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Influence of prostaglandin A_2 on Bax, Bcl-2 and PCNA expression in MCF-7 cells

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ABSTRACT

The effects of 20 μg/mL exogenous prostaglandin A_2 (PGA_2) were determined on Bax, Bcl-2 and proliferating cell nuclear antigen (PCNA) expression levels in MCF-7 cells. Flow cytometric analysis indicated a pronounced increase in the S phase and a decrease in the G_1 phase, whereas a significant increase in the DNA content preceding the G_0/G_1 peak was also observed after 48 h of exposure to PGA_2. Confirmation of apoptosis was determined after 12 h, 36 h and 48 h of PGA_2 exposure employing the mitosensor reagent that detects potential changes in the mitochondrial membrane. Twenty-eight percent of PGA_2-exposed cells were in apoptosis when compared to the 7.1% vehicle-treated cells after 48 h. PGA_2 exposure led to statistically significant increase (1.25-fold) over vehicle-treated controls in Bax expression levels. Decreases in Bcl-2 (0.79-fold), as well as PCNA (0.69-fold) expression levels over vehicle-treated controls were observed. The Bax/Bcl-2 ratio for PGA_2-exposed cells was 2.7. The present study suggests that an accumulation in the S phase, a decrease in expression levels of PCNA, as well as an altered ratio in favor of Bax, could lead to the induction of apoptosis in these cells.

Research has shown that prostaglandin A_2 (PGA_2), a cyclopentenone and endogenous metabolite derived from arachidonic acid, exhibits potent anti-proliferative activity *in vivo* (5, 14, 20, 22) and *in vitro* (1, 17–19, 21, 23). Concentration-dependent studies from previous research (12) revealed that 20 μg/mL results in optimal growth inhibition *in vitro*. This was confirmed in our laboratory where $IC_{50} = 20$ μg/mL was determined (results not shown). PGA_2 inhibited proliferation of tumor cells depending on dose, duration of exposure and cell type (12). In addition, we have demonstrated that the breast adenocarcinoma cells, MCF-7, were more susceptible to the anti-mitogenic effects of PGA_2 when compared to human epithelial cervix carcinoma (HeLa) cells (11). Furthermore, degree of differentiation of oesophageal carcinoma cells was also shown to influence tumor cell susceptibility to the anti-mitogenic effects of PGA_2. More differentiated and normal cells appeared to be less affected, while more pronounced effects were observed in less differentiated cell lines (12).

Since PGA_2 targets active proliferating cells and plays an active role in the induction of apoptosis, especially in cells that present with carcinogenic properties (12, 25), the aim of this study was to confirm the anti-mitogenic effects of PGA_2 and to investigate the influence of PGA_2 on Bax, Bcl-2 and PCNA expression in MCF-7 cells in order to suggest a possible mechanism for induction of apoptosis.

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MATERIALS AND METHODS

Materials. PGA_2, minimum essential medium eagle with Earle's salts, L-glutamine and $NaHCO_3$ (MEM), trypsin-EDTA, crystal violet, propidium iodide (PI) and trypan blue were supplied by Sigma Chemical Co. (St. Louis, MO, USA). Heat inactivated fetal calf serum (FCS), sterile cell culture flasks and plates were obtained through Sterilab Services (Kempton Park, Johannesburg, SA). Phosphate buffered saline (PBS) and MEM alpha medium with glutamax-1 were purchased from Gibco BRL (USA). Penicillin, streptomycin and fungizone were obtained from Highveld Biological (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. Anti-PCNA-FITC (fluoroisothiocyanate) and IgG2b-FITC were supplied by Dako (Glostrup, Denmark). The BD ApoAlert™ Mitochondrial Membrane Sensor Kit was purchased from CLONTECH Laboratories, Inc. (Palo Alto, CA, USA). 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.

