European Journal of Pharmacology 628 (2010) 51–56

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Molecular and Cellular Pharmacology

The effect of Telmisartan on collagen biosynthesis depends on the status of estrogen activation in breast cancer cells

Beata Kociecka, Arkadiusz Surazynski, Wojciech Miltyk, Jerzy Palka *

Department of Medicinal Chemistry, Medical University of Bialystok, Kilinskiego 1, 15-230 Bialystok, Poland

ARTICLE INFO

Article history:
Received 11 March 2009
Received in revised form 29 October 2009
Accepted 10 November 2009
Available online 27 November 2009

Keywords:
PPAR-γ
PPAR-γ agonist
Collagen
Estrogen
Breast cancer cell

ABSTRACT

PPAR-γ and estrogen receptor belong to a family of nuclear hormone receptors that were shown to affect transcriptional activity of each other. The angiotensin II type 1 receptor antagonist Telmisartan is a well known PPAR-γ ligand. The effect of Telmisartan-induced PPAR-γ activation on collagen biosynthesis was studied in the estrogen-dependent (MCF-7 cells expressing α and β receptors) and estrogen-independent (MDA-MB 231, expressing only β receptor) cell lines. We have found that the presence of estrogen in growth medium (2 nM) augmented collagen biosynthesis in both cell lines. An addition to the growth medium of PPAR-γ agonist, Telmisartan, but not rosiglitazone or clofibrat, other PPAR-γ agonists, induced inhibition of collagen biosynthesis in MCF-7 cells, cultured in the presence of estrogen, while it had no effect on collagen biosynthesis in MDA-MB-231 cells. On the other hand, Telmisartan induced stimulation of collagen biosynthesis in MCF-7 cells cultured in the absence of estrogen (or in conditions of estrogen receptor removal by ICI 182-780-dependent degradation) and had no effect on similarly cultured MDA-MB-231 cells. The effect of Telmisartan on collagen biosynthesis was found specific for PPAR-γ and not for angiotensin II type 1 since Losartan (specific antagonist of angiotensin II type 1 receptor) in the presence of estradiol did not induce inhibition of this protein in MCF-7 cells. The mechanism of the inhibition was found at the level of NF-kB (known inhibitor of collagen gene expression) and MAPK signaling. PPAR-γ ligands stimulated expression of NF-kB, while they inhibited expression of p-38 but not ERK1/ERK2. The data suggest that the effect of Telmisartan on collagen biosynthesis in breast cancer cells depends on the status of estrogen receptor activation and the inhibitory effect of Telmisartan on the process requires functional alpha estrogen receptor.

1. Introduction

Breast cancer is one of the most common malignancies and is considered to be an estrogen-dependent disease (Osborne et al., 1980). It has been documented by epidemiological data and the therapeutic efficacy of anti-estrogen therapy (Jordan, 1992). The evidence was obtained with estrogen receptor positive breast cancer cell lines (as for instance MCF-7 cells) in which estrogens were found to stimulate the proliferation of these cells both in culture (Dickson and Lippman, 1988a,b) and in nude mice (Osborne et al., 1985). However, estrogen receptor positive tumor cells are poorly metastatic compared to estrogen receptor negative ones (Price et al., 1990) and more responsive to antiestrogens (Lerner and Jordan, 1990).

Estrogen receptor negative ones, such as MDA-MB-231 are highly invasive and metastatic in rodent models (Livasy et al., 2006). It may suggest a regulatory role of estrogens in breast cancer cell metastasis.

Estrogens are involved in regulation of many physiologic and pathologic processes. Their activity is regulated by multiple factors (Cordera and Jordan, 2006). Peroxisome proliferator-activated receptor-γ (PPAR-γ) may represent one of them since it has been reported that estrogen receptor is capable of interacting with PPAR-γ (Bishop-Bailey and Hla, 1999). PPAR-γ belongs to a family of nuclear hormone receptors that include estrogen and thyroid hormone receptors (Wang et al., 2006). They are known as a ligand-activated transcription factors. Among specific PPAR-γ agonists are natural lipophilic agents, mainly arachidonic acid metabolites and polyunsaturated fatty acids (Grommes et al., 2004) as well as synthetic thiazolidinediones, the new class of anti-diabetic drugs that improve insulin sensitivity in type 2 diabetes (Houseknecht et al., 2002; Mueller et al., 2002). They are represented by troglitazone, rosiglitazone, pioglitazone and ciglitazone. These, as well as natural agonists, when bound to PPAR-γ activate its association with retinoid X receptor (RXR). Then the complex is binding to specific recognition sites of target genes and regulates their expression (Willson et al., 2000). Therefore, the specific PPAR-γ ligands are of considerable interest.

