The influence of tamoxifen on the maturation index of vaginal epithelium

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Summary

Purpose: The estrogenic effect of tamoxifen on vaginal epithelium in postmenopausal women with breast cancer was evaluated over a period of more than 48 months.

Methods: The tamoxifen group consisted of 118 postmenopausal patients, control group I of 30 postmenopausal women with breast cancer receiving no tamoxifen or hormonal replacement therapy and control group II of 40 postmenopausal women without breast cancer taking no hormones. We determined the maturation index of the vaginal epithelium. Pearson's chi-square-test and t-test for independent samples were used in the statistical analysis.

Results: The maturation index increased under tamoxifen therapy within the first 24 months from 0.4026 before taking tamoxifen (n=64) to 0.6066 (n=162, $p<0.0001$) and in the following 24 months to 0.6325 (n=122, $p<0.0001$). Under tamoxifen intake of more than 48 months, an additional small increase of the maturation index to 0.6735 (n=42, $p<0.0001$) could be noticed. The maturation index in the tamoxifen group was statistically significantly higher ($p<0.0001$) than in the control groups (control group I: 0.3975, $p<0.0001$; control group II: 0.4102, $p<0.0001$).

Conclusion: An apparent increase in the maturation of the vaginal epithelium caused by the estrogenic effect of tamoxifen could be demonstrated.

Key words: Tamoxifen; Maturation index; Vaginal epithelium; Breast cancer.

Introduction

Tamoxifen is a synthetic nonsteroid triphenyl antiestrogen with a partial estrogenic effect. The antiestrogenic effects are explained in terms of a tamoxifen-estrogen receptor complex that has an antiproliferative effect on the DNA level by stopping cell division in the G1 phase. Irrespective of the receptor status, tamoxifen does not only affect autocrine and paracrine factors such as TGF-ß but also stimulatory factors, e.g. the EGF encoded by the oncogene Her 2. In postmenopausal receptor-positive patients with breast cancer, not only is the ten year mortality reduced in absolute terms, but significantly fewer recurrences and contralateral mammary carcinomas occur [1].

Besides the antiestrogenic properties of tamoxifen, the partial estrogenic effects should be considered. Cardiovascular protection and osteoprotection have been known for a long time. In recent years many studies have been published on the carcinogenic potency of tamoxifen. In animal experiments, indications were found for raised liver toxicity with secondary development of hepatocarcinomas [2]. Development of endometrial hyperplasia [3], endometrial polyps [4], endometriosis [5] and endometrial adenocarcinomas [6] has been described as a partial estrogenic effect of tamoxifen.

The primary purpose of the Papanicolaou stained cervical smear is to detect dysplastic or malignant cells and other significant abnormalities including hormonal activity. The recognition of an unexpected estrogen effect in the pap smear of postmenopausal women is important because of the frequency of a coexisting high level of estrogen effect in early endometrial carcinoma in these patients [7]. Bert *et al.* [8] showed that a postmenopausal woman with a high level of maturation of squamous cells has a 15 times greater chance of having a coexisting endometrial adenocarcinoma than a woman of the same age group with an atrophic pattern. A variety of drugs, hormonal and non-hormonal, as well as various therapeutic procedures may affect the cells in a cervical or vaginal pap smear. A pattern of high maturation is common in women receiving estrogens or digitalis, whereas progestins and tetracycline result in a less mature cell pattern [9, 10]. The aim of this study was to analyze the influence of tamoxifen on the maturation of the vaginal epithelium over a period of more than 48 months.

Patients and Methods

All patients in this prospective study were asymptomatic for gynecological disease. The study group consisted of 118 postmenopausal, estrogen-receptor positive patients. They had taken tamoxifen as adjuvant therapy for at least one year following surgical treatment. All patients were still taking tamoxifen during the study and none of them had received hormonal replacement treatment before starting tamoxifen therapy. Tamoxifen was given orally in doses of 30 mg daily. Control group I consisted of 30 postmenopausal, estrogen-receptor negative women with breast cancer. These patients were first treated more than two years from the beginning of the study.

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None of them had taken adjuvant hormone therapy. In control group II, the pattern of cytological smears of 40 postmenopausal women without primary breast cancer was examined. All patients were instructed to avoid local estrogen therapy in the last four weeks before taking smears. They all were selected by our outpatient clinic.