Cell culture. MCF-7 (human breast adenocarcinoma) cell line was supplied by Highveld Biological (Pty) Ltd. (Sandringham, SA). Cells were grown as monolayers in MEM, at 37°C in a humidified atmosphere containing 5% CO_2. Media were supplemented with 10% FCS, penicillin (100 µg/L), streptomycin (100 µg/L) and fungizone (250 µg/L). Stock solutions of PGA_2 were made in ethanol at concentrations of 1 mg/100 µL and stored at −20°C. The final ethanol concentrations in the medium did not exceed 0.2% (v/v). Controls included showed that 0.2% ethanol had no toxic effects on experiments conducted. Known numbers of viable cells were seeded in appropriate culture vessels and, after 24 h incubation, exposed to 20 µg/mL of PGA_2. Non-viable cells were excluded with the trypan blue staining procedure.

Cell cycle progression. Viable MCF-7 cells (500 000) were seeded into 25 cm^2 flasks, incubated for 24 h and exposed to 20 µg/mL of PGA_2 for 48 h at 37°C where optimal effects were observed. Cells were trypsinized in equal volumes of trypsin (0.25%) and EDTA (1 mM), fixed in 99.5% methanol and stored at −20°C. Methanol was removed by centrifugation at 200 × *g* for 10 min. The sediments were resuspended in 1 mL 1% $CaCl_2$ and 50 µg/mL propidium iodide and incubated for 20 min while shaking gently. Each analysis was based on at least 10 000 events employing a Coulter Epic-XS flow cytometer. The data were analyzed using a multicycle analysis program (MulticycleAV software).

ApoAlert™ Mitochondrial Membrane Sensor analysis. Confirmation of apoptosis was determined after 12 h, 36 h and 48 h of PGA_2 exposure by employing the ApoAlert™ Mitochondrial Membrane Sensor kit that detects potential changes occurring during apoptosis in the mitochondrial membrane. Cells were seeded, exposed to PGA_2 as described above and analysed according to the manufacturer's instructions. Briefly, cells were removed from culture flasks to flow cytometry tubes, centrifuged at 350 × *g* for 5 min and resuspended in 1 mL diluted MitoSensor reagent per tube. One mL incubation buffer was added after an incubation period of 20 min at 37°C in a 5% CO_2 incubator. The tubes were centrifuged at 350 × *g* for 5 min, resuspended in incubation buffer and analyzed employing a Coulter Epic-XS flow cytometer using a band-pass filter for detection of fluorescein and rhodamine.

Bax and Bcl-2 expression levels. 500 000 viable cells were seeded in 25 cm^2 culture vessels and incubated for 24 h. Cells were harvested after 24 h of exposure to vehicle controls and 20 µg/mL of PGA_2, respectively. Cells were homogenized 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 known protein concentration (0.1 mg 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 times in blocking buffer and incubated for 30 min with peroxidase-conjugated goat-anti-mouse IgG (heavy and light chain) 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

was monitored at 450 nm with a SLT 340 ATC scanner (SLT Labinstruments, Austria).

PCNA-FITC flow cytometry. Cells were seeded at 500 000 per flask after trypan blue exclusion and exposed to either 0.2% ethanol (control) or 20 µg/mL of PGA_2 for 48 h. After trypsinizing the cells and washing twice with PBS, ice-cold methanol was added. Cells were stored at −20°C overnight. Samples were centrifuged at $100 \times g$ for 5 min, resuspended in 2 mL PBS containing 20% goat serum and 3% bovine serum albumin for 30 min at room temperature and centrifuged as mentioned above. 10^6 cells were incubated with 10 µL FITC-conjugated anti-PCNA mouse monoclonal antibody diluted in 1 mL PBS containing 10% goat serum and 3% bovine serum albumin for 1 h at room temperature. Isotypic controls were exposed to irrelevant mouse-IgG2b-kappa-FITC. Subsequently, cell pellets were washed three times with PBS. PI (18 µg/mL) and RNase (40 µg/mL) in PBS were added and samples were analysed on a Coulter Epic-XS Flow cytometer. Data was analysed employing MulticycleAV software.

Statistics. Data obtained from independent experiments are shown as the mean ± SD and were statistically analyzed 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 < 0.05 were regarded as statistically significant and indicated by an * or number as indicated in the legends.

