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Effects of low dose tamoxifen on normal breast tissue from premenopausal women

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Abstract

The aim of this study was to determine the effects of low doses of tamoxifen (5 and 10mg/day) for 50 days compared with the standard dose (20 mg/day) on breast biomarkers measured in normal breast tissue from premenopausal patients. A randomised double-blind study was performed using tissue from 56 premenopausal women with a diagnosis of fibroadenoma of the breast. Excisional biopsy was performed on the 50th day of therapy. Normal breast tissue samples were collected during surgery. The patients were divided in groups: A (placebo, $n=11$); group B (5 mg, $n=16$), group C (10 mg, $n=14$) and group D (20 mg, $n=15$). In this cross-sectional study, differences in the expression of Oestrogen Receptor alpha (ERα), Progesterone Receptor (PR), Ki-67, apoptotic bodies and mitotic index between the different groups after treatment can be seen on the normal breast tissue. We believe that a lower dose of tamoxifen could reduce the side-effects associated with treatment without affecting its chemopreventive activity in the breast.

Keywords: Tamoxifen; Premenopausal; Breast biomarkers; Normal breast tissue

1. Introduction

Tamoxifen is a selective oestrogen receptor modulator (SERM), and is the endocrine therapy of choice for pre and postmenopausal Oestrogen Receptor (ER) positive breast cancer patients. The chemoprevention of breast cancer with tamoxifen has been one of the most recent advances in therapeutics. However, despite clinical and epidemiological evidence that shows a reduction of breast cancer incidence following tamoxifen treatment, it is still uncertain whether the drug acts to prevent carcinogenesis or to treat occult carcinoma [1].

Evidence for the prevention of breast cancer with tamoxifen was originally based on a 47% reduction of incidence of new primary contralateral cancer in women during adjuvant tamoxifen therapy [2]. Additionally, the study sponsored by the United States (US) National Cancer Institute found a reduction in the incidence of invasive carcinoma of up to 50% in 13,338 women randomised to 20 mg tamoxifen daily compared with placebo for 5 years [3]. When the National Surgical Adjuvant Breast and Bowel Project/National Cancer Institute (NSABP/NCI) chemoprevention trial analysed the incidence of ductal carcinoma *in situ* (DCIS) in the tamoxifen and placebo groups, there was also a 50% reduction in the risk of non-invasive breast cancer in the tamoxifen-treated group. A subset analysis of women at risk with a diagnosis of lobular carcinoma in situ (LCIS) demonstrated a 56% reduction in breast cancer when treated with tamoxifen. Finally, the most dramatic reduction seen in a US prevention trial was in women at risk with a diagnosis of atypical hyperplasia where the risk of breast cancer was reduced by 86%. Unfortunately, the benefits of tamoxifen must be balanced with its side-effects. An increased risk of endometrial cancer was observed in women aged 50 years and older. Besides that, more women aged 50 years and older in the tamoxifen group developed deep vein

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thrombosis, pulmonary emboli and had an increased incidence of stroke than in the placebo group. Additionally a small increase in cataracts was noted in the tamoxifen-treated group—a rate of 24.8 women per 1000 compared with 21.7 in the placebo group. Lastly, hot flashes were noted in 81% of the women on tamoxifen compared with 69% of the placebo group, and the tamoxifen-associated hot flashes appeared to be of greater severity than those in the placebo group. Moderately bothersome or severe vaginal discharge was reported by 29% of women in the tamoxifen group and 13% in the placebo group.

The use of a 20 mg dose of tamoxifen for chemoprevention of breast cancer was empirical and there is no reason to believe that lower doses could be evaluated with efficacy. In the case of chemoprevention, a low, but effective, dose could reduce public health costs and potentially reduce side-effects, thereby increasing the availability of chemoprevention for larger numbers of women who could potentially reduce their risk for breast cancer.

