Induction of tamoxifen resistance in breast cancer cells by ELF electromagnetic fields

Rainer Girgert a,*, Hartmut Schimming b, Wolfgang Körner c, Carsten Gründker a, Volker Hanf a

a Department of Obstetrics and Gynecology, University of Göttingen, D-37099 Göttingen, Germany
b Facility for Electronic Equipment, University of Ulm, D-89075 Ulm, Germany
c Bavarian Environmental Protection Agency, D-86179 Augsburg, Germany

Received 30 August 2005
Available online 9 September 2005

Abstract

The incidence of breast cancer in western societies has been rising ever since the Second World War. Besides the exposure to a multitude of new chemical compounds, electromagnetic field exposure has been linked to breast cancer through a radiation-mediated anti-melatonin pathway. We investigated, whether low-frequency electromagnetic field exposure interferes with the anti-estrogenic activity of tamoxifen. Two different clones of the breast cancer cell line MCF-7 were exposed to highly homogeneous 50 Hz electromagnetic fields and IC50 values were calculated from dose–response curves of tamoxifen at various field intensities. An intensity-dependent shift of tamoxifen dose–response curves to higher concentrations with a maximal response at 1.2 μT was observed. Hypothetically, electromagnetic field exposure could contribute to tamoxifen resistance observed in breast cancer after long-term treatment.

Keywords: Breast cancer; Estrogen receptor; Electromagnetic fields; Tamoxifen resistance; Dose-response

The effect of extremely low-frequency electromagnetic field (ELF/EMF) exposure on human health has been widely debated. A number of epidemiological studies have pointed to a slight increase in malignant diseases in populations exposed to electromagnetic fields through the vicinity of power lines. A significant positive association was observed between childhood leukemia and exposure of children to magnetic fields during the night [1]. In two studies, premenopausal women exposed to environmental fields stronger than 0.2 μT had an increased risk of breast cancer (BC) [2,3]. Conversely, studies from Finland and Taiwan did not find any increased BC risk in populations living in the proximity (100–500 m) of power lines [4,5].

These epidemiological observations prompted the examination of the impact of electromagnetic fields on breast cancer incidence in an animal model. Sprague–Dawley rats suffer of a high rate of mammary tumors if treated with the chemical carcinogen 7,12-dimethylbenz[a]-anthracene (DMBA). Exposure of these rats to a 100 μT electromagnetic field for 27 weeks increased the number of tumor bearing rats to 65% compared to 50% in sham exposed rats [6]. Although radiation energy of an extremely low-frequency magnetic field (50 Hz) is considered to be by far too low to induce DNA strand breaks, Lai and Singh [7] observed an increase in DNA single- and double-strand breaks in brain cells of rats exposed to electromagnetic fields as low as 10 μT. This effect was attributed to the generation of oxygen radicals in the presence of iron ions [7]. In addition, EMF was reported to suppress the nocturnal synthesis of melatonin in the pineal gland in animals and human [8]. As melatonin may physiologically inhibit estrogen production by the ovary, the EMF-suppressed melatonin secretion would favor the growth of estrogen-dependent BC [9]. A direct oncostatic effect of melatonin on breast cancer cells was first demonstrated by Blask and Hill...
Materials and methods

Table 1

Influence of low-frequency magnetic fields on tumor cell gene expression

<table>
<thead>
<tr>
<th>Gene</th>
<th>Alteration</th>
<th>No effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-myc</td>
<td>Increase [27]</td>
<td>[28,29]</td>
</tr>
<tr>
<td>ODC</td>
<td>Increase [30,31]</td>
<td>[32]</td>
</tr>
<tr>
<td>HSP70</td>
<td>Decrease [33]</td>
<td>[34]</td>
</tr>
</tbody>
</table>

[10] and many other investigators thereafter. Melatonin reduced the growth of the estrogen receptor positive breast cancer cell line MCF-7 in vitro by 18–27%. When the cells were exposed to a 60 Hz electromagnetic field of 1.2 μT flux density, this inhibitory effect of melatonin was completely blocked [11]. This surprising observation has been independently replicated by several other authors [12,13]. Since the oncostatic effect of melatonin was estrogen-dependent, Harland et al. tested whether the growth-inhibitory effect of the estrogen receptor (ER) antagonist tamoxifen was modulated by ELF/EMF exposure. Using the same experimental set-up a reduced growth inhibition by tamoxifen on MCF-7 cells was observed at 1.2 μT [14]. These results were also reproducible by other laboratories [12].

