00	102.500	0.039	0.009	5.925	0.186	0.372	0.202	–
3	00:00	75.622	0.033	0.000	4.015	0.079	0.210	0.205	–
4	14:00	94.694	0.120	0.000	4.884	0.230	0.311	0.204	–
4	00:30	55.257	0.029	0.051	4.956	0.192	0.270	0.122	–
5	13:00	100.421	0.000	0.097	5.234	0.311	0.249	0.245	–
5	23:00	76.274	0.456	0.222	5.870	0.144	0.390	0.259	–
6	14:00	109.677	0.474	0.074	7.506	1.091	0.345	0.232	0.152
6	23:00	134.021	0.489	0.506	9.727	0.231	0.477	0.436	1.119
7	14:30	134.595	0.565	0.202	8.568	0.439	0.402	0.443	0.000
7	23:30	117.091	0.598	0.489	9.032	0.579	0.545	0.518	0.000
8	14:30	147.347	0.961	0.318	11.843	0.972	0.379	0.385	0.273
8	23:30	148.625	1.168	0.289	14.677	0.501	0.603	0.636	1.038
9	14:30	114.834	0.384	0.310	10.221	0.622	0.391	0.453	0.000
9	23:30	107.125	0.565	0.270	9.919	1.746	0.322	0.593	0.350
10	14:30	186.992	0.666	0.298	14.100	0.739	0.655	0.617	0.486
10	23:30	193.822	1.553	0.802	21.931	1.267	0.686	1.026	0.421
11	15:30	160.204	1.385	0.441	14.839	0.782	0.818	0.905	0.630
11	23:30	195.854	1.502	0.525	18.287	0.377	1.008	1.082	1.632
12	14:30	119.094	0.847	0.456	14.233	1.235	0.553	0.570	0.689
12	23:30	114.783	1.052	0.363	14.705	1.227	0.470	0.623	1.682
13	14:30	85.176	0.949	0.468	15.734	1.510	0.300	0.527	0.979
13	23:30	77.324	1.289	0.546	13.133	2.009	0.324	0.402	0.194
14	14:30	105.216	1.651	0.391	14.400	1.361	0.413	0.716	0.000
14	23:30	163.962	1.825	0.509	20.845	1.295	0.579	1.063	0.752
15	14:30	149.523	1.027	0.559	19.728	1.648	0.472	0.899	1.612
15	23:30	101.477	1.060	0.254	14.472	1.100	0.477	0.654	0.609
16	n.r.	52.078	1.543	0.535	13.182	0.666	0.208	0.404	1.285
17	n.r.	57.275	1.581	0.636	22.829	1000	0.251	0.461	1.265
18	n.r.	82.978	1.810	1.070	26.859	1.325	0.368	0.728	3.598
19	n.r.	174.090	2.115	0.747	23.636	0.497	0.628	0.925	3.857
20	n.r.	177.147	2.615	1.167	29.156	1.195	0.911	1.123	2.755
21	n.r.	122.332	2.195	0.841	15.795	0.892	0.715	1.266	1.551
22	n.r.	172.546	0.912	0.442	23.767	0.707	0.736	1.062	2.352
23	n.r.	214.041	2.459	0.797	36.577	0.243	0.813	1.283	4.945

Concentrations are expressed in nanomolar (nM).
Time refers to milk collection time.
NDtam, N-desmethyl-tamoxifen; NNDDtam, N,N-D-desmethyl tamoxifen; n.r, not recorded; TamNoX, tamoxifen N-oxide; Z-4'-endoxifen, Z-4'-endoxifen; Z-endoxifen, Z (cis) stereoisomer of endoxifen; Z-4OHtam, Z-4-hydroxy-tamoxifen; Z-4'-OHtam, Z-4'-hydroxytamoxifen.

the range 4.1% to 14% at low concentrations and accuracies were in the range 99% to 111% (table 1).

RESULTS

Quantifiable tamoxifen levels (40.9 nM) were measured in breast milk starting from the day after the first day of tamoxifen treatment and the concentrations increased significantly to 214 nM on day 23 by an average of 3.4 nM/day (p=0.02). The major metabolite of tamoxifen, N-desmethyl tamoxifen (NDtam), was quantifiable by day 6 (7.51 nM) and was found at the highest level on day 23 (36.5 nM). The two active metabolites 4-hydroxy tamoxifen and Z-endoxifen also increased over time and reached quantifiable levels at day 18 (figure 1). The concentrations of all compounds at each time point throughout the 23 days can be found in table 2.