Previous studies have demonstrated that a reduction in the conventional dose of tamoxifen (i.e., 20 mg/day) does not affect the activity of the drug in a large number of biomarkers, most of which are circulating oestrogenic surrogate markers of cardiovascular disease [4–6]. However, we believe it is important to access markers of anti-oestrogenic activity in the breast. We have demonstrated, for the first time, that low doses of tamoxifen (5 and 10 mg/day) were able to promote the same response on the breast biomarkers [ERα, Progesterone Receptor (PR), Ki-67, apoptotic bodies and mitotic index] compared with 20 mg of tamoxifen per day in normal breast tissue. The study of the interaction of low doses of tamoxifen with the steroid receptors, the proliferation and the apoptosis of the mammary lobules are important to evidence the effects of the drug in non-neoplasic tissues.

2. Patients and methods

2.1. Patients and eligibility

Fifty-six patients with fibroadenoma of the breast were recruited from the Mastology Sector of the Gynecology Department of the Federal University of São Paulo (UNIFESP-EPM) during the period from April to August of 1999. The patients were all volunteers and were informed about the research study prior to signing a waiver approved by the Medical during long-term treatment Ethics Committee (no. 873/98) of UNIFESP-EPM. The patients were all premenopausal women with ages varying between 15 and 35 years. The number of black and white women was similar. Malignancy was excluded by clinical examination, ultra-sonography, cytology and later histopathology of the fibroadenoma. All patients showed regular cycles (cycles of 28 plus or minus 2 days) for at least 6 months. They did not show endocrine diseases and none had previous hormonal therapy; they did not report previous vascular thrombosis or pregnancy for at least 12 months prior to the study and were subject to non-hormonal contraceptive methods.

2.2. Treatment

The patients were randomised by computer in group A (placebo, $n=11$); group B (Tamoxifen [Tam] 5 mg, $n=16$), group C (Tam 10 mg, $n=14$) and group D (Tam 20 mg, $n=15$). The drug was administered for 50 days starting on the first day of the menstrual cycle. Tamoxifen citrate was supplied by ZODIAC laboratories and processed by the Natural Product Laboratory of the Cellular Pharmacology Department of the Pharmacology Department (INFAR) of the Federal University of São Paulo. The capsules with placebo and tamoxifen were prepared in doses of 5, 10 and 20 mg and kept in sealed, bar-coded containers. The groups A (placebo), B (5 mg), C (10 mg) and D (20 mg) were considered homogenous with regard to age, volume of the fibroadenoma and parity (Table 1).

2.3. Surgical method

Patients whose fibroadenoma did not show a volume reduction had an excisional biopsy on the 50th day of treatment (luteal phase of the menstrual cycle, which was confirmed by a blood sample for progesterone levels and the date of the last day of the menses; data not shown) under local anaesthesia without a vascular constrictor. The mammary tissue specimens were extracted out of the area adjacent to the fibroadenoma, absent of fat tissue or macroscopic alteration.

Table 1
Patient characteristics

Age (years)	A (placebo)	B (Tam = 5 mg)	C (Tam = 10 mg)	D (Tam = 20 mg)
Average	23.7	20.6	22.5	21.1
S.D.	1.4	1.1	1.5	1.5
$P=0.385$				
Size of fibroadenoma (cm)				
Average	3.1	3.3	2.8	3.2
S.D.	0.9	0.7	1.0	1.0
$P=0.981$				
Parity (%)				
Yes	69.2	87.2	92.9	80
No	30.8	12.5	7.1	20
$P=0.392$				

Tam, tamoxifen; S.D., standard deviation.

2.4. Immunohistochemistry for ERα, PR and Ki-67

All specimens were fixed in 10% neutral formalin for 48 h and then embedded in paraffin. Sections measuring 4 μm, taken from the paraffin-embedded blocks, were stained using the EnVision system (DAKO, Carpinteria). After deparaffinisation with xylene and hydration with downgraded ethanol, the sections were incubated in 0.3% H_2O_2 in methanol for 5 min at room temperature. Monoclonal mouse anti-human ERα (1:80 dilution) (NCL-ER-6F11, Novacastra laboratory Ltd.), monoclonal mouse anti-human PR (1:50 dilution) (PgR-636, DAKO Corporation) and monoclonal anti-Ki-67 antibody (1:100 dilution) (ready-to-use, DAKO) were used as primary antibodies. Antigen retrieval was performed using antigen retrieval solution (DAKO) before incubation for the primary antibodies. The sections were rinsed with buffer solution, and peroxidase labelled polymer was incubated for 30 min at room temperature. After the sections were washed with buffer solution, the peroxidase reaction was developed with diaminobenzidine tetrahydrochloride. To evaluate the ERα, PR and Ki-67 expression, slides were counterstained with haematoxylin, dehydrated, cleared, and mounted for examination by light microscopy. Immunostaining for ERα, PR and Ki-67 showed a few positive nuclear granules or a continuum to homogeneous nuclear staining. Any appearance of nuclear staining was considered as ERα, PR and Ki-67 positivity. The Ki-67 labelling index (LI) was determined by counting the numbers of stained and unstained cell nuclei.