The reduced tamoxifen activity in the presence of electromagnetic fields appears similar to a phenomenon, called “tamoxifen resistance.” Tamoxifen has been used for treatment of ER positive BC for nearly thirty years. While most patients with advanced estrogen-responsive BC initially profit from tamoxifen treatment, most of their tumors recur and respond no longer to tamoxifen treatment [15]. Numerous investigations on EMF-regulated gene expression in tumor cells yielded controversial results (Table 1).

The authors employed different cellular systems and exposure conditions making comparisons between reported results difficult. However, there is agreement in the necessity of further investigations. In order to minimize uncontrolled external interferences with exposure conditions, particular caution must be paid to the generation of a stable and reproducible magnetic field.

For the analysis of EMF-induced modulation of tamoxifen activity we developed and constructed a novel incubator for the reproducible exposure of cells to defined ELF/EMF. Maximum effort was employed to achieve highly homogeneous sinusoidal fields and control of exposure characteristics.

Results

Influence of EMF on the anti-proliferative effect of tamoxifen in MCF-7 cells

Dose–response curves of tamoxifen were calculated for two different subclones (MCF-7 p40 and MCF-7 p181) and compared at various field intensities.

The tamoxifen dose–response curves in either a shielded and an unshielded configuration excluding surrounding environmental fields (0 μT), at the ambient (~0.2 μT) field, and at sinusoidal artificial fields of 1.2 and 100 μT intensity are shown in Fig. 1. The results of the measurements at 10 μT are not included in Fig. 1 for a better clarity but the calculations for IC50 of tamoxifen are listed in Table 2.

The dose–response curves of tamoxifen differ clearly in the two MCF-7 subclones examined (Fig. 1). The dose–response curves of clone MCF-7 p40 (Fig. 1A) show an inhibitory effect of tamoxifen on the growth of the BC cells at concentrations >10⁻⁷ M. In the absence of any alternat-
ing EMF (ambient field shielded by a container of mu-metal), the IC$_{50}$ of tamoxifen was calculated at 1.4 \times 10^{-6}$ M. If this mu-metal shielding were omitted, cells in culture plates would be exposed to an average ambient magnetic flux density of \(\approx 0.2 \mu T\) present in the laboratory. The dose–response curve of tamoxifen at 0.2 \(\mu T\) resembled the one recorded under shielded conditions. At 1.2 \(\mu T\) the dose–response curve is slightly shifted to the right, resulting in an IC$_{50}$ value of 2.3 \times 10^{-6}$ M. A at substantially higher field intensity of 100 \(\mu T\) this shift of the dose–response curve is no longer observed and the IC$_{50}$ of tamoxifen is reduced to about 0.9 \times 10^{-6}$ M (Fig. 1A).

In the cell clone MCF-7 p181, the described effects of magnetic fields on the dose–response curves of tamoxifen were more pronounced.

In the shielded situation (0 \(\mu T\)), the dose–response curve of tamoxifen in MCF-7 p181 cells showed a similar sigmoideal pattern as the one seen with the p40 clone. Even weak ambient flux densities (0.2 \(\mu T\)) resulted already in a marked proliferative activity of tamoxifen at concentrations around 10^{-7} M.

The maximal proliferative gain in MCF-7-p181 cells at 0.2 \(\mu T\) and a tamoxifen concentration of 10^{-7} M was 26% compared to the absence of tamoxifen.

Already at 0.2 \(\mu T\) the dose–response curve of tamoxifen was clearly shifted to higher concentrations. This shift was even more pronounced at a field intensity of 1.2 \(\mu T\). The maximal proliferative effect of tamoxifen at 1.2 \(\mu T\) was observed at a concentration close to 1 \(\mu M\). At higher field intensities (10 and 100 \(\mu T\)) the shift of the dose–response curve was lower as compared to 1.2 \(\mu T\), but did not return to the values measured in the absence of the EMF (Fig. 1B).

These measurements clearly show a “window effect” of the applied EMF with a maximum between 1.2 and 10 \(\mu T\) as has also been observed in other biological systems [17].

