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The growth inhibitory properties of a dopamine agonist (SKF 38393) on MCF-7 cells

DE Johnson,[1] J Ochieng[2] and SL Evans[1]

Departments of [1] Pharmacology and [2] Biochemistry, Meharry Medical College, Nashville, TN 37208, USA.
Tel: (+1) 615 327-6293; Fax: (+1) 615 327-6632.

Dopamine agonists have been indicated as treatment for disorders such as Parkinson's disease, cardiogenic shock and dopamine insufficiency. A unique relationship exists between dopamine and carcinogenicity. Chronic prolactin stimulation has been identified as a promoter of carcinogenicity. Prolactin secretion is regulated through dopamine receptor activation. Dopaminergic agonists inhibit prolactin release and antagonists increase release. High levels of prolactin have been shown to suppress production of estrogen and progesterone. As a result of these findings, a series of experiments were designed to examine the effects of a specific dopamine agonist, SKF 38393, against MCF-7 cells. MDA-MB231 and MCF-10 cells were used as negative controls. The breast cancer *in vitro* screening procedure involved the plating of MCF-7, MDA-MB231 and MCF-10 cells in a 96-well plate assay. After 1 day, the cells were exposed to SKF 38393 for 2 days and cell growth was determined by the Alamar blue dye reagent method. The optical density data was analyzed and IC_{50} values determined. The results indicated that SKF 38393 caused a significant decrease in proliferation of MCF-7 cells. The IC_{50} value was 0.1 ± 0.03 μM. The results also indicated no significant effect on MDA-MB231 and MCF-10 cells.

Key words: Antiestrogenic, antiproliferative, dopamine agonists, prolactin.

Introduction

Tamoxifen, a non-steroidal antiestrogen, is used for treatment of advanced breast cancer in post-menopausal women and adjuvant therapy in pre-menopausal women.[1,2] Tamoxifen is a mixed estrogen agonist and antagonist, and has been reported to act by binding to the estrogen receptor (ER) and therefore blocking the effect of endogenous estradiol.[3] Besides its antiestrogenic properties, it has also demonstrated other positive biological effects such as: (i) estrogenic properties maintaining bone matrix fixation (prevention of osteoporosis) and (ii) lowering circulating cholestrol by reducing low density lipoprotein levels (prevention of coronary heart disease).[1,2] The partial estrogen agonist activity of tamoxifen causes several undesirable effects in breast cancer patients such as (i) stimulation of ovarian estrogen production and (ii) an increased incidence of endometrial carcinoma.[4,5] The idea of the existence of antiestrogenic binding sites (AEBS) involved in the control of proliferation of breast cancer cells has been theorized for several years. The indirect relationship between dopamine receptor stimulation and estradiol production motivated our laboratory to examine the possible antiestrogenic phenomena of a specific dopaminergic agonist, SKF 38393, for its anticancer activity on ER-positive MCF-7 breast cancer cell line. SKF 38393 was also screened against (ER-negative) MDA-MB231 breast cancer and MCF-10 normal breast epithelium cell lines in order to establish specificity. Haloperidol, a dopamine antagonist, was also screened against the above cell line in order to prove the antiproliferative effect of SKF 38393 against MCF-7 cells was purely an agonist effect. In an attempt to elucidate a possible mechanism of action for the antiproliferative effects of SKF 38393 on MCF-7 cells, tamoxifen, a known antiestrogen, and SKF 38393 were tested against MCF-7 cells in the presence and absence of estradiol.

Materials and methods

Cell culture methods

The ER-positive MCF-7 human breast cancer cell line was obtained from American Type Culture Collection (Rockville, MD). MCF-7 cells were grown in T-75 tissue culture flasks as monolayer cultures in RPMI 1640 medium (phenol red) supplemented with 2 mM glutamine, penicillin (30 000 U ml), insulin (2500 U ml), 10% calf serum and fungizone (250 μg ml). Cultures were grown at 37°C in a humid 5% CO_2 atmosphere and fed every 3 days. When cultures reached confluency (usually every 5–7 days), they were subcultured using a 1 : 2 split-

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Correspondence to SL Evans

ting ratio every third day. Culture medium was changed every 3 days until the cells were confluent.