2.5. Apoptotic

The identification and quantification was performed double blinded in 10 fields at 400× magnification. The clinician and 2 analysts were blinded, and all analyses were performed before breaking the code. Only cells identified by the eosin-like cytoplasm and the chromatic condensation with or without blebs were considered. In addition, only cells without inflammatory reaction were considered (in around 1000 cells).

2.6. Mitotic index

Mitosis was identified at 400× magnification (different cellular phases) in around 1000 cells. To count the cells, an image digital system, *Image-Pro® Plus version 3.0 for Windows™* (Media Cybernetics, L.P) was used.

2.7. Statistical analysis

All histological parameters were evaluated on coded samples to avoid bias. The Levene's test [7] was used to evaluate the homogeneity among the groups (A, B, C and D) regarding age, menarche, parity, lactation, volume of the fibroadenoma and body mass index. The oneway ANOVA analysis was used to evaluate differences between multiple groups. All P values less than 0.05 were considered significant. We also conducted a Tukey test for trends (there was a greater effect of the higher dosages than the lower dosage of tamoxifen in certain parameters).

3. Results

3.1. Expression of ERα and PR [means ± standard deviation (S.D.)]

The percentage of cells expressing ERα on the normal breast tissue in group A (placebo) was 42.85%. When the patients were treated with tamoxifen, there was a decrease of ERα expression. For group B (Tam 5 mg/day), it was 7.02% and groups C (Tam 10 mg/day) and D (Tam 20 mg/day) 5.55 and 4.80%, respectively. The results for groups B, C and D were significantly lower ($P < 0.001$) than group A (Fig. 1).

The percentage of cells expressing PR on the normal breast tissue in group A (placebo) was 27.05%. Patients treated with tamoxifen had a lower PR expression. For group B (Tam 5 mg/day), it was 7.85% and groups C (Tam 10 mg/day) and D (Tam 20 mg/day) 7.45 and 7.74%, respectively. According to variance analysis, the results for groups B, C and D were significantly lower ($P < 0.001$) than group A (Fig. 2).

There was no difference among the groups B, C, and D for both ERα and PR expressions.

3.2. Immunohistochemistry for Ki-67 labelling index (LI)

The percentage of cells expressing Ki-67 LI on the normal breast tissue in group A (placebo) was 2.04%. When the patients were treated with tamoxifen, there

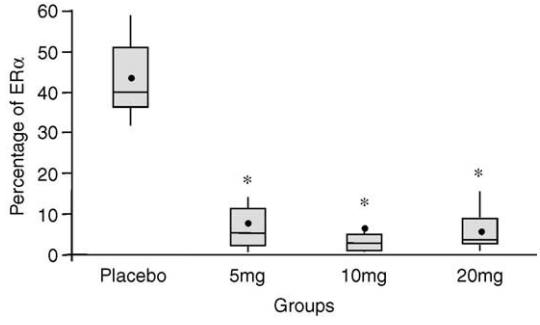

Fig. 1. Boxplots of Oestrogen Receptor (ERα) expression in normal epithelial breast tissue from premenopausal patients by groups (means are indicated by solid circles). Group A (placebo) presented higher ERα expression compared with tamoxifen-treatment groups. Tamoxifen decreased the ERα expression at all doses compared with the placebo group (*$P < 0.001$, oneway ANOVA). Group A was 42.85% (±8.74) positive for ERα compared with 7.02% (±7.88), 5.55% (±10.74), and 4.80% (±4.28) in groups B, C and D, respectively.

