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Modulation of tamoxifen-induced apoptosis by peripheral benzodiazepine receptor ligands in breast cancer cells

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Abstract

The peripheral benzodiazepine receptor (PBR), an integral protein of the mitochondrial membrane, is involved in the formation of mitochondrial permeability transition (MPT) pores. The opening of the MPT—leading to the dissipation of the inner-mitochondrial transmembrane potential ($\Delta\Psi_m$)—is considered to be an early apoptotic event. Therefore, we investigated the effect of the high-affinity PBR ligands Ro5-4684 and PK 11195 on tamoxifen (TAM)-induced apoptosis in MCF-7 and BT-20 breast cancer cell lines. Application of 100 nM TAM led to induction of apoptosis in both cell lines. Estrogene receptor (ER)-positive MCF-7 cells arrested in $G_{2/M}$ by TAM treatment showed no general dissipation of $\Delta\Psi_m$, but reduction of $\Delta\Psi_m$ was observed in a population of cells with high $\Delta\Psi_m$. In ER-negative BT-20 cells TAM treatment induced no arrest of the cell cycle but dissipation of $\Delta\Psi_m$. In both cell lines, nanomolar concentrations of the PBR ligands, which showed minor pro-apoptotic action themselves, reduced TAM-induced decrease of $\Delta\Psi_m$ and apoptosis. In MCF-7 cells, a reduction of bcl-2 protein expression by TAM treatment was abolished by a combination of TAM with PBR ligands. Bax protein expression in BT-20 cells showed a significant increase in TAM-treated cells after 24 hr but was not increased when treated with TAM and PBR ligands. From these findings, we concluded that binding of PBR ligands in nanomolar concentrations protects cells against apoptosis.

Keywords: PBR; MCF-7; BT-20; Tamoxifen; Apoptosis; Breast cancer

1. Introduction

The peripheral benzodiazepine receptor (PBR) is a 18 kDa protein, distinct from the central benzodiazepine receptor and present in peripheral tissues as well as in the CNS. It binds benzodiazepines such as Ro5-4864 and isoquinoline carboxamide derivatives like PK 11195 as well as the putative endogenous ligand diazepam binding inhibitor (DBI) with nanomolar affinities (for review see [1]). PBRs are predominantly located in the outer mitochondrial membrane associated with voltage-dependent anion channels [2] and seem to be involved in several mitochondrial functions like oxidative phosphorylation [3], translocation of cholesterol from the outer to the inner membrane in endocrine tissues [4] and regulation of apoptosis [3,5,6].

Mitochondria are considered to be the regulatory center of the apoptotic process [7]. The opening of the mitochondrial permeability transition (MPT) pore can cause the dissipation of the inner mitochondrial transmembrane potential ($\Delta\Psi_m$), leading to the release of pro-apoptotic inter-membrane proteins from the mitochondrion, provided that the pore opening is extensive and prolonged. As PBR is located in the mitochondrion membrane and probably is a constituent of the MPT pore, its involvement in the regulation of $\Delta\Psi_m$ and apoptosis is debated. Recently, several studies have demonstrated pro- as well as anti-apoptotic effects of PBR ligands. An induction of apoptosis in leukaemic cells (HL 60) by micromolar concentrations of a series of pyrrolo-1,5-benzoxazepines as well as by the more commonly used PBR ligands PK 11195 and Ro5-4864 was found [8,9]. In thymocytes, PK 11195 had no apoptotic effect but enhanced the apoptotic activity of agonists of glucocorticoid receptor agonists, chemotherapeutic agents, gamma irradiation and ceramide [10]. Ro5-4864 and PK 11195 also failed to induce apoptosis in

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Abbreviations: PBR, peripheral benzodiazepine receptor; TAM, tamoxifen; ER, estrogen receptor; FL, fluorescence.

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cell lines of hematopoietic and epithelial origin [11]. Anti-apoptotic activities of Ro5-4864 concentrations in the receptor binding range (nanomolar range) were demonstrated in lymphoblastoid U937 cells as well as in PBR-transfected leukaemic Jurkat cells [3]. In contrast, PK 11195 enhanced the apoptotic effect of TNF-α in nanomolar concentrations and inhibited the anti-apoptotic effect of Ro5-4864 [5]. After UV exposure PBR-transfected Jurkat cells were more resistant to apoptosis and exhibited a delayed mitochondrial transmembrane potential drop [12].

Tamoxifen (TAM) is a non-steroidal anti-estrogen which shows anticancer activity in many types of estrogen receptor (ER)-positive as well as ER-negative tumor cells. The mechanism of its action is not well understood, and anti-proliferative as well as pro-apoptotic actions of TAM are described in ER-positive and ER-negative cell types [13–15]. Our previous experiments have shown that ER-positive breast cancer cell lines exhibit low PBR expression and ligand binding capacities while ER-negative breast cancer cells show the opposite characteristics [16–18]. Therefore, we choose one cell line of each type and evaluated further characteristics concerning these two parameter. In the present study, we investigated the induction of apoptosis by TAM and the influence of nanomolar concentrations of the PBR ligands Ro5-4864 and PK 11195 on the effect of TAM in ER-positive MCF-7 and ER-negative BT-20 cells.