The ER-negative MDA-MB231 human breast cancer cell line was obtained from American Type Culture Collection. The MCF-10 normal breast epithelial cell line was obtained from the Michigan Cancer Foundation (Detroit, MI). MDA-MB231 cells were grown under cell culture conditions which were similar to MCF-7 cells. MCF-10 cells were grown in T-75 tissue culture flasks as monolayer cultures in DMEM (phenol red 14.8 g/l), F-12 HAM BASE supplemented with glutamine (200 mM), amino acid (50×), fungizone (5 ml), insulin (4 mg/ml), hydrocortisone (17 ml), EGCF (2 ml), sodium bicarbonate (2.2 g), penicillin/streptomycin (10 000 U/ml), cholera toxin (0.49 ml) and 10% bovine serum. The MCF-10 cell line was grown in a similar manner to MCF-7 cells, except they reached confluency in 7–9 days and were subcultured using a 1:3 splitting ratio every fifth day.

Cell proliferation studies

In each experiment the exponentially growing cells were trypsinized, counted and plated in a multiwell plates at a density of 2×10^4 cells per well in 200 μl of media. After 2 days of incubation when the cells were in an exponential growth phase (Figure 1), the test compounds were added. The test compounds were dissolved in dimethylsulfoxide and added to cell cultures following dilutions in culture medium. Control wells received the same amounts of vehicle alone. Exponentially growing viable cells were counted by a hemacytometer using the Trypan blue exclusion method. On the third day of administration, the treated cells were measured for optical density (OD) at 570 nm using a microwell plate reader. The OD was determined by using alamar blue dye reagent. Alamar blue measures the chemical environment surrounding cells in media. Dying cells undergo more extensive oxidation reactions which turns the media into a blue color. Viable cells undergo more extensive reduction reactions which tends to leave the media a light pink color. The microplate reader quantitates these color changes into numbers. The antiproliferative activity of the test compounds was calculated as a percent of control as follows:

$$\text{Antiproliferative activity} = \frac{\text{viable cells(control)} - \text{viable cells(treated)}}{\text{viable cells(control)}} \times 100$$

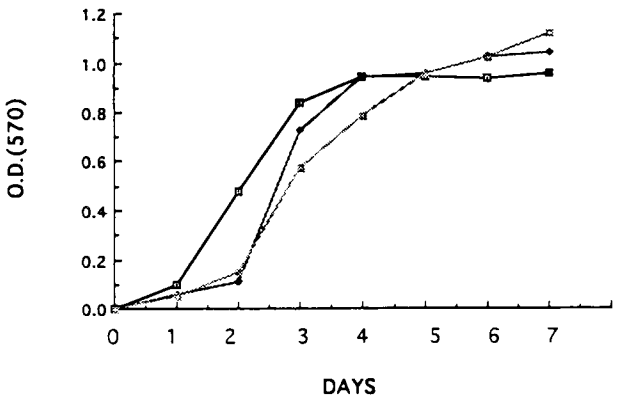

Figure 1. Time course growth curves for MCF-7 (□), MDA-MB231 (◆) and MCF-10 (■) cell lines. The three different cell lines were plated in 96-well plates and optical densities were read each 24 h interval. Log phase of each cell line was determined based on the linear portion of the curve.

