

Influence of prostaglandin A₂ and 2-methoxyestradiol on Bax and Bcl-2 expression levels in cervical carcinoma cells

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ABSTRACT

Proteins of the Bcl-2 family are key regulators of apoptosis. Bax can be regarded as pro-apoptotic, whereas Bcl-2 is perceived as anti-apoptotic. It has been proposed that an increased ratio of pro-apoptotic Bax to anti-apoptotic Bcl-2 can be associated with apoptosis. Since prostaglandin A₂ (PGA₂) and 2-methoxyestradiol (2-ME) play an active role in the induction of apoptosis, the influence of 20 µg/ml PGA₂ and 1 µM 2-ME was investigated on Bax and Bcl-2 expression levels in cervical carcinoma cells. Both PGA₂ and 2-ME exposure led to statistically significant increases in Bax expression levels. Cells were shown to be more susceptible to the effects of 2-ME than to the effects caused by PGA₂. In contrast, no statistically significant effects were observed on Bcl-2 expression levels after exposure to PGA₂ and 2-ME. The Bax/Bcl-2 ratios for PGA₂- and 2-ME-exposed cells were 2.06 and 1.87 respectively, normalised against Bcl-2 levels. Further investigation of the function and regulation of the Bcl-2 family will allow researchers to consider potential pathways of apoptosis signaling mechanisms for diseases where apoptosis can potentially be controlled.

Proteins of the Bcl-2 family are key regulators of apoptosis. Bax can be regarded as pro-apoptotic, whereas Bcl-2 is perceived as anti-apoptotic. Cytochrome c release from mitochondria is regulated by the pro- and anti-apoptotic Bcl-2 family proteins responsible for either the induction or prevention of mitochondrial membrane permeability. The opening of the mitochondrial permeability transition pore triggers the release of cytochrome c, therefore resulting in apoptosis. Pro-apoptotic proteins including Bax promote release of cytochrome c from the mitochondria resulting in apoptosis. The latter is antagonised by Bcl-2 and Bcl-XL, which inhibit

cytochrome c release from the mitochondria, thus opposing the induction of apoptosis (1, 8).

It has been suggested that an increased ratio of pro-apoptotic Bax to anti-apoptotic Bcl-2 can be associated with apoptosis. An increase in Bax and a decrease in Bcl-2 were observed in apoptosis-induced rat pheochromocytoma PC-12 cells (6). Liu *et al.* (2004) concluded that an increased mitochondrial Bax/Bcl-XL ratio led to induced Bax activation in the human leukemic K562 cell line (7), and Zhang and Raveche (12) revealed that mitotic spindle inhibitors can induce apoptosis in malignant B-1 cells by also altering the Bax/Bcl-2 ratio. Exposure of human melanoma cells to 2-methoxyestradiol (2-ME), another anti-mitotic drug and tubulin poison, illustrated higher levels of Bax and undetectable levels of Bcl-2 in cells treated with 2-ME compared to untreated controls (2, 11).

Since we and other researchers have previously

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shown that 2-ME and prostaglandin A₂ (PGA₂), an endogenous metabolite derived from arachidonic acid, play a vital role in the induction of apoptosis and especially in cells that present with carcinogenic properties (5, 10), the influence of these two endogenous metabolites was investigated on the Bax/Bcl-2 ratio in human cervicarcinoma cells.

HeLa (human epithelial cervix carcinoma) was purchased through Sterilab Services (Johannesburg, SA) from the American Tissue Culture Collection (ATCC) (Maryland, USA). PGA₂, 2-ME, Eagle's Minimum essential medium (EMEM) with Earle's salts, L-glutamine and NaHCO₃, trypsin-EDTA and trypan blue were supplied by Sigma Chemical Co. (St. Louis, USA). Heat-inactivated fetal calf serum, sterile cell culture flasks and plates were obtained through Sterilab Services. Phosphate buffered saline (PBS) was purchased from Gibco BRL through Laboratory Specialist Services (Johannesburg, SA). Penicillin, streptomycin and fungizone were obtained from Highveld Biological (Pty) Ltd. (Sandringham, SA). Goat-anti-mouse IgG (H+L) peroxidase conjugate, mouse anti-Bcl-2 (clone Bcl-2 100) antibody and mouse anti-Bax (clone 2DC concentrate) antibody were provided by Sterilab Services. The Bio-Rad Dye Reagent Concentrate protein assay was purchased from Bio-Rad Laboratories (München, Germany) and supplied by S.A. Scientific Inc. (Midrand, SA). All other chemicals were of analytical

grade and supplied by Sigma Chemical Co. (St. Louis, USA).