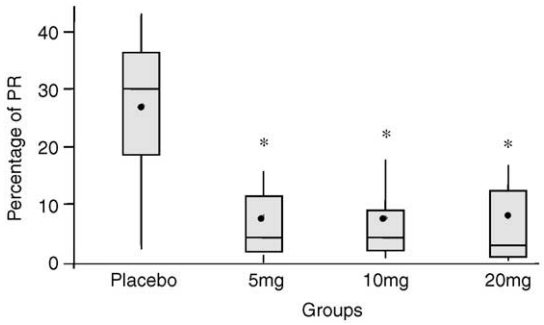

Fig. 2. Boxplots of Progesterone Receptor (PR) expression in normal epithelial breast tissue from premenopausal patients by groups (means are indicated by solid circles). Group B (Tam = 5 mg), group C (Tam = 10 mg) and group D (Tam = 20 mg) had a lower expression of PR when compared with the placebo group (*$P < 0.001$, oneway ANOVA). Group A was 27.05% (±12.58) positive for PR compared with 7.85% (±8.89), 7.45% (±8.80), and 7.74% (±9.60) in groups B, C and D, respectively.

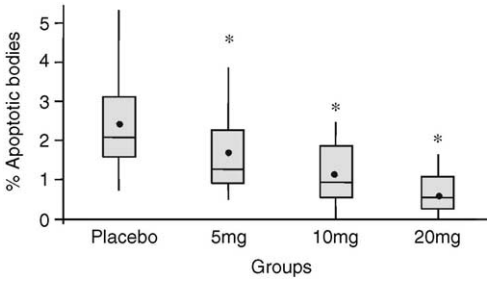

Fig. 4. Boxplots of the amount of apoptosis in normal epithelial breast tissue from premenopausal patients by groups (means are indicated by solid circles). The number of apoptotic bodies was diminished by tamoxifen at all doses when compared with placebo (*$P < 0.03$, oneway ANOVA). Group A had 2.4% (±0.36) of apoptotic bodies compared to 1.50% (±0.29), 1.14% (±0.18) and 0.65% (±0.11) in groups B, C and D, respectively.

was a decrease in Ki-67 LI expression. For group B (Tam 5 mg/day), it was 0.70% and for groups C (Tam 10 mg/day) and D (Tam 20 mg/day) it was 0.42 and 0.09%, respectively. The results for groups B, C and D were significantly lower ($P < 0.001$) than group A (Fig. 3). There was no difference among the groups B, C, and D in their expression of Ki-67 LI.

3.3. Identification of apoptotic bodies

The percentage of apoptotic bodies on the normal breast tissue in group A (placebo) was 2.40 (apoptotic bodies = 25). When the patients were treated with tamoxifen there were fewer apoptotic bodies. For group B (Tam 5 mg/day), it was 1.60% (apoptotic bodies = 16.5) and groups C (Tam 10 mg/day) and D (Tam 20 mg/day) 1.14% (apoptotic bodies = 11.23) and 0.65% (apoptotic bodies = 6.25), respectively. The results for groups B, C and D were significantly lower ($P < 0.03$) than group A (Fig. 4). There was no difference between groups C and D in the presence of apoptotic bodies, but group B was significantly different from D ($P = 0.004$).

3.4. Mitotic index

The mitotic index in the normal breast tissue for group A (placebo) was 0.012. When the patients were treated with tamoxifen, there was a decrease of the mitotic index in the normal breast tissue. For group B (Tam 5 mg/day), it was 0.003 and groups C (Tam 10 mg/day) and D (Tam 20 mg/day) 0.001 and 0, respectively. The results for groups B, C and D were significantly lower ($P < 0.001$) than group A (Fig. 5). There was no difference between the groups C and D in the presence of mitotic index, but group B was significantly different from D ($P < 0.05$).