Smears of the vaginal wall were taken in the standard fashion using a cotton swab. The smears were fixed immediately in 95% alcohol and stained by the Papanicolaou method. The estrogenic effect of tamoxifen was studied by using the maturation index. The maturation index [11] reports the percentage of parabasal, large and small intermediate and superficial squamous cells and was obtained by counting 200 squamous cells per smear in a representative area (Figure 1). The maturation index represents a modification of the original estrogen level and is especially suitable for follow-up observations of the proliferation degree, also when there is little estrogen effect on vaginal epithelium. All cytological smears were screened by two cytopathologists (M. F., A. W.-H.). Pearson's chi-square-test and t-test for independent samples were used in the statistical analysis.

Figure 1. — Calculation of the maturation index (MI):
MI = [1.0 x percentage of superficial cells + 0.6 x percentage of large intermediate cells + 0.5 x percentage of small intermediate cells + 0.0 x percentage of (parabasal cells + basal cells)] / 100.

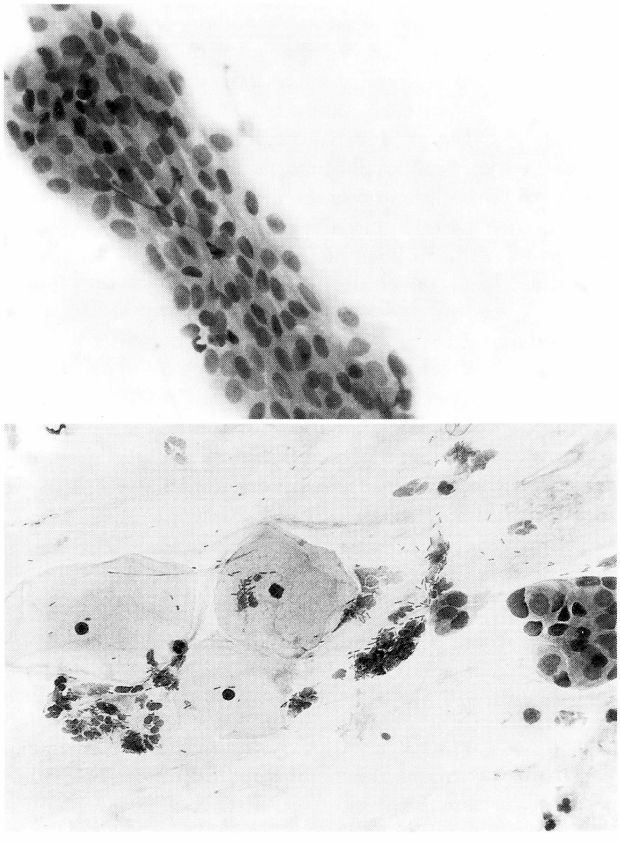

Figure 2. — Cytological changes under tamoxifen therapy. Atrophic cell picture before tamoxifen therapy (**a**) and increase of the maturation index under tamoxifen therapy (**b**).

Table 1. — *General data of the study group and both control groups.*

	Tamoxifen group median (range)	Control group I median (range)	Control group II median (range)	p-value
age (years)	65.74 (52-89)	67.65 (54-87)	63.21 (51-81)	n.s.
menopausal (months)	183.53 (12-480)	191.79 (24-453)	178.42 (20-426)	n.s.

n.s. = not significant

Table 2. — *Maturation index (MI) correlated with the duration of tamoxifen intake.*

Duration of tamoxifen intake (months)	Smears (n)	MI (means ± SD)	p-value
0	64	0.4026±0.11	
1-24	162	0.6066±0.13	<0.0001
25-48	122	0.6325±0.06	<0.0001
>48	42	0.6735±0.04	<0.0001

Results

The tamoxifen group and the control groups showed no significant difference in median age of the patients (Table 1). The median duration of tamoxifen intake was 36.42 months with a range from 8 to 82 months. Internal concomitant diseases appeared in equal parts in all three groups. There was no difference in taking drugs with an estrogenic partial effect. No significant differences in the clinical findings of the vaginal wall were observed among the three groups.

In the tamoxifen groups, smears of 64 patients were taken immediately before breast cancer surgery. Altogether 390 smears were studied in the tamoxifen group; 24 patients had a hysterectomy. In the control groups no patient had a hysterectomy; 96 smears were studied in control group I and 40 smears in control group II. The indications for all hysterectomies were leiomyomas.

The maturation index increased during the first 24 months from 0.4026 before taking tamoxifen (n=64) to 0.6066 (n=162, $p<0.0001$). In the following 24 months, the maturation index increased to 0.6325 (n=122, $p<0.0001$) and to 0.6735 (n=42, $p<0.0001$) after an intake of more than 48 months. Compared with the initial levels, a statistically high-significant increase ($p<0.0001$) of the maturation index could be observed every time. A low-significant increase ($p=0.005$) of the maturation index from 0.6066 to 0.6735 emerged within the first 48 months of therapy (Table 2). In the tamoxifen group the maturation index was statistically significantly higher than in the control groups (control group I: 0.3975; control group II: 0.4102, $p<0.0001$).