### Calculation of IC$_{50}$ and EC$_{50}$ values of tamoxifen at different field intensities

The dose–response curves of tamoxifen in MCF-7 (p181) cells (Fig. 1B) were separated into a proliferative branch (10^{-8}–10^{-7} M) and an anti-proliferative branch (10^{-7}–5 \times 10^{-6} M) and EC$_{50}$ values of the proliferative effect at low tamoxifen concentrations (clone p181 only) and the IC$_{50}$ values of the anti-proliferative effect of tamox-

<table>
<thead>
<tr>
<th>Magnetic field</th>
<th>Cell line</th>
<th>IC$_{50}$ (M)</th>
<th>EC$_{50}$ (M)</th>
<th>IC$_{50}$ (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shielded</td>
<td>MCF-7 p40</td>
<td>1.4 \times 10^{-6}</td>
<td>6.8 \times 10^{-7}</td>
<td></td>
</tr>
<tr>
<td>0.2 (\mu T) (environment)</td>
<td></td>
<td>1.6 \times 10^{-6}</td>
<td>1.9 \times 10^{-6}</td>
<td></td>
</tr>
<tr>
<td>1.2 (\mu T)</td>
<td></td>
<td>2.3 \times 10^{-6}</td>
<td>3.9 \times 10^{-6}</td>
<td></td>
</tr>
<tr>
<td>10 (\mu T)</td>
<td></td>
<td>2.3 \times 10^{-6}</td>
<td>3.7 \times 10^{-6}</td>
<td></td>
</tr>
<tr>
<td>100 (\mu T)</td>
<td></td>
<td>0.9 \times 10^{-6}</td>
<td>3.1 \times 10^{-6}</td>
<td></td>
</tr>
</tbody>
</table>

IC$_{50}$: tamoxifen concentration for half-maximal growth inhibition.
EC$_{50}$: tamoxifen concentration for half-maximal growth stimulation.

---

Table 2 IC$_{50}$ and EC$_{50}$ values of tamoxifen at various field intensities

---

Fig. 1. Dose–response curves of tamoxifen at various intensities of 50 Hz electromagnetic fields. (A) Clone MCF-7 p40. (B) Clone MCF-7 p181. Cells were grown at increasing concentrations of tamoxifen either in a shielded configuration (0 \(\mu T\)) (closed circle) or at 0.2 \(\mu T\) (open square) or at 1.2 \(\mu T\) (upright triangle) or 100 \(\mu T\) (diamond). Cell number was estimated after 7 days of culture by a colorimetric assay. Control: cell number achieved in the absence of tamoxifen = 100%. Means of at least three independent experiments with six replicates at each concentration.
if en at high concentrations were calculated from the separate dose response curves for all applied field intensities (Table 2).

In the shielded configuration, clone p181 was double as sensitive to the inhibitory effect of tamoxifen as clone p40 (Table 2). If p181 cells were exposed to the ambient EMF of about 0.2 μT, a threefold higher tamoxifen concentration was needed to achieve 50% growth inhibition as compared to the shielded situation. In cells of clone p40, sensitivity to tamoxifen was only slightly reduced at 0.2 μT. A strong shift in the IC50 occurred in both cell clones at 1.2 μT and similarly high concentrations of tamoxifen were needed for a half-maximal inhibition at a magnetic field of 10 μT. Surprisingly, at 100 μT the effect on tamoxifen inhibition was clearly lower than at 10 μT. From the data in Table 2 it can be seen that ELF/EMF clearly reduce the growth-inhibitory effect of tamoxifen with a maximum efficacy between 1.2 and 10 μT, and that this effect is waning at higher field intensities.

A marked estrogen-like proliferative effect at low tamoxifen concentrations was only observed in clone p181 in the presence of EMF. The proliferative EC50 is reduced with increasing field intensities, reaching its strongest effect at 10 μT.

Discussion

Here we show that the anti-estrogenic activity of tamoxifen is reduced in two subclones of MCF-7 cells under the influence of ELF/EMF to different extent. Dose–response curves of the growth-inhibitory effect of tamoxifen are shifted towards higher concentrations leading to a reduced growth inhibition at a given concentration. Our observation confirms results from a previous report describing a reduced inhibitory effect of tamoxifen at 10^-7 M from 40% to only 17% by exposure to an EMF of 1.2 μT [14]. More relevant from a therapeutic point of view, in our experiments tamoxifen even enhanced growth of the MCF-7 cells at concentrations below 10^-6 M if cells were exposed to EMF. The behavior of breast cancer cells exposed to EMF appears similar to the frequently observed tamoxifen resistance in tamoxifen-treated patients.

About 40% of ER-positive breast tumors fail to respond to anti-estrogen therapy by tamoxifen from the beginning (intrinsic resistance), while most of the residual tumors that initially respond to tamoxifen develop resistant relapse in the course of treatment (acquired resistance—AR) [15].