Cells were grown and maintained as monolayer cultures in minimum essential medium, containing 10% heat inactivated fetal calf serum and a 10% mixture of 10 µg/ml penicillin, 10 µg/ml streptomycin and 25 µg/ml fungizone at 37°C in a humidified atmosphere containing 5% CO₂. Stock solutions of PGA₂ and 2-ME were prepared in ethanol and dimethyl sulfoxide (DMSO), respectively. The solvent concentrations in the media never exceeded 0.05%.

500 000 viable cells were seeded in 25 cm² culture vessels and incubated for 24 h. Cells were harvested after 24 h of exposure to vehicle controls, 1 µM 2-ME or 20 µg/ml PGA₂. Cells were homogenised in saline (150 mM NaCl, pH 7.4). Protein concentrations of each cell extract were determined by means of the Bio-Rad Dye Reagent Concentrate protein assay according to the manufacturer's instructions. Samples of total protein concentration (0.1 mg total protein per well) were coated onto a 96 well microtiter plate, dried under a 150 W lamp in a stream of air generated by an electric fan and subsequently blocked in 300 µl of PBS (pH 7.4) containing 0.5% casein, for 60 min at 37°C. Blocking medium was replaced with cell culture supernatant containing the monoclonal antibody (diluted 1:100 in blocking buffer) and incubated at 37°C for 45 min after which the plates were washed three

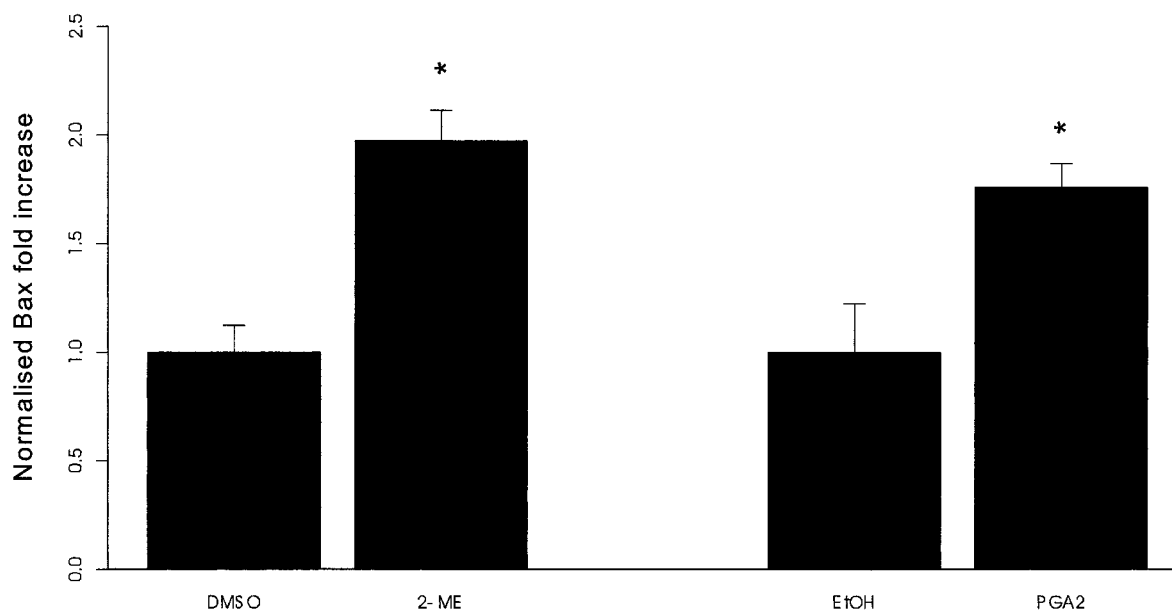


Fig. 1 Bax expression levels of either PGA₂- or 2-ME-exposed HeLa cells and vehicle-treated control cells after 24 h. Bax expression levels were normalised with regard to vehicle-treated control cells. Both PGA₂ and 2-ME exposure led to a statistically significant increase (indicated by an * on the graph) in Bax expression levels over vehicle-treated controls ($P < 0.05$).