DISCUSSION

Tamoxifen±Gn RH analogue represents the standard adjuvant treatment for premenopausal women with low-risk ER-positive breast cancer.[3–5] This also applies to women diagnosed after delivery, a situation that is becoming more frequent due to the widely reported increase in age at first pregnancy. Until now, no data has been available on the transfer of tamoxifen into breast milk during nursing. In this case report we have shown that during oral administration of tamoxifen, clinically significant levels of the parent compound and of its metabolites are present in human milk.

Similarly to what has been described for plasma kinetics,[7 14 15] we found that tamoxifen concentrations in milk increased for at least 3 weeks, reaching 214 nM at the last day of sample collection. It should be noted that, even if individual variations may occur, plasma steady state of tamoxifen is usually not reached until 28 days,[13 16] thus it is possible that the concentration in breast milk could have been higher if we had measured it after 4 to 5 weeks. However, parallel analysis of plasma and milk samples would have been needed to confirm this.

Pharmacological data from the literature report that at steady state, NDtam levels are usually at higher concentrations than the parent drug.[13 16] In our study, we found that the concentration ratio between NDtam and tamoxifen in the milk was approximately 1:5 (37 and 214 nM, respectively). A possible explanation for this discrepancy is that plasma NDtam steady state was not yet reached, thus explaining the low concentration found at day 23. It is also possible that NDtam passes into milk at a lower rate than tamoxifen. Again, parallel analysis on plasma and milk samples would have been needed to confirm this.

The two active metabolites of tamoxifen, Z-4-hydroxytamoxifen (Z-4OHtam) and Z-endoxifen, were below the lower limit of quantification before day 18 after tamoxifen initiation. Z-4OHtam is a primary tamoxifen metabolite formed through hydroxylation of the parent drug. However, this metabolic pathway is less relevant (approximately 7% of the whole) compared with tamoxifen demethylation to NDtam. Conversely, Z-endoxifen is a secondary metabolite generated through hydroxylation of NDtam in plasma, while the latter is not the precursor of Z-endoxifen in milk. A possible explanation for low concentrations of the above-mentioned metabolites in the milk is the fact that they could not reach steady state in the plasma. Moreover, the pH of breast milk tends to be lower than in plasma, which would increase the retention of basic compounds in milk. The possibility that drug metabolites might accumulate in milk later than the parent compound, and reach higher concentrations, has already been described for antiproliferative drugs.[17 18] Thus, a complete analysis of the parent compound and all the active metabolites should be performed, as we did in our study.

If a woman is treated with tamoxifen during breastfeeding and if the baby is constantly exposed to it through breast milk, the drug may have negative effects on the infant as shown in preclinical studies.[19 20] The risk of adverse effects of drugs that are found in the milk of a nursing mother depends on the so-called 'infant dose'.[21 22] This is calculated from the concentration of the drug and its active metabolites in the milk and from the amount of milk ingested by the child per day. The steady state plasma concentration in the child depends on the amount of drug ingested per unit of time, oral bioavailability and drug clearance by metabolism and excretion. Of note, tamoxifen has a high oral bioavailability and it is mainly metabolised through cytochrome P450 2D6, which becomes active few days after birth.[12 23] In our study, we did not have steady state levels and other parameters to calculate the relative infant dose, but we can assume that it could be clinically significant.

As tamoxifen and its active metabolites Z-4OHtam and Z-endoxifen have potentially adverse effects on the growth and development of the child, breastfeeding should be discouraged while the mother is taking tamoxifen.

CONCLUSIONS

To our knowledge, this is the first report of tamoxifen and metabolite levels in human milk by analysis of repeated samples.

We have demonstrated that during oral administration of tamoxifen, clinically significant levels of the parent compound are present in human milk and therapeutically active tamoxifen metabolites are also significantly accumulating in milk over time. As tamoxifen has a complete oral bioavailability, a long half-life (>7 days) and might interfere with the normal development of the infant, mothers should not breastfeed during tamoxifen treatment.

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Contributors EB, HJ and FAP conceived this analysis. EB and BB collected and stored samples. GM, EA, LEB, SH, ETZ and TH performed the measurements. GCP and FP wrote the first draft. All authors contributed to the final version.