RESULTS

Cell cycle progression

The effects of 20 µg/mL PGA_2 after 48 h of exposure on MCF-7 cells were evaluated on cell cycle progression. Flow cytometry analysis showed an increase in the number of treated cells in the S- and G_2/M phases, as well as the DNA content preceeding the G_0/G_1 peak. The latter indicates reduced quantity of DNA or hypercondensed DNA that may represent apoptotic bodies, as others and we have previously illustrated (3, 8, 25). In addition, a marked decrease was observed in the G_1 phase (Fig. 1).

ApoAlert™ Mitochondrial Membrane Sensor analysis

PGA_2-induced apoptosis was confirmed in a time-dependent study of 12 h, 36 h and 48 h employing the ApoAlert™ Mitochondrial Membrane Sensor analysis procedure. 28.0% of PGA_2-exposed MCF-7 cells were in apoptosis when compared to the 7.1% vehicle-treated cells after 48 h (Fig. 2).

Bax and Bcl-2 expression levels

The effects of 20 µg/mL PGA_2 were evaluated on the expression levels of Bax and Bcl-2 in MCF-7 cells compared to vehicle-treated controls after 24 h of exposure. PGA_2 exposure led to statistically significant increase (1.25-fold) ($P < 0.05$, indicated by an * on the graph) over vehicle-treated controls in Bax expression levels. In contrast, a decrease in Bcl-2 expression levels (0.79-fold over vehicle-treated controls) was observed. The Bax/Bcl-2 ratio for PGA_2-exposed cells was 2.7, normalized against Bcl-2 levels (Fig. 3).

PCNA-FITC flow cytometry

The influence of 20 µg/mL PGA_2 on PCNA induction in MCF-7 cells was measured by means of flow cytometry. Cells were exposed to 20 µg/mL PGA_2 for 48 h. In each analysis 10 000 events were counted. In the negative control (isotypic) only 1.6% of cells were labelled with PCNA and PI. 61.6% cells stained positive for PCNA and PI in the vehicle-treated control. PGA_2 caused a decrease in PCNA levels (0.69-fold) when compared to the vehicle-treated control. The percentage of cells labelled with PCNA and PI is given in Fig. 4.

DISCUSSION

Previous research has revealed that PGA_2 plays an active role in the induction of apoptosis, especially in cells with carcinogenic properties (12, 25). We have shown that 20 µg/mL PGA_2 significantly decreased cell numbers of HeLa (cervical carcinoma) and MCF-7 cells, with the latter cell line being more susceptible to PGA_2's anti-mitogenic effects (11). These anti-mitogenic effects were confirmed by morphological studies, mitotic indices and flow cytometry, as well as propidium iodide and Hoechst 33342 cell staining conducted after 48 h of exposure to PGA_2. Results suggested that the decrease in cell numbers initially observed was probably not due to inhibition of proliferation, but could be ascribed to an induction of apoptosis resulting in cell death (11). The aim of this study was therefore to confirm the anti-mitogenic effects of PGA_2 and to investigate the influence of PGA_2 on Bax, Bcl-2 and PCNA expression levels in MCF-7 cells in order to suggest a

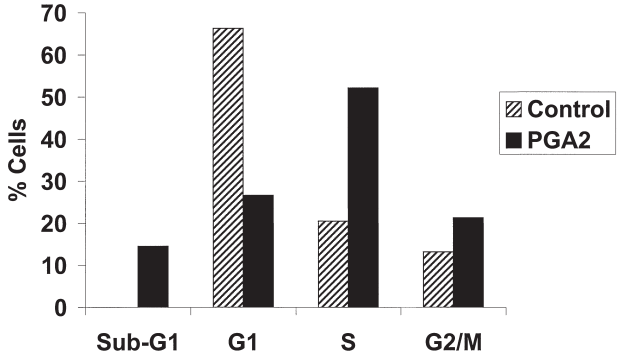

Fig. 1 Flow cytometry analysis of MCF-7 cells after 48 h of exposure to 20 µg/mL exogenous PGA_2. Cell cycle analysis showed an increase in both S- and G_2/M phases, as well as a marked decrease in the G_1 phase in the amount of PGA_2-treated cells. The DNA content below the G_0/G_1 peak increased after exposure to PGA_2 and is presented as the apoptotic fraction. Each analysis was based on at least 10 000 events employing a Coulter Epic-XS flow cytometer. The data were analyzed using a multicycle analysis program (MulticycleAV software).

