2,2′,4,6,6′-Pentachlorobiphenyl-Induced Apoptosis Is Limited by Cyclooxygenase-2 Induction

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Polychlorinated biphenyls (PCBs), a group of persistent and widespread environmental pollutants, are considered to be immunotoxic, carcinogenic, and to induce apoptosis. However, the cellular mechanisms underlying the action of PCBs have not been established. Here, we investigated the effects of PCBs on the induction of cyclooxygenase-2 (COX-2). Among the several congeners examined, only 2,2′,4,6,6′-pentachlorobiphenyl (PeCB) specifically increased the COX-2 promoter activity, and the levels of COX-2 mRNA and protein, and thereby enhanced prostaglandin E2 (PGE2) synthesis in Rat-1 cells. By conducting mutation analyses of the COX-2 promoter and its transcription factor, we found that the CRE site in a number of congeners, including 2,2′,4,6,6′-PeCB, is important for increased COX-2 promoter activity induced by 2,2′,4,6,6′-PeCB. In addition, 2,2′,4,6,6′-PeCB-stimulated COX-2 induction was reduced by the specific MAPK kinase (MEK) inhibitor, PD98059, and in p53-deficient cells, implying that COX-2 induction requires the activation of ERK1/2 MAPK and p53. The selective COX-2 inhibitor, NS-398, potentiated the 2,2′,4,6,6′-PeCB-induced mitochondrial apoptotic pathway involved in Bcl-xL attenuation, cytochrome c release and the subsequent activation of caspase-3. Furthermore, the cell death was prevented by PGE2 treatment, suggesting that 2,2′,4,6,6′-PeCB-induced apoptosis is restricted by prostaglandin upregulation by COX-2. Taken together, these results demonstrate that 2,2′,4,6,6′-PeCB-induced COX-2 expression may be an important compensatory mechanism for abating 2,2′,4,6,6′-PeCB toxicity. Among the several congeners examined, only 2,2′,4,6,6′-pentachlorobiphenyl (PeCB) specifically increased the COX-2 promoter activity, and the levels of COX-2 mRNA and protein, and thereby enhanced prostaglandin E2 (PGE2) synthesis in Rat-1 cells. By conducting mutation analyses of the COX-2 promoter and its transcription factor, we found that the CRE site in a number of congeners, including 2,2′,4,6,6′-PeCB, is important for increased COX-2 promoter activity induced by 2,2′,4,6,6′-PeCB. In addition, 2,2′,4,6,6′-PeCB-stimulated COX-2 induction was reduced by the specific MAPK kinase (MEK) inhibitor, PD98059, and in p53-deficient cells, implying that COX-2 induction requires the activation of ERK1/2 MAPK and p53. The selective COX-2 inhibitor, NS-398, potentiated the 2,2′,4,6,6′-PeCB-induced mitochondrial apoptotic pathway involved in Bcl-xL attenuation, cytochrome c release and the subsequent activation of caspase-3. Furthermore, the cell death was prevented by PGE2 treatment, suggesting that 2,2′,4,6,6′-PeCB-induced apoptosis is restricted by prostaglandin upregulation by COX-2. Taken together, these results demonstrate that 2,2′,4,6,6′-PeCB-induced COX-2 expression may be an important compensatory mechanism for abating 2,2′,4,6,6′-PeCB toxicity.

Key Words: polychlorinated biphenyl; cyclooxygenase-2; compensation.

Polychlorinated biphenyls (PCBs), a group of halogenated aromatic hydrocarbons (HAHs), are widely spread environmental contaminants. The high lipophilicity and chemical stability of PCBs have added to this widespread environmental contamination, and there is significant evidence of PCBs accumulation in biological system (Koopman-Esseboom et al., 1994; Safe, 1993). The toxic effects of PCBs range from carcinogenesis and immunotoxicity to disruption of the nervous, endocrine, and reproductive systems. PCBs can form 209 possible congeners with respect to the number and position of chlorine atoms on the biphenyl ring. The toxic effects and physical properties of PCB congeners are structure-dependent. Moreover, these PCB congeners seem to have distinct modes of action. Planar congeners without chlorine(s) substituted at ortho-position have relatively high affinity for aryl hydrocarbon receptor, the endogenous dioxin receptor, and thus, considered to exhibit toxic effects through the receptor. However, the other congeners with ortho-substituted chlorine(s) have negligible binding affinity for this receptor and therefore, their toxic effects probably involve a different mechanism (Kodavanti and Tilson, 1997).

