

Original Article

Effects of COX-2 Inhibitor on the Proliferation of MCF-7 and LTED MCF-7 Cells

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Abstract Inflammation is now considered as well-established cancer risk factor. Breast cancer is common among women especially after menopause, suggesting that estrogen deprived tissues are sensitive to estrogen exposure. In many cancers, cyclooxygenase-2 (COX-2) enzymes are highly expressed. Since COX inhibitors possess anti-inflammatory activity by inhibition of COX enzymes resulting in inhibition of prostaglandin biosynthesis, they may play a role as antitumor agents. This study was performed *in vitro* and aimed to investigate the effect of celecoxib, a COX-2 inhibitor, on the proliferation of MCF-7 breast cancer cells and long-term estrogen deprived (LTED) MCF-7 cells. LTED MCF-7 cells used in the present study were wild-type MCF-7 cells being cultured over a prolonged period in estrogen-free medium for at least 3 months. The effect of celecoxib at concentrations of 10^{-10} to 10^{-4} M on the proliferation of MCF-7 and LTED MCF-7 cells was assessed on day 2 and day 6 of incubation using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method. Morphological features of these cells were visualized on day 2 of incubation after Ho33342 staining and apoptosis was confirmed by DNA fragmentation. The results indicated that celecoxib at higher concentrations could exhibit antiproliferative effect in MCF-7 and LTED MCF-7 cells in a similar manner. Celecoxib at the highest concentration (10^{-4} M) could markedly induce apoptosis. This study provides the *in vitro* evidence to support the beneficial effect of COX-2 inhibitors against breast tumors whether or not at the estrogen-deprived stage. ©All right reserved.

Keywords: aspirin, celecoxib, cyclooxygenase inhibitor

INTRODUCTION

Inflammation is now considered as well-established cancer risk factor.^{1,2} Breast cancer is common among women especially after menopause, suggesting that estrogen deprived tissues are sensitive to estrogen exposure. The first clinical evidence for “adaptive hypersensitivity” in women was initiated from the finding that initial requirement for pre-menopausal levels of estradiol for tumor growth was higher, *i.e.* 50-600 pg/ml but later, the requirement for that was only 10-15 pg/ml.^{3,4} Overexpression of cyclooxygenase-2 (COX-2) has been described for several different malignancies.⁵⁻⁷ Since COX inhibitors possess antiinflammatory activity by inhibition of COX enzymes resulting in inhibition of prostaglandin biosynthesis, they

may play a role as antitumor agents.⁷⁻⁹ This study aimed to investigate the effect of celecoxib, a COX-2 inhibitor, on the proliferation of MCF-7 breast cancer cells and long-term estrogen deprived (LTED) MCF-7 cells.

MATERIALS AND METHODS

Cell Culture

MCF-7 and LTED MCF-7 cells were used. LTED MCF-7 cells used in the present study were wild-type MCF-7 cells being cultured over a prolonged period in estrogen-free medium for at least 3 months. The cells were grown in T-25 flasks (25 cm²) in culture medium consisting of Dulbecco’s modified Eagle’s medium (DMEM; Invitrogen, USA)

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with phenol red, supplemented with 5% fetal bovine serum (FBS; Gibco BRL, USA), 100 units/mL penicillin G sodium, and 100 µg/mL streptomycin sulfate (Gibco BRL, USA) in a humidified atmosphere containing 5% CO₂ at 37°C.

Effects of COX-2 Inhibitor on Cell Proliferation

The experiments were carried out in a quadruplicate manner and were repeated four times. During the proliferation assay, "treated cell culture medium" consisting of phenol red-free DMEM added with 5% charcoal-dextran-treated FBS (charcoal-dextran-treated FBS being used instead of normal FBS in order to remove endogenous steroids), 100 units/mL penicillin G sodium, and 100 µg/mL streptomycin sulfate was used. The effect of celecoxib at concentrations of 10⁻¹⁰ to 10⁻⁴ M on the proliferation of MCF-7 and LTED MCF-7 cells was assessed on day 2 and day 6 of incubation using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method, as previously described by Mosmann.¹⁰ The absorbance was then measured using a microplate reader (Molecular Devices Thermomax Absorbance Reader, USA).