Discussion

Tamoxifen is widely used as adjuvant therapy for breast cancer in women with positive estrogen receptors. Its benefits include an improvement in disease-free survival in postmenopausal women with estrogen receptor positive tumors confined to the breast and axillary nodes, decreased recurrences in node negative cancers and

decreased incidence of second primaries. Tamoxifen has also been reported to produce estrogenic changes in the vaginal epithelium of postmenopausal breast cancer patients by increasing the karyopycnotic index as well as having stimulatory effects on the endometrium [12, 13]. In athymic mice, the contrasting actions of tamoxifen on the growth of breast and endometrial carcinoma cell line EnCa 101 and a resistant MCF-7 breast carcinoma cell line have been demonstrated [2, 3, 14, 15]. Gallo *et al.* [16] explain the agonistic effects of tamoxifen as a function of the estrogen receptor complex present in a particular cell or tissue. In their opinion, tamoxifen is antagonistic if a cell type requires activating factors 1 and 2 of the estrogen receptor to be functioning concurrently. However tamoxifen is agonistic if a tissue requires only activating factor 1 to interact with transcription factors at the promoter. Metabolites of tamoxifen such as metabolite E, formed by removal of the aminoethane side chain, are described as a weak estrogen agonist [17].

The relationship between tamoxifen and cancer of the uterine corpus is currently receiving great interest in the medical literature [1, 4, 16, 17]. In a randomized trial of adjuvant tamoxifen treatment in 1,846 postmenopausal women with early breast cancer Fornander *et al.* [6] reported a 6.4-fold increase in the relative risk of endometrial cancer in 931 tamoxifen-treated patients, compared to 915 patients in the control group. They suggested a dose-effect relation of tamoxifen exposure for the induction of endometrial cancer. However, the tamoxifen dosages in their report (40 mg daily) were higher than in some other trials. Adami *et al.* [18] reported an overall relative risk for developing endometrial cancer in 1.72 in women with a history of breast cancer. This ranged from a relative risk of 1.0 in women with a diagnosis of breast cancer before age 50 to a relative risk of 2.4 for those over 70 years. In the analysis of the NSABP Study [1], in which the data of 2,843 breast cancer patients (including 1,220 who received randomized treatment with 20 mg tamoxifen per day) with a positive estrogen receptor status in negative lymph node status were analyzed, there was no significant increase in the incidence of second carcinomas under tamoxifen therapy, such as for example gastrointestinal tumors, hepatocarcinomas and malignancies of the urogenital tract. On the other hand, the relative risk of developing an endometrial malignancy under tamoxifen intake rises significantly to 7.5, whereas over an observation period of five years the incidence of second carcinomas in the contralateral breast fell from 40.5 per 1,000 in the placebo group to 23.5 per 1,000 in the group treated with tamoxifen. Estrogenic action on vaginal epithelium has been noted with 20 mg tamoxifen daily. Thus, a significant estrogenic effect may also occur with relatively low doses of tamoxifen [1].

In the present study, an apparent increase of the maturation of the vaginal epithelium caused by tamoxifen therapy could be demonstrated. Factors, that may effect endogenous estrogen production such as age and menopausal age, did not differ among the groups. It is well known that hormonal substances and non-hormonal drugs (e.g. digitalis and tetracycline) may affect maturation of vaginal squamous epithelium [9, 10]. Concerning this fact, there were no differences between the three groups. The mechanism of the mild proliferative effect of tamoxifen on vaginal cells in premenopausal women may be due to increased levels of serum estradiol levels reported in patients taking tamoxifen [19]. In postmenopausal women, these changes are attributed to the estrogenic activity of tamoxifen.

Conclusion

The most relevant conclusion for the clinicians is the importance of monitoring the effect of tamoxifen on the genital tract of breast cancer patients. The maturation index seems to be a helpful instrument. In future, antiestrogens of the second and third generation with selectively antagonistic properties will possibly provide an application which is weaker in secondary effects. The proven global benefit of adjuvant tamoxifen therapy must be considered - not only with regard to the reduction in the risk of second breast carcinoma by 40%, to the increase in the length of the recurrence-free interval and to the overall survival, but also with regard to a reduction of cardiovascular diseases and development of osteoporosis.

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