Tamoxifen is known as a partial estrogen antagonist because it can either stimulate or inhibit ER-dependent tumor growth in a tissue-, cell-, and promoter-specific manner. Like other selective estrogen response modifiers (SERMs) tamoxifen acts estrogen antagonistic in certain tissues, e.g., breast tissue, and agonistic in other tissues like bone and uterus [18]. Resistant tumors behave like tissues where tamoxifen acts as an estrogen agonist.

Several mechanisms have been hypothesized as to how AR to tamoxifen could arise. AR may be due either to a selection process favoring cells in the tumor that are already sensitized to growth stimulation by tamoxifen or are at least insensitive to the growth inhibition or to cellular alterations induced by the drug or other environmental factors. Wiseman et al. [19] observed a sensitization of tumor cells to the proliferative activity of IGF-I after treatment with tamoxifen. Tamoxifen treatment would select for these IGF-1-dependent cells ultimately producing a tamoxifen-stimulated tumor.

The modulated tamoxifen effects that we observed in p181 cells under the influence of ELF/EMF are incompatible with a selection process because the time of exposure was too short to allow a hypothetically tamoxifen-stimulated or at least tam-insensitive subpopulation to overgrow the majority of tamoxifen-sensitive tumor cells.

One further hypothesis for the development of tamoxifen resistance in breast tumors suggests that this resistance is associated with an inappropriate expression of receptor interacting proteins (RIPs) [20]. A multitude of receptor interacting proteins (RIPs) regulate gene transcription by nuclear hormone receptors, e.g., ER, for review, see [21]. In a preliminary clinical study, high levels of SRC-1 were detected in breast tumors showing good response to tamoxifen treatment [22].

In a comparison of the expression of various RIPs in wild type MCF-7 breast cancer cells and MCF-7/TAMR-1 cells that acquired a tamoxifen resistant phenotype after permanent treatment with tamoxifen revealed no differences in the expression of TIF-1, SUG-1, and SMRT but RIP140 expression was lower in non-stimulated cells of the resistant strain. Stimulation of the resistant cells by E2 or tamoxifen increased the level of RIP140 mRNA but not in the parental MCF-7 cells [20].

When expression levels of the corepressor N-CoR are low, patients receiving tamoxifen therapy experience poor outcomes. This observation suggests that tamoxifen antagonism requires high levels of N-CoR function [23].

Tamoxifen can act as an agonist through ERα/ERβ heterodimers, thus, in breast cancer cells where sufficient concentrations of ERα and ERβ are present, tamoxifen could induce cell proliferation [24]. An imbalance of ERα and ERβ-expression may determine a breast tumor to become resistant to tamoxifen.

Exposure to ELF/EMF is omnipresent in our electrified environment but the strength of the EMF generated by the electric wiring in usual households varies between 0.01 and 1 μT, in occupational situations exposure values of 1 μT and more are occasionally achieved [25]. At 1.2 μT the enhancing/augmenting influence of ELF/EMF on the proliferative effect of tamoxifen is strongest and is surprisingly waning at higher field intensities. Such kind of “window effect” of EMF activity has also been observed in other experimental settings [17].

In the clinical situation where BC is frequently treated with tamoxifen, it could be speculated that EMF exposure
may also contribute to the induction of a tamoxifen-resistance-like behavior in some breast tumors.

From a medical point of view it is disturbing that maximal induction of cell proliferation by tamoxifen at a field strength of 1.2 μT is observed at a concentration of 10^{-6} M. This is exactly the serum concentration achieved in BC patients under standard oral therapy [26]. Given the great number of BC patients under long-term oral tamoxifen treatment and more so in the light that in October 1998 the US Food and Drug Administration (FDA) approved the use of tamoxifen to reduce the incidence of breast cancer in healthy women at increased risk of the disease, clearly more research efforts are warranted to exclude the fact that EMF exposure could induce breast epithelial proliferation in tamoxifen users. Such research is continuously being supported by the German Radiation Protection Agency—BFS.

Our results confirming earlier reports on the modulation of tamoxifen activity through exposure of BC cells to ELF/EMF suggest that clones of MCF-7 cells are suitable models to study cellular changes associated with the induction of tamoxifen resistance.

Acknowledgment

This work was supported by Grant StSch4219 of the Federal Ministry for the Environment, Nature Conservation, and Nuclear Safety.

References