Cyclooxygenase (COX) is the rate-limiting enzyme that catalyzes the oxygenation of arachidonic acid to prostaglandin endoperoxides, which are converted enzymatically into prostaglandins (PGs) and thromboxane A2 (Marnett et al., 1999; Warner and Mitchell, 2004). Two distinct isoforms of COX have been identified. COX-1 is constitutively expressed in most tissues, but in contrast, COX-2, the product of a related inducible gene, is absent in most normal tissues. However, it is expressed rapidly in response to proliferative and inflammatory stimuli such as growth factors, cytokines, and tumor promoters (Kroll et al., 1999; Matsuura et al., 1999; Xie and Herschman, 1996). Ihas been well established that COX-2 is linked to cell survival (Adderley and Fitzgerald, 1999; Subbaramaiah et al., 2000; Van Putten et al., 2001; Xie and Herschman, 1995; Yang et al., 2000). For example, COX-2 protects (1) macrophages from superoxide-induced apoptosis (Address von et al., 1999); (2) neuronal cells from nerve growth factor (NGF) withdrawal-induced apoptosis (Chang et al., 2000; McGinty et al., 2000); and (3) renal cells from hypertonicity-induced cell death (Yang et al., 2000). Intriguingly, recent reports have suggested that p53 can induce the sustained activation of the Ras/Raf/ERK cascade. And, such activation induces COX-2 expression, which counteracts p53- or genotoxic stress-induced apoptosis (Han et al., 2002; Lee et al., 2000).

In this study, we show that 2,2′,4,6,6′-PeCB-induced COX-2 expression is mediated by the CRE site, c-Jun, p53 and ERK1/2.
MAPK activation. Moreover, the selective COX-2 inhibitor, NS-398, potentiated the 2,2'-4,6,6'-PeCB-induced mitochondrial apoptotic pathway. These results suggest that COX-2 expression by 2,2'-4,6,6'-PeCB may be a compensatory mechanism designed to abate its toxicity by reducing apoptotic susceptibility.

MATERIALS AND METHODS

Materials. PCBs (>99% pure) were purchased from AccuStandard (New Haven, CT). FuGENE transfection reagent was from Roche Molecular Biochemicals. Luciferase activity assay reagent was from Promega (Madison, WI). NS-398 (N-(2-cyclohexyloxy-4-nitrophenyl) methanesulfonanilide) and PD98059 were from Calbiochem (La Jolla, CA). COX-2 polyclonal antibody was from Cayman Co. (Ann Arbor, MI). Polyclonal antibodies to ERK1/2 MAPK (Thr-202/Tyr-204) was from New England Biolabs, Inc. (Beverly, MA). Monoclonal antibody to cytochrome c was from PharMingen (La Jolla, CA).

Cell culture. Rat-1 fibroblasts and mouse embryonic fibroblasts (MEFs) were maintained in DMEM supplemented with 10% fetal bovine serum at 37°C (La Jolla, CA). COX-2 promoter-luciferase wild type and deletion constructs were kindly provided by Dr. Herschman (UCLA-Los Angeles Center for the Health Sciences, Los Angeles, CA). A c-Jun dominant negative expression vector was a gift of Dr. Tom Curran (St. Jude Children’s Research Hospital, Memphis, TN).

Transient transfection. Rat-1 cells were transfected plasmids using FuGENE transfection reagent according to a procedure recommended by the manufacturer. The transfected cells were cultured for one day, starved with medium without serum for one day. Plasmids. p53-Luc- and AP-1-Luc plasmid was obtained from Stratagene (La Jolla, CA). COX-2 promoter-luciferase wild type and deletion constructs were maintained in DMEM supplemented with 10% fetal bovine serum at 37°C in a humidified, 5% CO2-controlled incubator. Before stimulation, the cells were starved with medium without serum for one day.

 Luciferase reporter gene assay. The cells were lysed with lysis buffer (20 mM Tris-HCl, pH 7.8, 1% Triton X-100, 150 mM NaCl, 2 mM DTT). The cell lysate (5 μl) was mixed with luciferase activity assay reagent (25 μl) and luminescence produced for 5 s was measured using luminoskan (labsystems). Data are presented as the fold of relative light unit relative to vehicle-treated control cultures.

Western blot analysis. For Western blot analysis, whole cell lysate were prepared in lysis buffer (20 mM Tri-HCl, pH 7.4, 150 mM NaCl, 20 mM NaF, 1 mM EDTA, 1 mM PMFSF). Proteins (30 μg/lane) were separated on a denaturing 10% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. Primary antibody was incubated with the membranes overnight at 4°C. Secondary antibody linked to horseradish peroxidase was used at 1:10,000, and signals were visualized by the ECL technique.