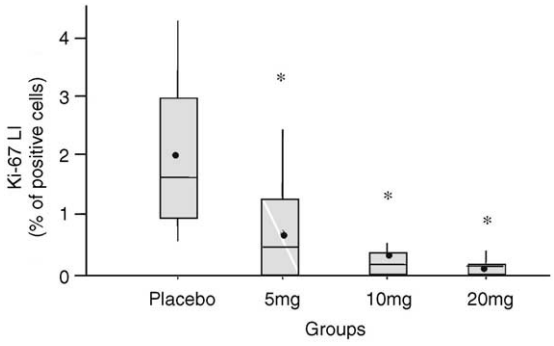

Fig. 3. Boxplots of Ki-67 Labelling Index (LI) in normal epithelial breast tissue from premenopausal patients by groups (means are indicated by solid circles). Tamoxifen decreased the nuclear proliferative marker in all of the tamoxifen-treatment groups compared with the placebo group (*$P < 0.001$, oneway ANOVA). Group A was 2.04% (±0.34) positive for Ki-67 LI compared with 0.70% (±0.19), 0.42% (±0.24), and 0.09% (±0.04) in groups B, C and D, respectively.

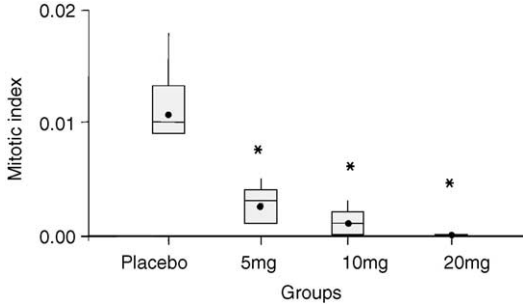

Fig. 5. Boxplots of Mitotic index in normal epithelial breast tissue from premenopausal patients by groups (means are indicated by solid circles). The mitotic index was decreased by tamoxifen in group B (5 mg), C (10 mg) and D (20 mg) compared with group A (placebo) (*$P < 0.001$, oneway ANOVA). Group A had 0.012 (±0.0007) of mitotic index bodies compared with 0.003 (±0.0004), 0.001 (±0.0003), and 0 in groups B, C and D, respectively.

4. Discussion

Tamoxifen expresses oestrogenic activity in select tissues of a women body's. Tamoxifen increases the incidence of endometrial cancer by 3–4-fold in postmenopausal patients and increases the rates of stroke, pulmonary embolism, and deep-vein thrombosis more frequently in women aged 50 years or older [3]. Several studies have shown that the endometrial effect of tamoxifen is associated with treatment duration and cumulative dose [3,8–10]. On the other hand, recent data support the notion that the dose of tamoxifen may be lowered without affecting its activity [4,5]. In addition, it is well known that after the use of conventional doses of tamoxifen in premenopausal women an increase in blood oestradiol levels occurs. However, when a lower dose is used a smaller enhancement of oestradiol occurs (data not shown). This is an important finding related to low dose tamoxifen because in addition to the lower incidence of side-effects, the premenopausal women are not at risk of high levels of oestradiol, which is safer for the women.

The current dose of tamoxifen to treat and prevent breast cancer in the United States is 20 mg/day, but there are different clinical treatment trials that use 30 [11] or 40 mg [12,13] for breast cancer therapy. Although the best way to determine the optimal dose of tamoxifen is to conduct a randomised trial comparing different doses of treatment, no trials have been conducted. Nevertheless, indirect comparisons suggest (overview analysis) that there are no differences in response using 20, 30 or 40 mg per day. However, this does not rule out that the fact that a dose lower than 20 mg per day might be equally effective for either treatment or prevention.

A cross-sectional study has shown that 10 mg/day of tamoxifen can reduce the incidence of hip fracture among older nursing home residents compared with non-users and women taking 20 mg/day [14]. Additionally, comparable activity of lower doses of tamoxifen has been shown on several surrogate biomarkers of cardiovascular disease and breast cancer, including Insulin-like Growth Factor-I (IGF-I) [4]. Although low tamoxifen concentrations induce a comparable modulation of the IGF family relative to the conventional dose, the lower decrements in the IGF-I/IGFBP-3 ratio observed at low drug concentrations might be associated with a reduced preventive activity [15].