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REFERENCES

1 Paluch-Shimon S, Cardoso F, Partridge AH, *et al*. ESO–ESMO 4th International consensus guidelines for breast cancer in young women (BCY4). *Ann Oncol [Internet]* 2020;31:674–96.
2 Amant F, Loibl S, Neven P, *et al*. Breast cancer in pregnancy. *Lancet* 2012;379:570–9.
3 Burstein HJ, Lacchetti C, Anderson H, *et al*. Adjuvant endocrine therapy for women with hormone receptor-positive breast cancer: ASCO clinical practice guideline focused update. *J Clin Oncol* 2019;37:423–38.
4 Burstein HJ, Curigliano G, Loibl S, *et al*. Estimating the benefits of therapy for early-stage breast cancer: the St. Gallen international consensus guidelines for the primary therapy of early breast cancer 2019. *Ann Oncol* 2019;30:1541–57.
5 Lambertini M, Blondeaux E, Perrone F, *et al*. Improving adjuvant endocrine treatment tailoring in premenopausal women with hormone receptor-positive breast cancer. *J Clin Oncol* 2020;38:1258–67.
6 Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet* 2005;365:1687–717.
7 Buckley MM, Goa KL. Tamoxifen. A reappraisal of its pharmacodynamic and pharmacokinetic properties, and therapeutic use. *Drugs* 1989;37:451–90.
8 Schuurman TN, Witteveen PO, van der Wall E, *et al*. Tamoxifen and pregnancy: an absolute contraindication? *Breast Cancer Res Treat* 2019;175:17–25.
9 Braems G, Denys H, De Wever O, *et al*. Use of tamoxifen before and during pregnancy. *Oncologist* 2011;16:1547–51.
10 Buonomo B, Brunello A, Noli S, *et al*. Tamoxifen exposure during pregnancy: a systematic review and three more cases. *Breast Care* 2020;15:148–56.
11 Shaaban MM. Suppression of lactation by an antiestrogen, tamoxifen. *Eur J Obstet Gynecol Reprod Biol* 1975;4:167–9.
12 Hale TW. *Hale's Medications & Mothers' Milk*™. New York, NY: Springer Publishing Company, 2020: 928 p. http://connect.springerpub.com/lookup/doi/10.1891/9780826150356
13 Helland T, Hagen KB, Haugstøyl ME, *et al*. Drug monitoring of tamoxifen metabolites predicts vaginal dryness and verifies a low discontinuation rate from the Norwegian prescription database. *Breast Cancer Res Treat* 2019;177:185–95.
14 Patterson JS, Settatree RS, Adam HK, *et al*. Serum concentrations of tamoxifen and major metabolite during long-term Nolvadex therapy, correlated with clinical response. *Eur J Cancer* 1980;1:89–92.
15 Jordan VC. New insights into the metabolism of tamoxifen and its role in the treatment and prevention of breast cancer. *Steroids* 2007;72:829–42.
16 Helland T, Henne N, Bifulco E, *et al*. Serum concentrations of active tamoxifen metabolites predict long-term survival in adjuvantly treated breast cancer patients. *Breast Cancer Res* 2017;19:1–13.
17 Codacci-Pisanelli G, Honeywell RJ, Asselin N, *et al*. Breastfeeding during R-CHOP chemotherapy: please abstain! *Eur J Cancer* 2019;119:107–11.
18 Egan PC, Costanza ME, Dodion P, *et al*. Doxorubicin and cisplatin excretion into human milk. *Cancer Treat Rep* 1985;69:1387–9.
19 Karlsson S. Histopathology and histomorphometry of the urogenital tract in 15-month old male and female rats treated neonatally with SERMs and estrogens. *Exp Toxicol Pathol* 2006;58:1–12.
20 Chamness GC, Bannayan GA, Landry LA, *et al*. Abnormal reproductive development in rats after neonatally administered antiestrogen (tamoxifen). *Biol Reprod* 1979;21:1087–90.
21 Pistilli B, Bellettini G, Giovannetti E, *et al*. Chemotherapy, targeted agents, antiemetics and growth-factors in human milk: how should we counsel cancer patients about breastfeeding? *Cancer Treat Rev* 2013;39:207–11.
22 Begg EJ, Duffull SB, Hackett LP, *et al*. Studying drugs in human milk: time to unify the approach. *J Hum Lact* 2002;18:323–32.
23 O'Hara K, Wright IMR, Schneider JJ, *et al*. Pharmacokinetics in neonatal prescribing: evidence base, paradigms and the future. *Br J Clin Pharmacol* 2015;80:1281–8.