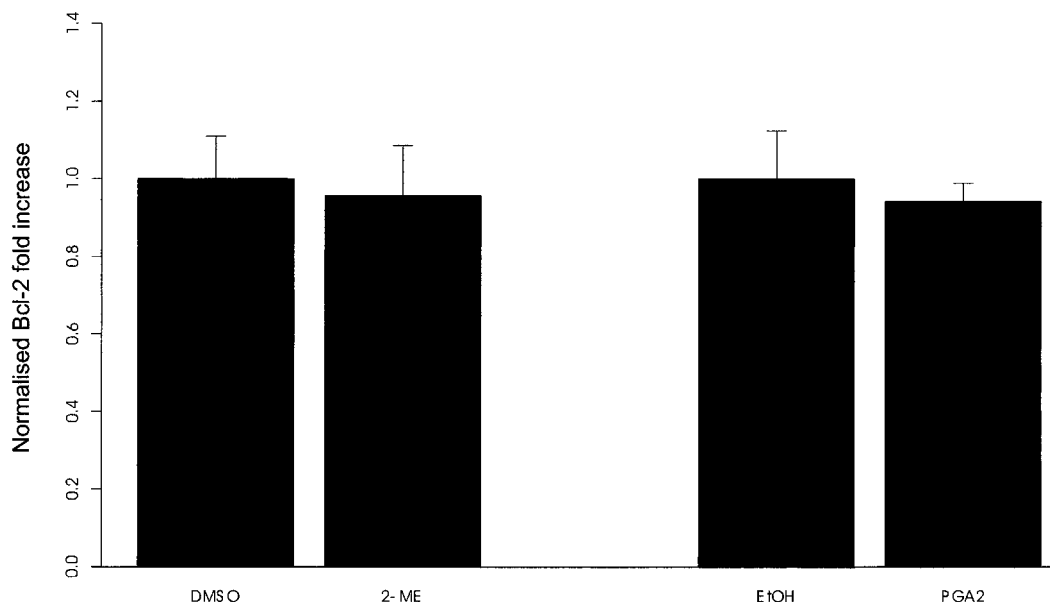


Fig. 2 Bcl-2 expression levels of either PGA_2 - or 2-ME-exposed HeLa cells and vehicle-treated control cells after 24 h. Bcl-2 expression levels were normalised with regard to vehicle-treated control cells. No statistically significant effect on Bcl-2 expression levels was observed after exposure to PGA_2 and 2-ME respectively.

times in blocking buffer and incubated for 30 min with goat-anti-mouse IgG (heavy and light chain) peroxidase conjugate at a 1 : 500 dilution with blocking buffer. After a second washing step, 100 μl of developing buffer (10 ml citrate, 10 mg o-phenylene diamine and 8 mg hydrogen peroxide, pH 4.5) was added and the reaction monitored at 450 nm with a SLT 340 ATC scanner (SLT Labinstruments, Austria).

Data obtained from independent experiments are shown as the mean \pm SD and were statistically analysed for significance using the analysis of variance (ANOVA)-single factor model followed by a two-tailed Student's t-test. Means are presented in bar charts, with T-bars referring to standard deviations. *P*-values of < 0.05 were regarded as statistically significant.

The effect of 20 $\mu\text{g/ml}$ PGA_2 and 1 μM 2-ME was evaluated on the expression levels of Bax and Bcl-2 in HeLa cells compared to vehicle-treated controls after 24 h of exposure (Figs. 1, 2). In both PGA_2 - and 2-ME-exposed cells, statistically significant increases (indicated by an * on the graph) were observed in Bax expression levels when compared to vehicle-treated controls ($P < 0.05$) (Fig. 1). Cells were shown to be more susceptible to the effects of 2-ME than to PGA_2 . In contrast, no statistically significant effects on Bcl-2 expression levels were observed after exposure of HeLa cells to PGA_2 and

2-ME respectively (Fig. 2). The Bax/Bcl-2 ratios for PGA_2 - and 2-ME-exposed cells were 2.06 and 1.87 respectively, normalised against Bcl-2 levels.

Our previous research has already revealed that PGA_2 and 2-ME caused significant decreases in cell growth, overexpression of extracellular signal regulated protein kinase (ERK1/2) and subsequent induction of apoptosis in HeLa cells (3, 4, 9). These results suggested that the increased ratio of pro-apoptotic Bax to anti-apoptotic Bcl-2 appear to be associated with both the PGA_2 - and 2-ME-induced apoptosis observed in HeLa cells.

Deregulation of Bcl-2 proteins has been implicated in several pathological conditions (1) and further investigation of the function and regulation of the Bcl-2 family will allow researchers to consider potential pathways of apoptosis signaling mechanisms for diseases where apoptosis can potentially be controlled.

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