RT-PCR. cDNA was reverse-transcribed from total cellular RNA using oligo (dT) primers and murine leukemia virus reverse transcriptase. cDNA was amplified for 35 cycles using the following rat COX-2 gene-specific primers: 5'-ACTTGGCTCAGCTTTGTAATCT-3' (sense) and 5'-TTTGTAGTTGCTGTAGTTCTTTGC-3' (antisense) and rat COX-1 gene-specific primers: 5'-AAATGCAAGGCTGCTGAAGGC-3' (sense) and 5'-GTTTCATCTTATCAGGAGGAGG-3' (antisense). The cycling parameters were the following: 1 min at 94°C for denaturation, 1 min at 60°C for primer annealing, and 1 min at 72°C for polymerization. Meanwhile, the same amount of DNA was amplified for 25 cycles using specific β-actin primers: 5'-GAGACCCTCCTACATCAACCC-3' (sense) and 5'-GTTGGTGTTGAGCTGTAGAAGCC-3' (antisense). The products were visualized after electrophoresis on a 2% agarose gel containing ethidium bromide.

Cyclooxygenase activity. Rat-1 cells were plated in 24-well plate. One day later, the medium was replaced with serum free DMEM for one day, the cells were then incubated with or without PCBs for 6 h. Media were collected from each well and analyzed for PGE2 by enzyme-linked immunoassay as described previously (Yan et al., 2000).

Caspase-3 activity assay. Rat-1 cells were harvested and sonicated. Following centrifugation at 15,000 rpm for 10 min, 20 μg protein of supernatant in buffer (100 mM Hepes, 10% sucrose, 5 mM dithiothreitol, 10% NP-40, and 0.1% CHAPS at pH 7.25) was added to each well of a 96-well plate along with 50 μM DEVD-aminomethylcoumarin (AMC). After incubation at 37°C for 1 h, the cleaved free AMC (excitation of 355 nm, emission of 460 nm) was detected using fluorometer.

Trypan blue exclusion and MTT assay. For the trypan blue exclusion assay, 1 × 10⁶ cells/1 ml were plated and treated with NS398 and PCB. The cells were then harvested and resuspended in medium, and an equal volume of trypan blue was added. More than 200 cells were scored on a hemocytometer. The percentage of cell survival was calculated by taking the number of trypan blue-excluding cells following treatment and dividing it by the number of DMSO-treated control cells and multiplying by 100.

To determine cell viability, 1 × 10⁴ cells/well was subcultured in a 96-well plate. After treatment with chemical in serum-free DMEM for the indicated times, the cells were incubated for 2 h in the presence of 0.5 μg/ml MTT reagent in a 37°C incubator. After removal of MTT reagent and disruption of cells with DMSO, the absorbance was measured at 570 nm using a 96-well plate reader. Data are presented as the percentage of viability relative to vehicle-treated control cultures.

Statistical analysis. The results are expressed as means ± SE. Statistical significance was determined using the Student’s t-test.

RESULTS

2,2',4,6,6'-PeCB Increases COX-2 Induction

To investigate the effect of PCBs on COX-2 expression, we transiently transfected a COX-2 promoter construct ligated to luciferase into Rat-1 cells. The transfected cells were treated with each 10 μM PCB congener for 6 h as indicated in Figure 1A. Among the congeners examined, 2,2',4,6,6'-PeCB specifically caused about a three-fold increase in COX-2 promoter activity. Moreover, treatment of Rat-1 cells with 2,2',4,6,6'-PeCB produced a time- and dose-dependent increase in COX-2 protein level (Figs. 1B and 1C, respectively). Maximal effects were observed at 6 h and 10 μM 2,2',4,6,6'-PeCB, respectively. RT-PCR analysis was done to determine whether 2,2',4,6,6'-PeCB induces COX-2 mRNA. Treatment with 2,2',4,6,6'-PeCB resulted in the increase in COX-2 mRNA but not of COX-1 (Fig. 1D). As shown in Figure 1E, such COX-2 mRNA induction was inhibited by actinomycin D, transcription inhibitor, implying that 2,2',4,6,6'-PeCB stimulates COX-2 transcription. Furthermore, the level of prostaglandin E2 (PGE2), one of the major enzymatic products of COX-2, was caused about an 8-fold increase by 2,2',4,6,6'-PeCB (Fig. 1F). However, 2,3,3',4,4'-PeCB, an isomer of 2,2',4,6,6'-PeCB with same number of chlorines, but in different positions, had no effect on COX-2 induction or PGE2 synthesis.