Determination of Morphological Changes of Cells

MCF-7 and LTED cells (3 x 10⁵ cells/well) were incubated for 48 hours with 500 µl of celecoxib at different concentrations (10⁻¹⁰ to 10⁻⁴ M) in 24-well plates. The living and apoptotic cells were examined microscopically in their natural state without being killed under an inverted microscope (Zeiss, Germany) at 400× magnification 48 hours (on day 2) after treatment. After that, the experimental media were aspirated and cells were washed with 50-100 µl of PBS. Ho33342 staining solution (1 µg/ml) at the amount of 200 µl was then added to each well and further incubated at 37°C for 30 minutes in the dark. Apoptotic cells were visualized through a blue filter by fluorescence inverted microscope at 400× magnification.

Detection of DNA Fragmentation

MCF-7 and LTED cells (1.8 x 10⁶ cells/60 mm dish) were pre-incubated in DMEM for 24 and 72 hours, respectively, and then cultured without or with various concentrations of celecoxib (10⁻⁸, 10⁻⁶ and 10⁻⁴ M) in treatment media for 48 hours. At the end of the incubation, DNA fragmentation was analyzed by agarose gel electrophoresis as described previously by Ueda *et al.*^{11,12}

Statistical Analysis

Data were expressed as means of % viability (compared with control untreated cells) obtained from four separated quadruplicate experiments. ANOVA was used to assess the significance of differences between means and *p*-values of less than 0.05 were considered to be significant.

RESULTS AND DISCUSSION

The proliferation rate of LTED MCF-7 cells was much slower than that of wild-type MCF-7 cells. However, the patterns of proliferation of MCF-7 and LTED MCF-7 cells, treated with different concentrations of celecoxib (10⁻¹⁰ to 10⁻⁴ M), were similar when expressed as % viability compared with the control untreated cells, either investigated on day 2 or day 6 of incubation (Figure 1). The antiproliferative effect of celecoxib at lower doses (10⁻¹⁰ to 10⁻⁶ M) could be observed only on day 2 of incubation but not on day 6, and not in a dose-related manner. The effect of celecoxib at the highest concentration used in the present study (10⁻⁴ M) was extremely pronounced. Up to now, there was no informative data about the effects of celecoxib on proliferation of MCF-7 cells or LTED MCF-7 cells. With other cell types, *e.g.* ovarian cancer cell lines (CAOV3, OVCAR3 and SKOV3), head and neck squamous cell carcinoma (HNSCC), and human chronic myeloid leukemia cells, COX-2 inhibitors could inhibit the cell proliferation.¹³⁻¹⁵

In addition to the antiproliferative effect, celecoxib at the highest concentration (10⁻⁴ M)