2. Materials and methods

2.1. Cell culture

The human breast cancer cell lines MCF-7 and BT-20 were purchased from Cell Line Service (CLS). BT-20 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM, Gibco) and MCF-7 cells in RPMI-1640 (Gibco) supplemented with penicillin/streptomycin (180 IU/mL) in 10% (v/v) heat-inactivated fetal calf serum (FCS) and 1% (v/v) L-glutamine. They were incubated in a humidified atmosphere with 5% CO_2 in air at 37°.

2.2. Chemicals

Ro5-4864 (4′-chloro-diazepam) was purchased form Fluka Chemie. PK 11195 (Isoquinoline carboxamide), and propidium iodide (PI) were supplied by Sigma–Aldrich. 5,5′,6,6′-Tetrachloro-1,1′,3,3′-tetraethylbenzimidazolcarbocyanine iodide (JC-1) was obtained from Molecular Probes.

2.3. Induction of apoptosis

For each experiment, 1×10^6 cells were plated in 10-cm petri dishes and allowed to attach for 24 hr before treatment. Subsequently, culture medium was substituted with phenol-red-free media containing drugs in the concentrations indicated in the results.

2.4. Western blotting experiments

Apoptosis was induced as described. A total of 2×10^6 cells were collected and incubated in NET-NP 40 lysis buffer (10 mM NaCl, 2 mM EDTA, 50 mM Tris–HCL pH 7.0, 0.3% NP-40, 1 μM microcystin, 10 μg aprotinin per mL, 20 μM leupeptin) for 20 min at 4°. Lysate was cleared by centrifugation at 16,000 *g* for 20 min at 4°. Protein concentration was determined by Bio-Rad protein assay (Biorad laboratories). Total protein (50 μg) were separated by SDS–PAGE and transferred to PVDF-membranes (NEN Life Science). Membranes were blocked in phosphate buffered saline/0.05% Tween-20/5% (w/v) non-fat dry milk and probed with specific antibodies for β-actin (Sigma) and bax (Santa Cruz) or bcl-2 (Santa Cruz), respectively, and visualized by specific HRP-coupled secondary antibodies (Santa Cruz) using the chemiluminescence method. Signal intensity was determined by Kodak Image Station 440 CF running Kodak image analysis software 1D. The quotient i = probe: β-actin was used for further analysis.

2.5. Mitochondria potential

Data acquisition was performed using a FACScan (Becton Dickinson) flow cytometer in all experiments. A minimum of 10^5 events was collected on each sample. Data were acquired using Cell Quest software (Becton Dickinson). ModFIT software (Becton Dickinson) was used for cell cycle determination and Cell Quest for all other evaluations. In all experiments, cells were harvested after 72 hr in culture by trypsination and stained for flow cytometric analysis. JC-1 staining was carried out according to [19]. In short, cells were resuspended in 1 mL of culture medium containing the respective drugs and 10 μg JC-1 from a stock solution in dimethyl sulfoxide (DMSO). After 10 min of incubation at 37°, cells were transferred to ice for FACS analysis. Forward and side angle scatters (FSC and SSC) were used to gate and exclude cellular debris. Cells were excited at 488 nm and green and red fluorescence was collected on FL1 and FL2 channels at 530 and 585 nm, respectively. Green fluorescence (FL1) represents the monomeric form of JC-1, corresponding to mitochondria mass. Red fluorescence (FL2) corresponds to the J-aggregates from JC-1 and is proportional to $\Delta\Psi_m$.

2.6. Apoptotic cells

DNA fragmentation was measured using FlowTACS Apoptosis Detection Kit (R&D Systems). Labeling was performed according to the manufacturer's protocol. In short, cells were harvested, fixed in 3.7% formaldehyde and permeabilized. In the first staining step biotinylated