Results

Antiproliferative activity

The experimental standard estradiol (0.1 μM) stimulated the growth of MCF-7 cells 50% above control. The antiestrogen standard tamoxifen (0.1 μM) and SKF 38393 (0.1 μM) significantly ($p < 0.05$) inhibited the growth of MCF-7 cells by 50 and 58% of the control, respectively (Figure 2). Over the concentration range of 0.01–10 μM, tamoxifen and compound SKF 38393 inhibited the growth of MCF-7 cells in a dose-dependent manner (Figure 3). The antiproliferative effect of SKF 38393 was found to be comparable with that of tamoxifen. Estradiol

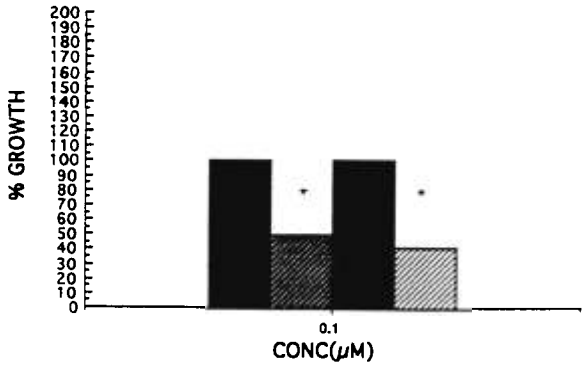

Figure 2. Tamoxifen (0.1 μM) and SKF 38393 (0.1 μM) inhibit proliferation of MCF-7 cells. * Statistically significant. ■, Control; ▩, tamoxifen; □, control; ▨, SKF 38393.

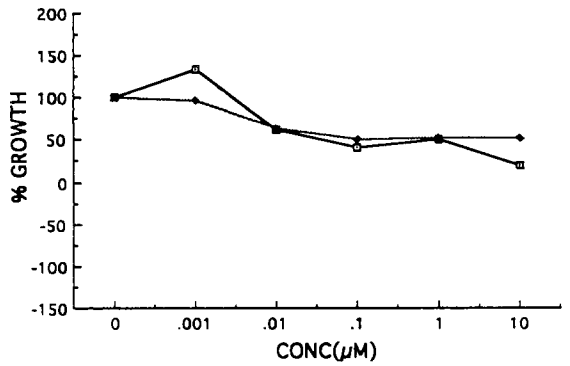

Figure 3. SKF 38393 (□) and tamoxifen (◆) exhibited a dose-dependent inhibition of growth against the MCF-7 cell line.

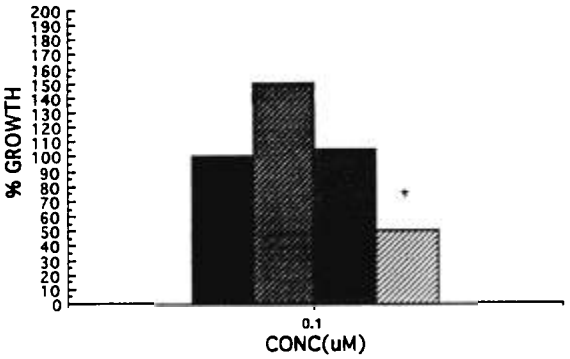

Figure 4. The inhibitory growth effects of tamoxifen on MCF-7 cells in the presence and absence of estradiol. Inhibitory growth effects were determined by alamar blue dye assay method. Tamoxifen and estradiol concentrations = 0.1 μM. Each value is the mean of quadruplet samples ± SEM. * Statistically significant. ■, Control; ▨, estradiol; □, tamoxifen, estradiol; ▨, tamoxifen.

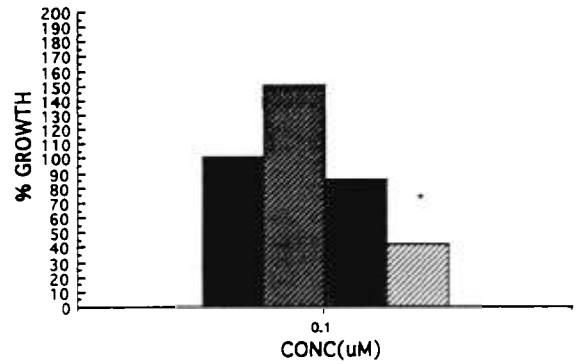

Figure 5. Inhibitory growth effects of SKF 38393 (0.1 μM) on MCF-7 cells in the presence and absence of estradiol (0.1 μM). Inhibitory growth effects were determined by alamar blue dye assay method. Each value is the mean of quadruplet samples ± SEM. * Statistically significant. ■, Control; ▨, estradiol; □, SKF 38393, estradiol; □, SKF 38393.