Reducing the dose of tamoxifen could reduce side-effects, but must retain pharmacological activity as a preventive. Up to a 75% reduction in the conventional dose of tamoxifen (i.e. 20 mg/day) does not affect the activity of the drug on a large number of biomarkers, most of which are surrogate markers of cardiovascular disease [4]. Additionally, an 80% reduction in blood concentrations does not seem to affect the activity of tamoxifen on biomarkers of cardiovascular (lipid profile, blood cell count, fibrinogen, antithrombin III, osteocalcin) or breast cancer risk (IGF-I) and may in fact have a more favourable safety profile, because the side-effects of the drug can be diminished [5]. Nevertheless, no studies have been reported on the action of low doses of tamoxifen on the normal epithelial breast tissue of the premenopausal patients. This is a prerequisite before prevention trials can be considered safe.

A precise interpretation of ER regulation by hormones in non-neoplasic breast tissue is unclear because of their heterogeneity. ERs are downregulated during the luteal phase, while PRs remain at a high level throughout the menstrual cycle [16]. According to most studies, in vivo proliferation of normal breast epithelial cells is higher during the luteal phase in the vast majority of women [16]. The mammary lobule undergoes variations during the menstrual cycle with its peak and largest number of apoptotic bodies in the luteal phase [17,18]. Battersby and colleagues [19] demonstrated ERα positivity in 35% of normal epithelial breast tissue during the luteal phase of the menstrual cycle, while Khan and colleagues [20] showed 31% of ER positivity in the normal breast tissue of postmenopausal women.

In a previous small study, we demonstrated that short-term tamoxifen therapy increased breast cancer ERα expression in 10% of the patients and decreased in 40% [21]. Brotherick and colleagues [22] also showed that ERα levels in breast cancer patients treated with tamoxifen 3 weeks before surgery are significantly lower than in a comparative group of patients who received no drug. In addition, a decrease in ERα and a rise in PR after 14 days of treatment with tamoxifen was observed in another study in breast cancer tissue [23]. Similarly with breast cancer tissue, the ER content seemed to be reduced in normal breast tissue following tamoxifen treatment (Fig. 1).

After limited exposure to tamoxifen, the PR appeared to be increased in normal breast tissue, and longer treatment caused the PR to go down to pretreatment levels or below [24]. The observation that PR status is different in response to tamoxifen depending on the normal and tumour breast tissue is highly important. In normal epithelial breast tissue, tamoxifen downregulates PR, while in breast cancer tissue it upregulates the receptor, which indicates that tamoxifen plays a greater agonistic effect in tumour tissue than in normal tissue.

In the present study, ERα and PR expressions decreased significantly on the normal breast tissue (epithelium) of patients receiving 5, 10 or 20 mg/day of tamoxifen for 50 days compared with the placebo group. The important finding was that low doses of tamoxifen decreased ERα and PR expression to levels observed with the standard dose of tamoxifen.

Ki-67, a nuclear proliferation marker, is useful to evaluate the prognosis of patients with breast cancer.

Tamoxifen inhibits breast tumour cell proliferation, so when measurements of proliferative activity (Ki-67 LI) are analysed after a short treatment period, the result could be considered a reasonable approach to predict response. One study showed that tamoxifen-treated patients had a median Ki-67 LI of 5.6% in the first biopsy (pre-treatment) falling to 3.0% in the second biopsy (after 21 days of treatment) ($P < 0.001$), whereas placebo-treated patients had a median Ki67 LI of 5.4% in the first biopsy and 5.75% in the second (non significant difference) [25]. In the same study, no significant differences were observed when the median%ERα or%PR staining before and after treatment were compared [25]. A decrease in Ki-67 LI and ERα and a rise in PgR after 14 days of treatment with tamoxifen was observed by Makris and colleagues [23] and these factors were related to subsequent response. In addition, Ki-67 LI significantly decreased after 30 days of tamoxifen treatment [21]. In the present study, Ki-67 LI expression on normal breast tissue decreased significantly in patients receiving 5, 10 or 20 mg/day of tamoxifen for 50 days compared with placebo group. The relevant finding was that lower doses of tamoxifen decreased Ki-67 LI as much as the 20 mg/day dose of tamoxifen.