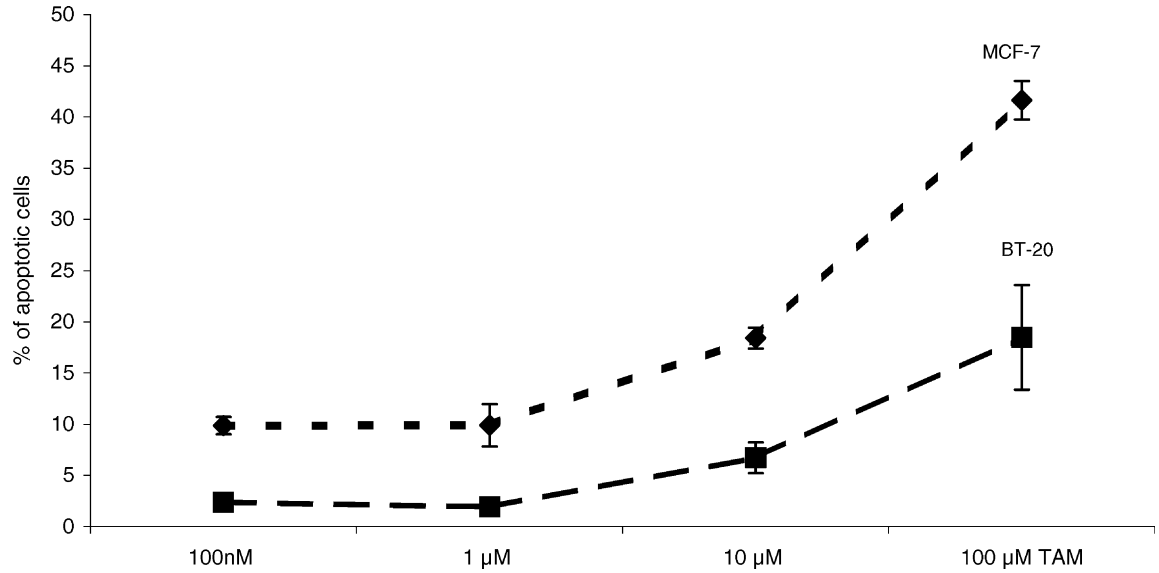

Fig. 1. TAM-induced apoptosis in MCF-7 and BT-20 cells. Cells were incubated with the indicated concentrations of TAM for 24 hr. Apoptosis was measured using flow cytometry. Results are expressed as a percentage of cells which show fluorescin stained DNA fragments. Each point represents the mean ± SD of three parallel cultures in two independent experiments.

nucleotides were added to the 3'-ends of the DNA fragments by terminal deoxynucleotidyl transferase (TdT). In the second step streptavidin-conjugated fluorescein (FITC) was bound to the biotinylated DNA fragments, which were detected by flow cytometry. For positive controls cells were treated with TACS-Nucleases provided in the kit.

2.7. Statistics

Results represent the mean of 3–5 independent experiments. Alterations in cell cycle phases, apoptotic cells, mitochondrial membrane potential, and protein expression were analyzed by Student's *t*-test.

3. Results

The antiestrogen TAM (10^{-7} M) induced apoptosis in ER-positive MCF-7 as well as in ER-negative BT-20 breast cancer cells in a dose-dependent manner, but the proportion of cells which showed DNA fragmentation, the measure of apoptosis, was higher in ER-positive MCF-7 cells after 24 hr of treatment (Fig. 1). In both cell lines, no significant

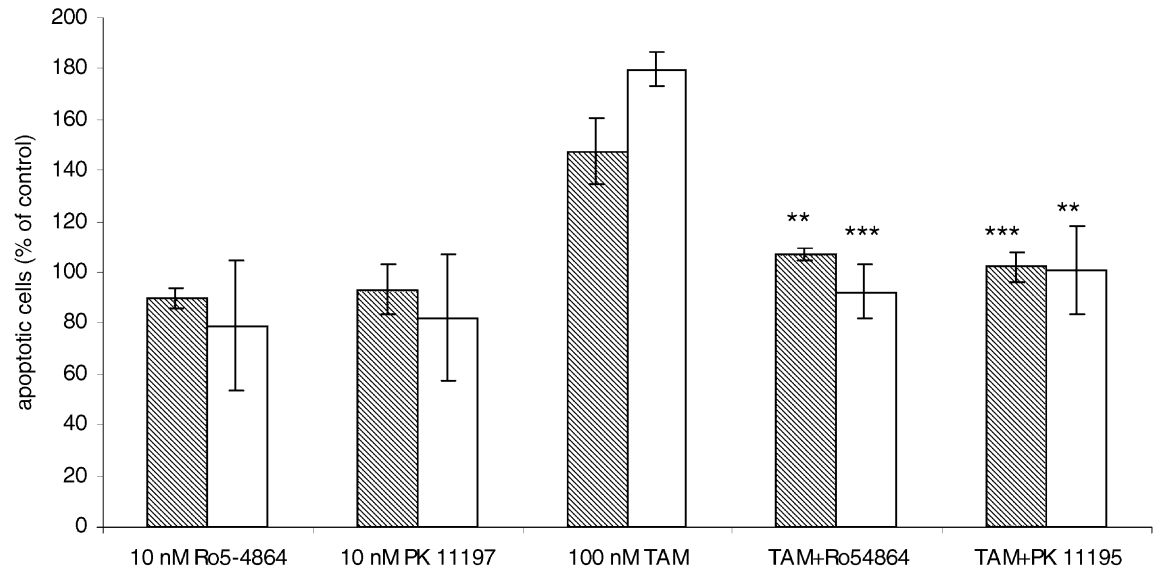

Fig. 2. Apoptosis in MCF-7 and BT-20 cells after 72 hr of treatment. Apoptosis was measured using flow cytometry of TdT stained cells. Results are expressed as a percentage of cells treated with PBR ligands, TAM or a combination of both vs. control cells as 100%. The results are mean ± SD of four separate experiments done in triplicate. Ro5-4864 (10^{-8} M), PK 11195 (10^{-8} M), TAM (10^{-7} M), $^{***}P < 0.001$ vs. TAM-treated cultures, blank columns BT-20 cells, hatched columns MCF-7 cells.