It has been known for the last few years that Telmisartan, a structurally unique angiotensin II receptor antagonist used for the

* Corresponding author. Tel.: +48 85 7485706; fax: +48 85 8795703.
E-mail address: pal@amb.edu.pl (J. Palka).

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doi:10.1016/j.ejphar.2009.11.043

treatment of hypertension, can function as a partial agonist of PPAR-γ (Benson et al., 2004).

PPAR-gamma is expressed in many types of cancer and it is well established that activation of the receptor by either natural or pharmacologic ligands may inhibit cell proliferation and induce apoptosis (Wang et al., 2006). However, there is important cross-talk between PPAR-γ and estrogen receptor (Keller et al., 1995). It suggests that knowledge of the mechanism of PPAR-γ activation in cancer cells may contribute to novel approaches for pharmacotherapy of neoplastic diseases.

Estrogens are well known stimulators of collagen biosynthesis and cell growth in several cell types (Baldekas et al., 1982; Schmid and Froesch, 1988; Surazynski et al., 2003). Collagens are not only structural tissue proteins but also they play important role in the interaction with integrin class of surface adhesion receptors (Surazynski et al., 2005). The interaction between cells and collagen can regulate cellular gene expression, differentiation, growth and plays an important role in tumorigenicity and invasiveness (Carey, 1991; Ruoslahti, 1992). Therefore any alterations in collagen metabolism may influence cell growth and metabolism.

The mechanism of PPARγ function is similar to that of estrogen receptor. Both are bound to corepressor proteins or silencing mediators (Guan et al., 2005). For instance, PPARγ activation by ligands induce its heterodimerization with retinoid x receptor alpha (RXRα), and its subsequent interaction with co-activators like steroid receptor co-activators (SRCs) followed by binding to PPARγ response elements (PPREs) within target gene promoters (Glass, 2006). PPARγ is sharing a similar pool of cofactors with estrogen receptor β which provides a platform for mutual interactions between these two nuclear hormone receptor family (Glass, 2006; Gronemeyer et al., 2004). Therefore, ligands of PPARγ may contribute to cross-talk between these two receptors, representing molecular mechanism of its metabolic response.

The current study was therefore undertaken to characterize the effect of Telmisartan-induced PPAR-γ activation on estrogen-dependent stimulation of collagen biosynthesis in the estrogen-dependent, MCF-7 and estrogen-independent, MDA-MB 231 cell lines.

2. Materials and methods

2.1. Materials

L-glycyl–L-proline, L-proline, and Dulbecco's modified Eagle's medium with or without phenol red (DMEM), bacterial collagenase, sodium bicarbonate, penicillin, streptomycin, fetal bovine serum (FBS) used in cell culture, Dulbecco's phosphate buffered saline (DPBS), 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium liquid substrate reagent (BCIP/NBT), Estradiol, Losartan, monoclonal (mouse) anti-phospho-MAPK (p-38, ERKs) antibody anti-Rabbit IgG antibody, and anti-Mouse IgG antibody, were purchased from Sigma Chemicals as were most other chemicals used. Polyclonal (rabbit) anti-NF-kB antibody and polyclonal (rabbit) collagen type I antibody were obtained from Santa Cruz Biotechnology, INC.

Telmisartan was purchased from LKT Laboratories, rosiglitazone, clofibrat were purchased from Alexis Biochemicals, ICI 182,780 was received from Tocris Bioscience.