Table 1. Influence of estradiol, tamoxifen, SKF 38393 and haloperidol on MDA-MB231 and MCF-10 cell lines

Treatment	Per cent of control (viable cells/well ± SEM)	
	MDA-MB231	MCF-10
Estradiol (0.1 μM)	109 ± 0.09	99 ± 0.06
Tamoxifen (0.1 μM)	107 ± 0.08	99 ± 0.01
SKF 38393 (0.1 μM)	89 ± 0.02	95 ± 0.03
Haloperidol (0.1 μM)	83 ± 0.06	108 ± 0.05

MDA-MB231 and MCF-10 treated cells were counted on the third day of drug administration. Each value represents the mean of quadruplet samples ± SEM.

(0.1 μM) significantly reversed ($p < 0.05$) the inhibition of MCF-7 cell proliferation caused by either tamoxifen (0.1 μM) or compound SKF 38393 (0.1 μM) on the third day of administration (Figures 4 and 5). Estradiol, tamoxifen nor SKF 38393 significantly changed the growth or inhibition profile of MDA-MB231 or MCF-10 cells. Haloperidol showed no significant effect on the antiproliferation growth of MCF-7, MB231 or MCF-10 cell lines (Table 1).

Discussion

Antiestrogens, such as tamoxifen, are effective in controlling the growth of ER-dependent breast tumors.[10] However, specific *in vitro*, antiestrogenic effects of tamoxifen on the growth of ER-positive tumors are believed to exist at a range of 1 μM or lower, above this concentration the antiproliferative effects of tamoxifen on cell growth is believed to be due to cytotoxic action.[11,12] Therefore, compound SKF 38393 was initially evaluated at a concentration of 0.01–1 μM to examine its specific comparative antiestrogenic properties. Tamoxifen and estradiol were used as standard antiestrogen and estrogen, respectively, in the evaluation of MCF-7 cell proliferation responsiveness. Estradiol (0.1 μM) stimulated and tamoxifen (0.1 μM) clearly inhibited the growth of MCF-7 cells by 50% of control. Compound SKF 38393 was observed to be as potent as tamoxifen on the inhibition of MCF-7 cell proliferation.

Although the antiproliferative activity of compound SKF 38393 was observed on an ER-positive human breast cancer cell line, it may not be triggered through an ER-positive mechanism. One alternative explanation for the effects produced by SKF 38393 against MCF-7 cells could be the existence of antiestrogen binding sites that are linked to estradiol receptor activation or inactivation through

'cross-talk' mechanisms.[3] In order to determine whether the antiproliferative activity of SKF 38393 is an estrogen related phenomena, the activity of tamoxifen and compound SKF 38393 was studied in the absence and presence of estradiol. The dose-dependent inhibition of MCF-7 cells by tamoxifen as well as compound SKF 38393 at concentrations of 0.01–1 μM tends to indicate a similar potency for MCF-7 cells. In addition, the absence of an antiproliferative effect of SKF 38393 and tamoxifen against both MDA-MB231 and MCF-10 cell lines seem to indicate a selective specificity of SKF 38393 and tamoxifen towards estrogen-sensitive tumor cells. It appears that compound SKF 38393 inhibits the proliferation of ER-dependent human breast cancer cells (MCF-7) by an antiestrogenic mechanism of action. Thus, the results of the present study indicate the antiproliferative activity of the SKF 38393 against MCF-7 cell line comparable to that of tamoxifen. Tamoxifen and SKF 38393 did not show any *in vitro* estrogenic activity over a range of 0.01–1 μM. Therefore SKF 38393 may be a good candidate for clinical trials on women with ER-positive tumors. It also might be of value in the primary or adjuvant treatment and/or prevention of breast cancer in patients whose tumors are ER-positive.

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