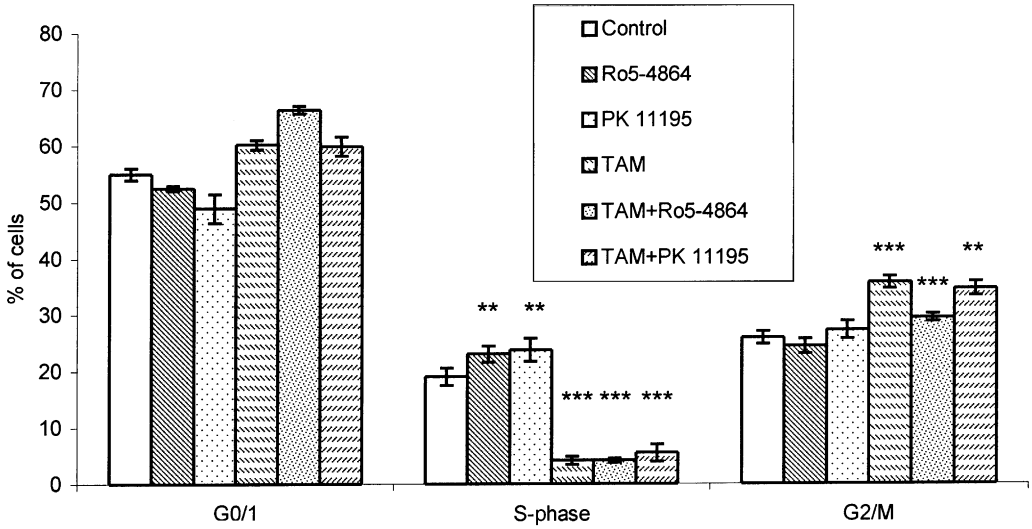

Fig. 3. Effects of PBR ligands and TAM on the cell cycle of MCF-7 cells after 72 hr of treatment. Analysis of cell distribution in the respective phases of the cell cycle was performed by flow cytometry of PI stained cells. The results are given in percentage of cells as mean ± SD of three different experiments run in triplicate $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control of the same cell cycle phase.

apoptotic effect was induced by nanomolar concentrations of the PBR ligands Ro5-4864 and PK 11195 (Fig. 2). In order to investigate whether the PBR ligands influence the apoptotic effect of TAM, we incubated both cell lines with TAM and the respective PBR ligand. When administered in combination with the PBR ligands Ro5-4864 (10^{-8} M) or PK 11195 (10^{-8} M), the induction of DNA fragmentation by 10^{-7} M TAM was reduced in both cell lines to nearly the level of control cells (Fig. 2).

The pro-apoptotic effect of 10^{-7} M TAM appeared in BT-20 much earlier than in MCF-7 cells. After 24 hr of incubation in BT-20 cell cultures the percentage of apoptotic cells was 15% over that in control cultures. After 48 hr, these proportion increased to 29% and to 56% over control after 72 hr. In MCF-7 cells, an induction of apoptosis became visible only after 72 hr of incubation with 10^{-7} M TAM.

Cell cycle analysis revealed an increase in S-phase cells in both cell lines after incubation with Ro-4864 (10^{-8} M), while PK 11195 (10^{-8} M) caused a higher percentage in S-phase cells only in MCF-7 cells (Figs. 3 and 4). The presence of TAM (10^{-7} M) in the culture medium caused a highly reduced percentage of cells in S-phase and an increased $G_{2/M}$ phase in MCF-7 cells (Fig. 3), while S-phase levels of BT-20 cells remained unchanged (Fig. 4). In experiments with MCF-7 cells using combined drugs, the

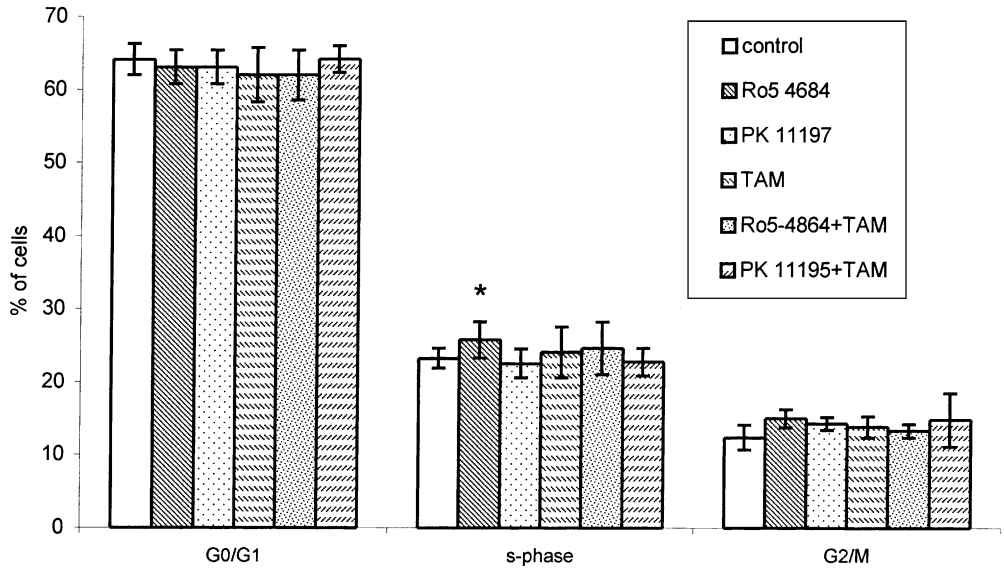

Fig. 4. Effects of PBR ligands and TAM on the cell cycle of BT-20 cells after 72 hr of treatment. Analysis of cell distribution in the respective phases of the cell cycle was performed by flow cytometry of PI stained cells. The results are given in percentage of cells as mean ± SD of three different experiments run in triplicate. $^{*}P < 0.05$ vs. control of the same cell cycle phase.