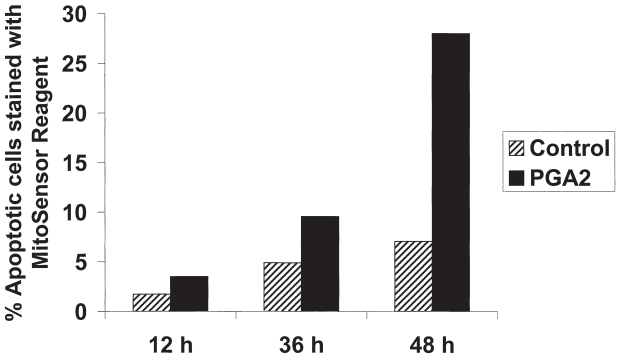

Fig. 2 Detection of mitochondrial transmembrane potential changes after PGA_2 exposure of MCF-7 cells. PGA_2-induced apoptosis was confirmed in a time-dependent study of 12 h, 36 h and 48 h. 28.0% of PGA_2-exposed MCF-7 cells were in apoptosis when compared to the 7.1% vehicle-treated cells after 48 h. Each analysis was based on at least 10 000 events employing a Coulter Epic-XS flow cytometer. The data were analyzed using a multicycle analysis program (MulticycleAV software).

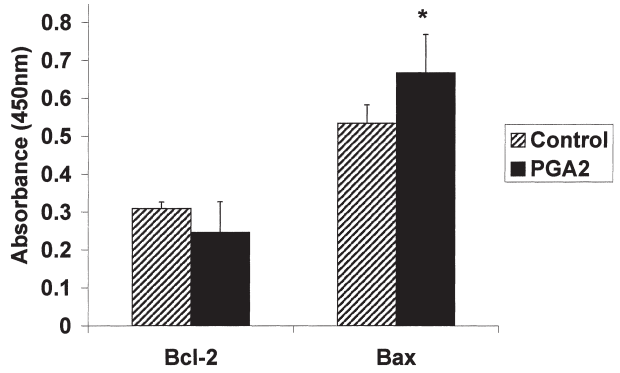

Fig. 3 Bcl-2 and Bax expression levels of PGA_2-exposed MCF-7 cells and vehicle-treated control cells after 24 h. PGA_2 exposure led to statistically significant increase (1.25-fold; indicated by an * on the graph) over vehicle-treated controls in Bax expression levels. In contrast, a decrease in Bcl-2 expression levels (0.79-fold over vehicle-treated controls, however not statistically significant) was observed. Means are presented in bar charts, with T-bars referring to standard deviations. *P*-values < 0.05 were regarded as statistically significant and indicated by an *.

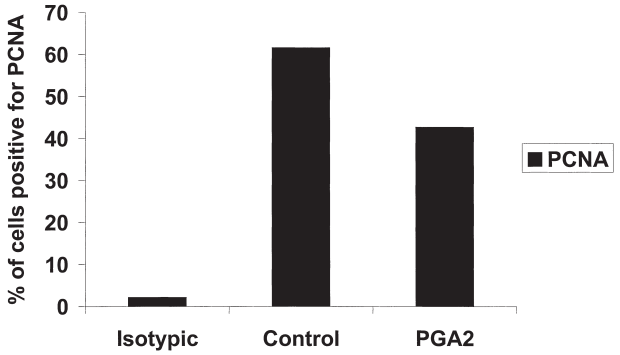

Fig. 4 Influence of vehicle-control and 20 µg/mL PGA_2 on PCNA levels in MCF-7 cells. Isotypic controls are also included. PGA_2 reduced expression levels of PCNA 0.69-fold when compared to vehicle-treated control cells after 48 h of exposure. Each analysis was based on at least 10 000 events employing a Coulter Epic-XS flow cytometer. The data were analyzed using a multicycle analysis program (MulticycleAV software).

possible mechanism for induction of apoptosis.