Nitrocellulose membrane (0.2 μm), sodium dodecylsulphate (SDS), polyacrylamide, molecular weight standards and Coomassie Briliant Blue R-250 were received from Bio-Rad Laboratories. L-5[^{3}H] proline (28 Ci/mmol) was purchased from Amersham.

2.2. Cell cultures

The studies were performed on estrogen-dependent MCF-7 cells, expressing α and β receptors and on estrogen-independent, MDA-MB 231 cells, expressing only β receptor. It is well known that estrogens stimulate collagen metabolism (Baldekas et al., 1982; Schmid and Froesch 1988; Surazynski et al., 2003). MCF-7 and MDA-MB 237 cells were maintained in DMEM without phenol red supplemented with 10% CPSR1, 50 U/ml penicillin, 50 μg/ml streptomycin at 37 °C in a 5% CO_2 incubator. Cells were cultured in Costar flasks and sub-confluent cells were detached with 0.05% trypsin, 0.02% EDTA in calcium-free phosphate buffered saline, counted in hemocytometers and plated at 5×10^5 cells per well of 6-well plates (Nunc) in 2 ml of growth medium. Cells reached about 80% of confluence at day 2 after plating and in most cases such cells were used for the assays.

2.3. Western blot analysis

Slab SDS/PAGE was used, according to the method of (Laemmli 1970).

After SDS-PAGE, the gels were allowed to equilibrate for 5 min in 25 mM Tris, 0.2 M glycine in 20% (v/v) methanol. The proteins were transfered to 0.2 μm pore-sized nitrocellulose at 100 mA for 1 h by using a LKB 2117 Multiphor II electrophoresis unit. The nitrocellulose was incubated with: polyclonal antibody against human Collagen type I at concentration 1:1000; monoclonal anti-MAPK antibody (p38 and ERK1/2) at concentration 1:1000 and polyclonal anti-NF-kB antibody at concentration 1:500 in 5% dried milk in Tris buffered saline with Tween 20 (TBS-T) (20 mmol/l Tris–HCl buffer, pH 7.4, containing 150 mmol/l NaCl and 0.05% Tween 20) for 1 h. In order to analyze Collagen and NF-kB, anti-Rabbit IgG (whole molecule) alkaline phosphatase conjugate was added at concentration 1:5000 in TBS-T, in order to analyze MAP-kinases second antibody-alkaline phosphatase conjugated, anti-Mouse IgG (whole molecule) was added at concentration 1:5000 in TBS-T and incubated for 30 min while slowly shaking. Then nitrocellulose was washed with TBS-T (5×5 min) and submitted to 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium liquid substrate reagent (BCIP/NBT).

2.4. Collagen synthesis

Incorporation of radioactive precursor into proteins was measured after labeling confluent cells in serum-free medium for 24 h with the 5-[^{3}H] proline (5 μCi/ml, 28 Ci/mmol). Incorporation of label into collagen was determined by digesting proteins with purified C. histolyticum collagenase according to the method of (Peterkofsky et al. 1982). Results are shown as combined values for cell plus medium fractions.

2.5. Statistical analysis

In experiments the mean values for six assays ± standard deviations (S.D.) were calculated. The results were analyzed using the ANOVA method, accepting $P<0.05$, as significant.

3. Results

In cultured cells, phenol red contained in medium mimics activity of estrogens (Devleeschouwer et al., 1992). Therefore, in the experiments the cells were cultured in medium without phenol red, containing 10% CPSR1 with or without estradiol. In order to test the effect of activated estrogen receptor on collagen biosynthesis, the MCF-7 and MDA-MB-231 cells were treated for 24 h with different concentration of estradiol (Fig. 1A and B). Collagen biosynthesis in the cells was measured by 5-[^{3}H] proline incorporation into proteins susceptible to the action of bacterial collagenase. It was found that estrogen stimulated collagen biosynthesis in both cell lines and was the most effective at concentration of 2 nM. However, the level of collagen biosynthesis stimulation by estradiol was more pronounced in estrogen-dependent, MCF-7 cells (Fig. 1A).

In order to evaluate the effect of PPAR-γ agonist on estrogen-dependent stimulation of collagen biosynthesis we used Telmisartan