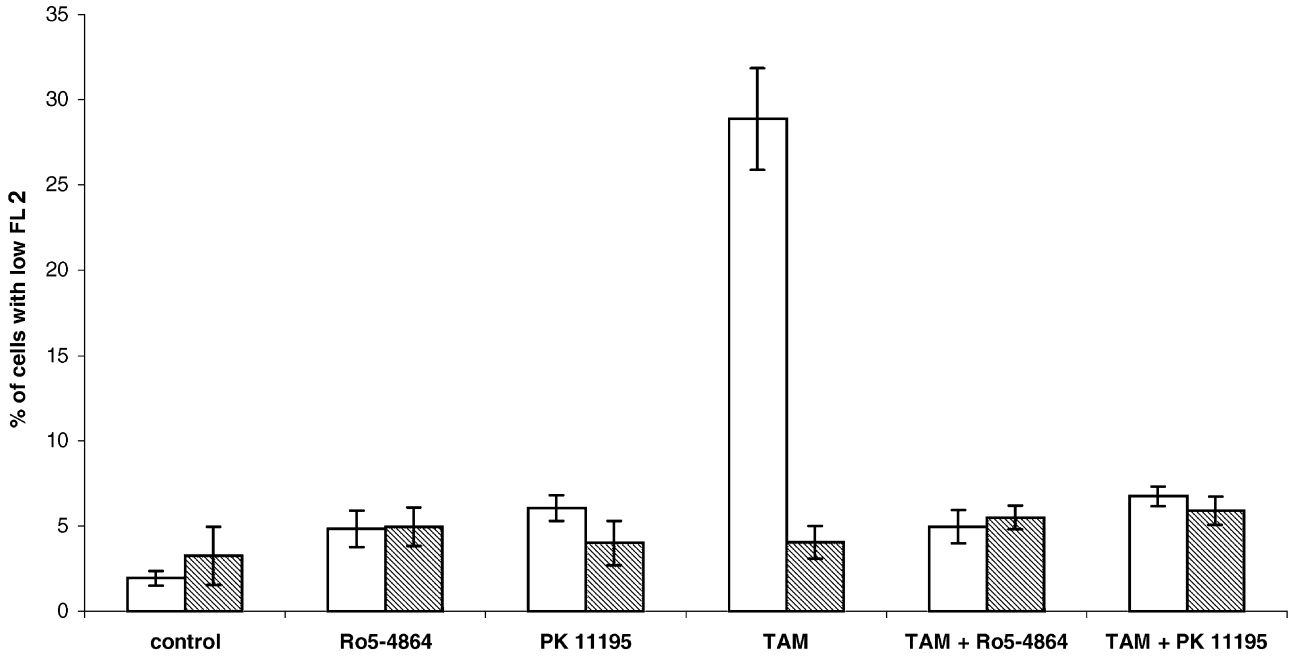

Fig. 5. Percentage of cells with low mitochondria membrane potential ($\Delta\Psi_m$) after 72 hr of treatment. Cells were stained with JC-1 as described in materials and methods and measured by flow cytometry. The definition of "low" red fluorescence (FL2-H) is below the level of 95% of control cells. Data represent one mean values of six experiments ± SD. Concentrations of drugs used in the experiments: Ro5-4864 (10^{-8} M), PK 11195 (10^{-8} M), TAM (10^{-7} M), blank columns BT-20 cells, hatched columns MCF-7 cells.

S-phase was reduced and $G_{2/M}$ was increased in the same range as observed with TAM (10^{-7} M) only. The combination of TAM (10^{-7} M) with Ro5-4864 (10^{-8} M) led to a higher percentage in $G_{0/1}$ cells and a lower percentage of $G_{2/M}$ cells as compared to TAM (10^{-7} M) + PK 11195 (10^{-8} M) treatment.

Because pre-apoptotic collapse of the $\Delta\Psi_m$ is a critical event in numerous cell types and different apoptosis inducers, the effect of TAM and PBR ligands on $\Delta\Psi_m$ was investigated using the mitochondrial potentiometric probe JC-1. In BT-20 cells, a decrease in the JC-1-aggregate formation—monitored as red fluorescence (FL2)—