In this study, PGA_2 caused an accumulation in both the S- and G_2/M phases, a marked decrease in G_1 phase and an increase in DNA content preceding the G_0/G_1 peak (indicative of apoptotic body formation) after 48 h of exposure thereby confirming previous results obtained (11). Similarly to our cell cycle progression status results obtained after PGA_2 exposure, Grzanka *et al.* (2005) also observed a decrease of K-562 leukemia cells in the G_0/G_1 phase, an increase in the G_2/M phase, as well as an increase in the number of apoptotic cells after exposure to another anti-mitogenic drug, doxorubicin. It was suggested that increased expression of cyclin A may have contributed to the induction of apoptosis in these cells (7).

In a study conducted by Distefano *et al.* (1997) it was shown that the anti-mitogenic drugs, paclitaxel, docetaxel and a series of new analogs synthesized from 14beta-hydroxy-10-deacetylbaccatin III also induced a cell cycle block in G_2/M (4). The latter re-

sult correlated significantly with apoptosis measured in the sub-G_1 region and occurrence of apoptosis was confirmed by DNA gel agarose electrophoresis (4). Premature activation of p34(cdc2) has been implicated as a prerequisite for apoptosis induction in cells arrested in G_2/M (27). In addition, Seegers *et al.* (2000) demonstrated a decrease in p34(cdc2) activity in PGA_2-treated WHCO3 oesophageal carcinoma cells (24). In this study confirmation of apoptosis was determined by employing the mitosensor reagent that detects potential changes in the mitochondrial membrane. 28.0% of PGA_2-exposed cells were in apoptosis when compared to the 7.1% vehicle-treated cells after 48 h.

Since the Bcl-2 family of proteins is regarded as key regulators of apoptosis, the influence of PGA_2 on Bax and Bcl-2 expression levels in MCF-7 cells were subsequently investigated. Bcl-2 is perceived as anti-apoptotic, whereas Bax can be regarded as pro-apoptotic and an increased ratio of pro-apoptotic Bax to anti-apoptotic Bcl-2 can be associated with apoptosis. The pro- and anti-apoptotic Bcl-2 family of proteins are responsible for either the induction or prevention of mitochondrial membrane permeability important in regulating cytochrome c release from mitochondria (2, 6, 13, 16, 28).

In this study, PGA_2 exposure led to statistically significant increase (1.25-fold) over vehicle-treated controls in Bax expression levels. In contrast, a decrease in Bcl-2 expression levels (0.79-fold over vehicle-treated controls) was observed. The Bax/Bcl-2 ratio for PGA_2-exposed cells was 2.7, normalized against Bcl-2 levels. It was previously reported that 15-deoxy-Delta (12,14)-prostaglandin J(2) (15d-PGJ(2)) also induced apoptosis in the human chondrosarcoma cell line, OUMS-27 by down-regulation of anti-apoptotic Bcl-xL and up-regulation of pro-apoptotic Bax (26). Furthermore, Joubert *et al.* (2005) obtained similar results after exposure of squamous oesophageal cancer cells (WHCO3) to 2-methoxyestradiol (2-ME), another anti-mitotic drug and tubulin poison. The Bax/Bcl-2 ratio for WHCO3-treated cells was determined as 1.45, normalized against Bcl-2 levels (9). Exposure of human cervical carcinoma cells (HeLa) to PGA_2 and 2-ME revealed a Bax/Bcl-2 ratio of 2.06 and 1.87 respectively, normalized against Bcl-2 levels in these cells, also suggesting that this altered ratio in favor of Bax could lead to the induction of apoptosis in these cells (10).

Furthermore, PGA_2 caused reduced expression levels of PCNA (0.69-fold) when compared to vehicle-treated control cells after 48 h of exposure. Liu *et al.* (2005) also revealed a decrease of PCNA together with nuclear changes that are characteristic of apoptosis, a decreased mitochondrial membrane potential and activation of caspase 3 in human promyelocytic leukemia HL60 cells (15).