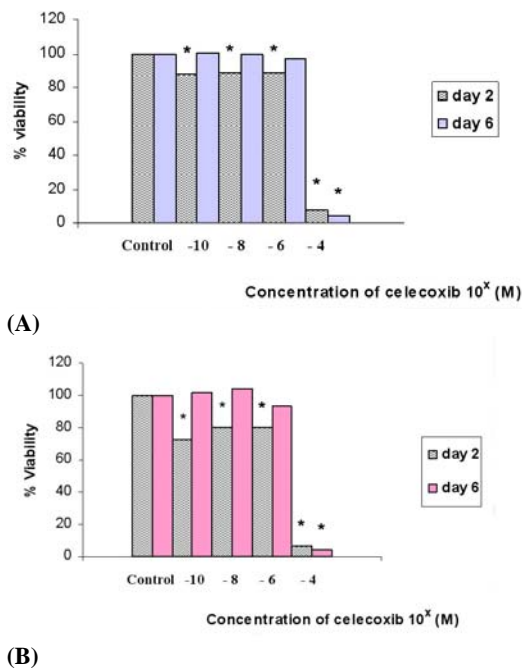


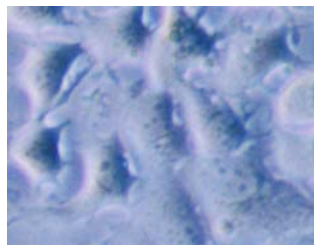
Figure 1. Inhibition of breast cancer cell growth by celecoxib, (A) MCF-7 cells and (B) LTED MCF-7 cells. The percentage of viable cells was calculated in comparison to control cells. The data were means of four separated quadruplicate experiments (S.D. values not shown).

could markedly induce apoptosis, characterized by changes in cell morphological features such as cell shrinkage and nuclear fragmentation, as being examined after Ho33342 staining (Figure 2) and confirmed by DNA fragmentation (Figure 3). Celecoxib-induced cell apoptosis was also evidenced in other cancer cells.¹⁴⁻¹⁶ In the present study, fragmented DNA was clearly observed in MCF-7 cells exposed to 10⁻⁶ M and 10⁻⁴ M of celecoxib while in LTED MCF-7 cells it was clearly observed only at 10⁻⁴ M of celecoxib.

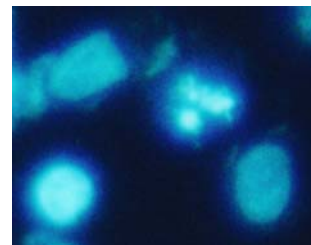
In conclusion, celecoxib had antiproliferative effect in MCF-7 and LTED MCF-7 cells at high concentrations. Celecoxib at the highest concentration used in the present study (10⁻⁴ M) could markedly induce apoptosis in these two cell types. This study provides the *in vitro* evidence to support the beneficial effect of COX-2 inhibitors against breast tumors whether or not at the estrogen-deprived stage.

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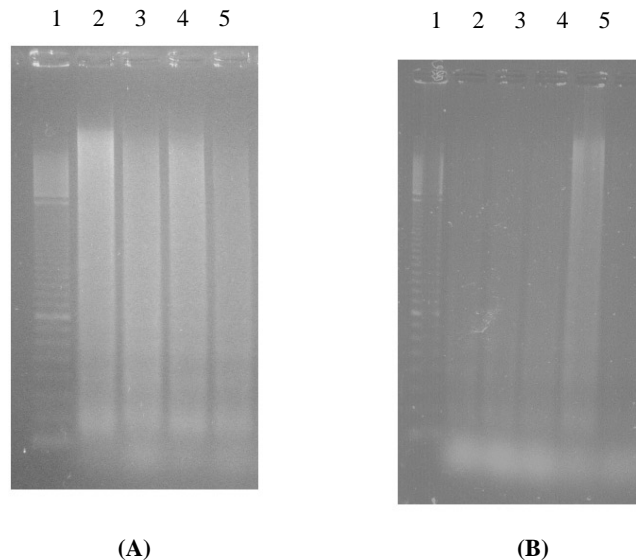


(A)



(B)

Figure 2. Morphological features of MCF-7 cells being observed under an inverted microscope at 400× magnification, (A) normal MCF-7 cells cultured in DMEM and (B) MCF-7 cells after benzimidazole Ho33342 staining—the characteristic morphological features of apoptosis, *e.g.* cell shrinkage and chromatin condensation were shown.



1 = marker; 2 = control; 3, 4 and 5 = celecoxib at 10^{-8} , 10^{-6} and 10^{-4} M, respectively

Figure 3. Effect of celecoxib on DNA fragmentation and ladders being detected by 1.5% agarose gel electrophoresis, (A) MCF-7 cells and (B) LTED MCF-7 cells.

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