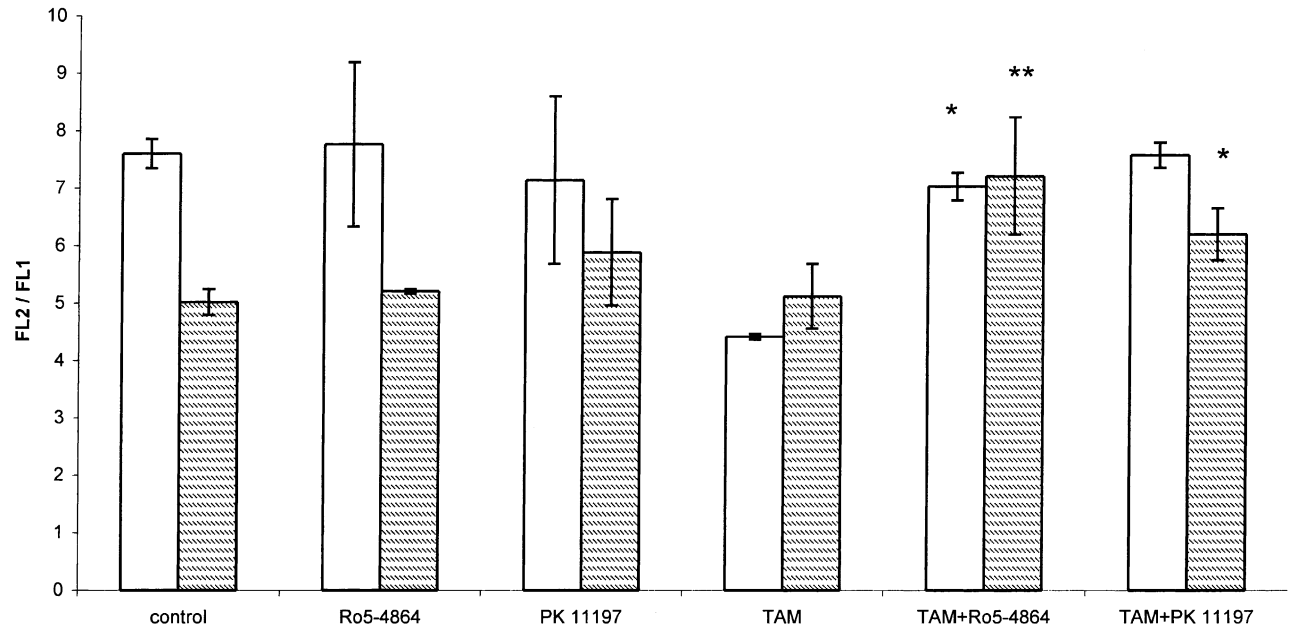

Fig. 6. Mitochondrial potential vs. mitochondrial mass after 72 hr of treatment with the respective drugs. JC-1 stained cells were measured by flow cytometry. The ratio of red fluorescence (mitochondrial potential) and green fluorescence (mitochondrial mass) of the same sample of cells was calculated using the geometrical mean as given by the flow cytometer. The results are mean ± SD of four separate experiments done in triplicate. Ro5-4864 (10^{-8} M), PK 11195 (10^{-8} M), TAM (10^{-7} M), $^{*}P < 0.05$, $^{**}P < 0.01$ vs. TAM-treated cultures, blank columns BT-20 cells, hatched columns MCF-7 cells.

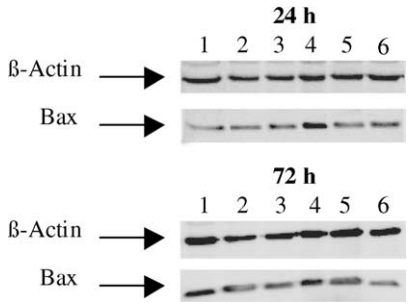

Fig. 7. The bax expression in BT-20 cells after 24 hr (a) and 72 hr (b) of treatment with the respective drugs. Whole cell lysates were fractionated on SDS–PAGE and immunoblotted with bax antibody. The results represent one of three independent experiments in each case. 1, control, 2, Ro5-4864, 3, PK 11195, 4, TAM, 5 Ro5-4864 + TAM, 6, PK 11195 + TAM.

occurred after treatment with TAM (10^{-7} M). An increase of $\Delta\Psi_m$ was observed after incubation with nanomolar concentrations of the PBR-ligands Ro5-4864 (10^{-8} M) or PK 11195 (10^{-8} M). In cells simultaneously incubated with TAM (10^{-7} M) and PBR ligands (10^{-8} M) a small proportion of cells (4–10%) showed dissipation of $\Delta\Psi_m$ while most of the cells kept values observed in control cells (Fig. 5). In PK 11195 (10^{-8} M) as well as in TAM (10^{-7} M) + PK 11195 (10^{-8} M) treated cultures, the proportion of cells with decreased $\Delta\Psi_m$ was somewhat higher than in Ro5-4864 (10^{-8} M) treated cells (data not shown). In MCF-7 cells no dissipation of the mitochondrial membrane potential after TAM (10^{-7} M) treatment below the level of control cells was observed, but a proportion of cells which showed high values of JC-1 red fluorescence under all tested conditions, decreased considerably in TAM-treated cells (data not shown). JC-1 green fluorescence (FL1), corresponding to mitochondrial mass, was decreased in TAM-treated cells of both cell lines. A significant increase in mitochondrial potential relative to mitochondrial mass was observed in MCF-7 cells treated with TAM (10^{-7} M) and PBR (10^{-8} M) ligands, while PBR ligands or TAM alone caused neither change in the ratio in MCF-7 cells nor in BT-20 cells (Fig. 6). A combination of TAM (10^{-7} M) and Ro5-4864 (10^{-8} M) increased the ratio of mitochondrial potential to mass in BT-20 cells (Fig. 6).