Although the exact mechanisms of apoptosis induction by PGA_2 and the role of key molecules influenced in MCF-7 cells require further investigation, the present study suggests that an accumulation in the S phase, a decrease in expression levels of PCNA, as well as an altered ratio in favor of Bax, could lead to the induction of apoptosis in these cells. More knowlegde regarding 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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REFERENCES

1. Bregman MD, Funk C and Fukushima M (1986) Inhibition of human melanoma growth by prostaglandin A, D, and J analogues. *Cancer Res* **46**, 2740–2744.
2. Chan SL and Yu VC (2004) Proteins of the bcl-2 family in apoptosis signalling: from mechanistic insights to therapeutic opportunities. *Clin Exp Pharmacol Physiol* **31**, 119–128.
3. De Kock M, Lottering M-L, Grobler CJS, Viljoen TC, Le Roux M and Seegers JC (1996) The induction of apoptosis in human cervical carcinoma (HeLa) cells by gamma-linolenic acid. *Prostaglandins Leukot Essent Fatty Acids* **55**, 403–411.
4. Distefano M, Scambia G, Ferlini C, Gaggini C, De Vincenzo R, Riva A, Bombardelli E, Ojima I, Fattorossi A, Panici PB and Mancuso S (1997) Anti-proliferative activity of a new class of taxanes (14beta-hydroxy-10-deacetylbaccatin III derivatives) on multidrug-resistance-positive human cancer cells. *Int J Cancer* **72**, 844–850.
5. Fukushima M, Sasaki H and Fukushima S (1993) Prostaglandin J2 and related compounds: mode of action in G1 arrest and preclinical results. *Ann NY Acad Sci* **744**, 161–165.
6. Ghosh R, Ott AM, Seetharam D, Slaga TJ and Kumar AP (2003) Cell cycle block and apoptosis induction in a human melanoma cell line following treatment with 2-methoxyoestradiol: therapeutic implications? *Melanoma Res* **13**, 119–127.
7. Grzanka A, Zuryn A, Styczynski J, Grzanka AA and Wisniewska H (2005) The effect of doxorubicin on the expression of cyclin A in K-562 leukemia cell line. *Neoplasm* **52**, 489–493.
8. Hendzel MJ, Nishioka WK, Raymond Y, Allis CD, Bazett-Jones DP and Th'ng JP (1998) Chromatin condensation is not associated with apoptosis. *J Biol Chem* **273**, 24470–24478.