Apoptosis (''programmed cell death'') is an active process characterised by prominent nuclear changes and DNA cleavage, which distinguishes it from cellular necrosis. Tamoxifen induces typical apoptosis in ER+or ER−human breast cancer cells. The induction of apoptosis by tamoxifen in MCF-7 cells (ER +) involves the ER, and requires the synthesis of new protein and mRNA. Tamoxifen-induced apoptosis in MDA-231 cells (ER-) depends primarily on protein synthesis. Tamoxifen-induced cytotoxicity and DNA damage appear to be explained, in part, by the induction of apoptosis [26]. Perry and colleagues [27] showed that, independent of the ER status, tamoxifen directly regulates TGF-beta 1 transcription, leading to an increase of cells in the G0/G1 phase of the cell cycle and apoptosis. However, oestrogen is involved [28] in the process of mammary apoptosis, and our data have shown that a low dose of tamoxifen was able to block the effects of oestrogen, as demonstrated by the reduced number of apoptotic bodies in the tamoxifen-treated tissue.

Ferlini and colleagues [29] performed studies *in vitro*, showing that tamoxifen promoted apoptosis after 8 h of drug exposure. In addition, another study demonstrated that an increased apoptotic cellular number was seen in the first 7 days of tamoxifen therapy in nude mice implanted with ER-positive breast cancer cell lines while after 28 days of drug treatment there was a significant reduction [30]. As a conclusion, during an acute phase of tamoxifen therapy there is an increase in apoptosis, however, there is an important reduction of cellular proliferative rate that intensifies later on. In the present study, we found a diminished number of apoptotic bodies after 50 days of tamoxifen therapy in doses of 5, 10 and 20 mg with an intense lobular atrophy. The reduction of the cellular population shows a relative decrease in the number of apoptotic bodies which we believe to be a secondary event. Earlier studies [29,30] agree with our results and the reduction in the number of apoptotic bodies is probably due to a reduction in the proliferative activity of the epithelium. It would be very useful to evaluate the molecular events of normal breast tissue to elicit this important observation that we found in our study.

In a previous study we demonstrated the action of tamoxifen on normal breast tissue from premenopausal patients. Tamoxifen given over 22 days, either 10 or 20 mg/day, significantly reduced proliferating cell nuclear antigen (PCNA) expression and therefore the proliferative activity of normal human breast tissue. In addition, increasing levels of oestradiol, progesterone and sex hormone-binding globulin (SHBG) were associated with tamoxifen therapy, given at doses of 10 or 20 mg/day [31]. In another study, tamoxifen (20 mg/day for 10 days) significantly reduced the nuclear volume and mitotic index (number of mitoses/1000 nuclei counted) of the normal breast tissue, which demonstrated an antagonistic action of tamoxifen on oestrogen even when administered for short periods of time [32]. Finally, tamoxifen administered after ovulation significantly decreases the number of lysosomes in cells of normal mammary epithelium, demonstrating the anti-oestrogenic effect of the drug on this target tissue [33]. In the present study, we have consistently demonstrated that low doses of tamoxifen could act as an anti-oestrogen with efficiency on normal epithelial breast tissue by lowering the nuclear proliferative marker (Ki-67), apoptotic bodies and the mitotic index.

The concept that tamoxifen has a favourable effect on human normal breast tissue is well known. A previous study by Walker and colleagues [34] indicates that normal breast tissue obtained from the perimeter of benign biopsies from 17 patients (15 postmenopausal and 2 premenopausal patients) receiving 40 mg of tamoxifen daily (4 days–3 weeks) did not show any adverse effect from tamoxifen treatment on the normal tissue. Most importantly, treatment with the anti-oestrogen does not appear to stimulate cell proliferation even when given as long-term therapy. In the present study, we have demonstrated that low dose tamoxifen is appropriate for women who are deemed to be at a high-risk of developing breast cancer.

In conclusion, our results indicate that breast biomarkers of premenopausal patients are modified with 5, 10 or 20 mg/day of tamoxifen for 50 days when compared with the placebo group. Since the adverse effects

of tamoxifen therapy may be dose-related, we support the view that testing low doses of tamoxifen may decrease the side-effects of tamoxifen treatment without compromising its chemopreventive effects on breast cancer.

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