To clarify the mechanism underlying apoptosis-modulating action of TAM and PBR ligands, we investigated the expression of the anti-apoptotic bcl-2 and the pro-apoptotic bax protein expression by Western blot analysis. The results indicate that TAM (10^{-7} M) up-regulates the expression of bax in BT-20 cells during the first 24 hr of treatment. In experiments with PBR ligands or TAM + PBR ligands, the bax expression did not exceed the range of controls. After 72 hr of TAM (10^{-7} M) treatment bax protein expression was also reduced to control levels (Fig. 7). The bcl-2 protein expression could not be detected in BT-20 cells. In MCF-7 cells bcl-2 expression was slightly though not significantly decreased under all conditions tested (Fig. 8). TAM treatment reduced bcl-2 levels to 50% of control values. In MCF-7 cells, no bax protein expression was found.

4. Discussion

In the presented study, we demonstrated the prevention of TAM-induced apoptosis in ER-positive MCF-7 as well as in ER-negative BT-20 breast cancer cell lines by nanomolar concentrations of the PBR ligands Ro5-4864 and PK 11195. While the induction of apoptosis by TAM is well known [14,15], the mechanism of action of the anti-estro-

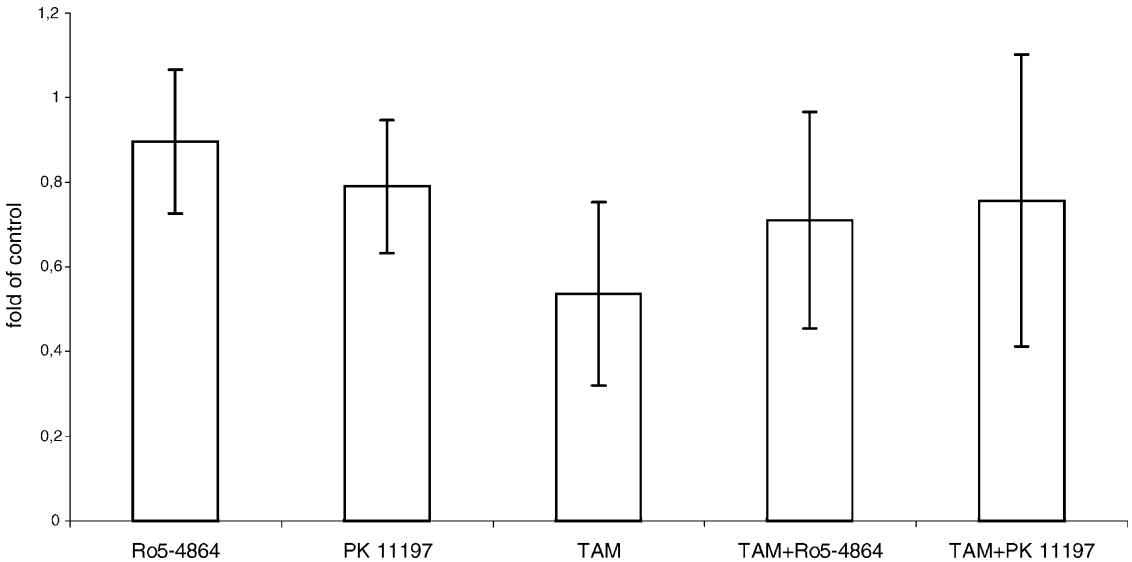

Fig. 8. Expression of anti-apoptotic bcl-2 protein in MCF-7 cells. The bcl-2 levels were quantitated using Western blot analysis after 72 hr of treatment. β-Actin expression was used to determine equivalent loading. Results were expressed as percent of controls. The results represent mean values ± SD of three separate Western blot experiments.

gen is still being debated. In ER-positive cells, TAM seems to act as an ER antagonist, but it was demonstrated that it shows either agonistic or antagonistic effects on ER-mediated gene regulation, depending on experimental conditions [20]. The involvement of up-regulation of c-*myc* expression [21], increase of intracellular calcium levels [13], generation of oxidative stress, and activation of the nuclear transcription factor kappa B [14] were shown in TAM-induced apoptosis. In isolated rat liver mitochondria, TAM-induced collapse of the mitochondrial membrane potential and inhibition of the electron transfer in the respiratory chain was described [22], while in the same system, it was found that TAM prevented the calcium-induced mitochondrial permeability transition [23]. These quite contradictory results may be due to the respective cell type and experimental conditions such as drug concentration and incubation time. In our experiments, we used TAM concentrations which were similar to those obtained in patients under TAM therapy. Under these conditions, TAM leads to dissipation of $\Delta\Psi_m$ in the two cell types in a quite different manner: in MCF-7 cells a cluster of cells with a high $\Delta\Psi_m$ present in control cultures disappeared after TAM administration, while in BT-20 cells a general decrease of $\Delta\Psi_m$ occurs. As for TAM-treated MCF-7 cultures, there was a concomitant increase of the proportion of cells which were arrested in the $G_{2/M}$ phase of the cell cycle. It can be suggested that this particular group may be sensitive for the action of TAM on mitochondria. An association between the ability to induce apoptosis and the growth inhibitory action of TAM and pure anti-estrogens was described for MCF-7 cells [15]. Several other tumor cell types exhibited significant apoptosis after $G_{2/M}$ arrest, as well [11].