9. Joubert A, Maritz C and Joubert F (2005) Bax/Bcl-2-expression levels of 2-methoxyestradiol-exposed esophageal cancer cells. *Biomed Res* **26**, 131–134.
10. Joubert A, Maritz C and Joubert F (2005) Influence of prostaglandin A_2 and 2-methoxyestradiol on Bax and Bcl-2 expression levels in cervical carcinoma cells. *Biomed Res* **26**, 87–90.
11. Joubert AM, Panzer A, Bianchi PC and Lottering M-L (2003) The effects of prostaglandin A_2 on cell growth, cell cycle status and apoptosis induction in HeLa and MCF-7 cells. *Cancer Lett* **191**, 203–209.
12. Joubert AM, Panzer A, Joubert F, Lottering M-L, Bianchi PC and Seegers JC (1999) Comparative study of the effects of polyunsaturated fatty acids and their metabolites on cell growth and tyrosine kinase activity in oesophageal carcinoma cells. *Prostaglandins Leukot Essent Fatty Acids* **61**, 171–182.
13. Kang CD, Jang JH, Kim KW, Lee HJ, Jeong CS, Kim CM, Kim SH and Chung BS (1998) Activation of c-jun N-terminal kinase/stress-activated protein kinase and the decreased ratio of Bcl-2 to Bax are associated with the auto-oxidized dopamine-induced apoptosis in PC12 cells. *Neurosci Lett* **256**, 37–40.
14. Kikuchi Y, Kita T, Miyaunchi M, Hirata J, Sasa H, Nagata I and Fukushima M (1992) Adjuvant effects of antineoplastic prostaglandins to cisplatin in nude mice bearing human ovarian cancer cells. *J Cancer Res Clin Oncol* **118**, 453–457.
15. Liu WK, Ho JC and Che CT (2005) Apoptotic activity of isomalabaricane triterpenes on human promyelocytic leukemia HL60 cells. *Cancer Lett* **230**, 102–110.
16. Moreau C, Cartron PF, Hunt A, Meflah K, Green DR, Evan G, Vallette FM and Juin P (2003) Minimal BH3 peptides promote cell death by antagonizing anti-apoptotic proteins. *J Biol Chem* **278**, 19426–19435.
17. Narumiya S and Fukushima M (1986) Site and mechanism of growth inhibition by prostaglandins. I. Active transport and intracellular accumulation of cyclopentenone prostaglandins, a reaction leading to growth inhibition. *J Pharmacol Exp Ther* **239**, 500–505.
18. Narumiya S, Ohno K, Fukushima M and Fujiwara M (1987) Site and mechanism of growth inhibition by prostaglandins. III. Distribution and binding of prostaglandin A_2 and d12-prostaglandin J_2 in nuclei. *J Pharmacol Exp Ther* **242**, 306–311.
19. Ohno K, Fujiwara M, Fukushima M and Narumiya S (1986) Metabolic dehydration of prostaglandin E_2 and cellular uptake of the dehydration product: correlation with prostaglandin E_2-induced growth inhibition. *Biochem Biophys Res Commun* **139**, 808–815.
20. Ohno K, Sakai T, Fukushima M Narumiya S and Fujiwara M (1988) Site and mechanism of growth inhibition by prostaglandins. IV. Effect of cyclopentenone prostaglandins on cell cycle progression of G_1-enriched HeLa S3 cells. *J Pharmacol Exp Ther* **245**, 294–298.
21. Santoro MG, Crisari A, Benedeto and Amici C (1986) Modulation of the growth of a human erythroleukemic cell line (K562) by prostaglandins: antiproliferative action of prostaglandin A. *Cancer Res* **46**, 6073–6077.
22. Sasaki H and Fukushima M (1994) Prostaglandins in the treatment of cancer. *Anticancer Drugs* **5**, 131–138.
23. Sasaki HK, Takeda K, Terashima Y, Ekimoto H, Takahashi K, Tsuruo T and Fukushima M (1991) Human ovarian cancer cell lines resistant to cisplatin, doxorubicin and L-phenylalanine mustard are sensitive to d7-prostaglandin A1 and d12-prostaglandin J_2. *Gynecol Oncol* **41**, 36–40.
24. Seegers JC, Joubert AM, Panzer A, Lottering M-L, Jordan CA, Joubert F, Maree J-L, Bianchi P, De Kock M and Gelderblom WCA (2000) Fumonisin B1 influenced the effects of arachidonic acid, prostaglandins E_2 and A_2 in cell cycle progression, apoptosis induction, tyrosine- and cdc2-kinase activity in esophageal cancer cells. *Prostaglandins Leukot Essent Fatty Acids* **62**, 75–84.
25. Seegers JC, Lottering M-L, Grobler CJ, Van Papendorp DH, Habbersett RC, Shou Y and Lehnert BE (1997) The mammalian metabolite, 2-methoxyestradiol, affects p53 levels and apoptosis induction in transformed cells but not in normal cells. *J Steroid Biochem Mol Biol* **62**, 253–267.
26. Shen ZN, Nishida K, Doi H, Oohashi T, Hirohata S, Ozaki T, Yoshida A, Ninomiya Y and Inoue H (2005) Suppression of chondrosarcoma cells by 15-deoxy-Delta 12,14-prostaglandin J2 is associated with altered expression of Bax/Bcl-xL and p21. *Biochem Biophys Res Commun* **328**, 375–382.
27. Shi L, Nishioka WK, Th'ng J, Bradbury EM, Litchfield DW and Greenberg AH (1994) Premature p34cdc-2 activation required for apoptosis. *Science* **263**, 1143–1145.
28. Zhang M and Raveche ES (1998) Apoptosis induction in fludarabine resistant malignant B-1 cells by G2-M cell cycle arrest. *Oncol Rep* **5**, 23–30.