The effect of PBR ligands on apoptosis seems to be dependent on ligand concentration and cell type. As apoptosis-inducing and antiproliferating actions of PBR ligands have been observed at concentrations far above the ligand binding range, the effects have been discussed as being independent of specific binding [1]. Nanomolar concentrations of Ro5-4864 and PK 11195 used in this study did not induce apoptosis but clearly prevented apoptosis when administered with apoptosis-inducing TAM. The only significant differences between the action of the two ligands was found in the decrease of $G_{2/M}$ cells and increase in $G_{0/1}$ cells in MCF-7 when treated with TAM (10^{-7} M) + Ro5-4864 (10^{-8} M). In BT-20 cells the proportion of S-phase cells was increased by Ro5-4864. The differences may be due to different affinities of the two ligands to PBR, with PK 11195 is more potent than Ro5-4864 [1]. In the previous experiments, we found a growth stimulating action of nanomolar concentrations of Ro5-4864 in breast cancer cell lines [16,18]. This effect is still visible in a high proportion of cells in S-phase in BT-20 cells treated with TAM (10^{-7} M) + Ro5-4864 (10^{-8} M), while it is totally abolished in MCF-7 cells. In this cell type, the reduction of the proportion of cells in S-phase by TAM was neither counteracted by Ro5-4864 (10^{-8} M) nor by PK 11195 (10^{-8} M). This difference may be due to the lower PBR content of MCF-7 cells compared to BT-20 cells, which is increased in the S-phase and $G_{2/M}$ phase cells [18]. Further investigations to analyze the sensitiveness to induction of apoptosis of mitotic cells or resting cells, respectively, are needed. The involvement of TAM in cell cycle regulation is obvious in ER-positive cells, but a connection to apoptosis cannot be deduced form our findings. Therefore, we conclude that prevention of apoptosis by PBR ligands may be due to other mechanisms like calcium channel regulation, which is discussed to be affected by PBR ligands as well as by TAM [1,13].

The benzodiazepine Ro5-4864 and the isoquinoline carboxamide PK 11195 have been classified as agonist and as antagonist of the PBR, respectively, and several studies suggest that they bind to different conformational states of the receptor or bind to different proteins (for review see [24]). However, both similar and opposite effects of the two ligands in several physiological processes were found. In lymphoblastoid cells, PK 11195 abrogated the anti-apoptotic activity of Ro5-4864 in TNF-α-induced apoptosis [5], and in thymocytes micromolar concentrations of PK 11195 enhanced susceptibility of cells to apoptosis induction by different agents [10]. In this study no significant differences in the action of the two PBR ligands were found. This is in accordance with previous studies where nanomolar concentrations of Ro5-4684 and PK 11195 had similar growth simulating effects on breast cancer cells, while micromolar concentrations of both ligands inhibited cell proliferation [16].

Clinical studies revealed a positive correlation between estrogen receptor content and bcl-2 protein level in tumor tissue [25,26]. In the present study, we found low levels of bcl-2 expression in ER-positive MCF-7, which were down regulated by TAM. Application of PBR ligands or TAM + PBR ligands led also to slightly decreased bcl-2 expression. The regulation of anti-apoptotic bcl-2 protein by TAM is controversially discussed. While some authors described down-regulation of bcl-2 in TAM-induced apoptosis in MCF-7 cells [27,28] others reported unchanged levels after TAM treatment [14,15,29]. In nitric oxide-mediated apoptosis in MCF-7 and BT-20 cells the expression of bax and bcl-2 protein was low and remained unchanged [30]. Pure antiestrogens and progestins were able to down regulate bcl-2 expression in ER-negative and ER-positive cells [14,15,29]. On the other hand, bcl-2 was found to be significantly increased in MCF-7 cells after estradiol administration and to counteract apoptosis [31]. In MCF-7 xenografts, reduction in both bcl-2 as well as bax protein appeared after 14 days of hormone withdrawal with a greater effect on bcl-2 reduction [32]. In our experiments, TAM-induced up-regulation of pro-apoptotic bax protein during the first 24 hr of treatment in BT-20 cells, while after 72 hr of culture bax protein levels were no longer increased in TAM-treated BT-20 cells.

Table 1

	Effect of TAM		Effect of TAM + PBR ligand	
	MCF-7 ER+++ PBR+	BT-20 ER− PBR+++	MCF-7 ER+++ PBR+	BT-20 ER− PBR+++
Apoptosis	↑	↑	↓	↓
Cycling cells	↓	–	↓	–
$\Delta\psi_m$	–	↓	–	–
Bcl-2 expression	↓	n.d.	–	n.d.
Bax expression	n.d.	↑	n.d.	–

↑ increased, ↓ decreased, – unaltered, n.d. not detectable.

The anticancer action of TAM in ER-positive and ER-negative cells, which includes tumor growth arrest as well as apoptosis, seams to be regulated by different mechanisms. While in ER-positive MCF-7 cells an arrest of mitotic activity precedes apoptosis, in ER-negative BT-20 cells proliferation is not impaired by TAM. A loss in mitochondrial membrane potential takes place in TAM-induced apoptosis in BT-20 cells but is of no significance in MCF-7 cells (Table 1). Despite these differences, in both cell lines co-incubation with the PBR ligands Ro5-4864 or PK 11197 prevented apoptosis. As MCF-7 cells have low PBR content and ligand binding capacity, the effect of PBR ligands may be unspecific, that is not receptor-mediated. In BT-20 cells, TAM may effect the PBR and cause apoptosis *via* mitochondrion membrane potential dissipation, which may be prevented by specific PBR-ligand binding.

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