Cyclic AMP Response Element and c-Jun Mediate 2,2',4,6,6'-PeCB-Stimulated COX-2 Induction

About 1000 bp of the 5' flanking region of the COX-2 gene contains various putative transcription response elements.
FIG. 1. COX-2 induction by 2,2',4,6,6'-PeCB. (A) Rat-1 cells transiently transfected with COX-2 promoter construct (11432/159) ligated to luciferase. Transfected cells were treated with DMSO (0.1%), 10 µM 2,2', 3,3', 4,4'-DiCB, 2,2',4-, 2,2',6-, 2,4,4'-, 3,3',4-TriCB, 2,2',4,4'-, 2,2',6,6'-, 3,3',4,4'-TeCB, 2,2',4,6,6'-, 2,3,3',4,4'-, 2,3',4,4',5-, or 3,3',4,4',5-PeCB for 6 h in serum-free medium. Luciferase activity in 1 µg of cell lysate was assayed as described in Materials and Methods. Luciferase activity represents data that have been normalized into cotransfected renilla luciferase activity. Data represent the means ± SE of three separate experiments, each conducted in duplicate. DiCB: dichlorobiphenyl, TriCB: trichlorobiphenyl, TeCB: tetrachlorobiphenyl, PeCB: pentachlorobiphenyl. (B and C) 10 µM 2,2',4,6,6'- or 2,3,3',4,4'-PeCB was incubated with Rat-1 cells for the indicated times or at the indicated concentrations. Each protein was separated by SDS-PAGE, and then Western blot analysis with specific antibodies against COX-2 or actin was performed. (D) Total RNA was isolated from cells that were treated with DMSO, 10 µM 2,2',4,6,6'- or 2,3,3',4,4'-PeCB for 1 h. RT-PCR with specific rat COX-1, COX-2 or actin primers was performed as detailed under Materials and Methods. (E) Rat-1 cells were pretreated with 1 µg/ml actinomycin D and then treated with 10 µM 2,2',4,6,6'-PeCB for 1 h. COX-2 mRNA levels were determined using RT-PCR analysis. (F) Cells were treated with DMSO, 10 µM 2,2',4,6,6'- or 2,3,3',4,4'-PeCB for 6 h. The medium was collected to determine the synthesis of PGE2. Production of PGE2 was determined by specific enzyme immunoassay. Data are representative of at least three separate experiments.
ERK1/2 MAPK and p53 Are Important for 2,2′,4,6′-PeCB-Mediated COX-2 Induction

Mitogen-activated protein kinase pathways mediate the regulation of COX-2 expression to a variety of extracellular stimuli (Guo et al., 2001; Subbaramaiah et al., 2000; Yan et al., 2000; Yang et al., 2000). Figure 3A demonstrated that 2,2′,4,6′-PeCB but not 2,3,3′,4,4′-PeCB induced ERK1/2 MAPK phosphorylation in a time-dependent manner. To investigate whether such activation of ERK1/2 MAPK is important for the induction of COX-2, PD98059, a specific inhibitor of MAPK kinase, was used to block the activation of ERK1/2 MAPK. Treatment of PD98059 reduced COX-2 protein induction and PGE2 production in response to 2,2′,4,6,6′-PeCB (Fig. 3B and data not shown).

Recent reports have suggested that p53 can induce the sustained activation of the Ras/Raf/ERK cascade. Such activation induces COX-2 expression, which counteracts p53- or genotoxic stress-induced apoptosis (Han et al., 2002; Lee et al., 2000). Also, we previously reported that 2,2′,4,6′-PeCB activates p53 by disrupting microtubule in NIH 3T3 cells (Shin et al., 2004). Therefore, we investigated whether COX-2 is induced in response to p53 in Rat-1 cells. As shown in Figure 3C, 2,2′,4,6, 6′-PeCB caused an increase in p53-dependent transcription. To investigate whether p53 acts upstream of ERK1/2 MAPK, we checked the effect of 2,2′,4,6,6′-PeCB on ERK1/2 MAPK activity in p53+/+ and p53−/− mouse embryonic fibroblasts (MEFs). Treatment of p53+/+ MEFs with 2,2′,4,6,6′-PeCB increased ERK1/2 MAPK phosphorylation. In contrast, ERK1/2 MAPK was not phosphorylated by 2,2′,4,6,6′-PeCB in p53−/− MEFs (Fig. 3D), indicating that p53 acts upstream of ERK1/2 MAPK. Moreover, treatment of p53+/+ MEFs with 2,2′,4,6,6′-PeCB increased the level of COX-2 protein.

FIG. 2. CRE and c-Jun is important for 2,2′,4,6,6′-PeCB-mediated COX-2 induction. (A) Transient transfection of Rat-1 cells was performed with either wild type (wt) or mutant (m) luciferase reporter plasmids. (B) Rat-1 cells were transiently transfected with AP-1-Luc plasmid. (C) Rat-1 cells were cotransfected with COX-2 promoter construct ligated luciferase and vector or dominant negative C-Jun. Transfected cells were treated with 2,2′,4,6,6′-PeCB for 6 h and then performed the assay for luciferase activity. Luciferase activity represents data that have been normalized into cotransfected renilla luciferase activity. Data represent the means ± SE of three separate experiments, each conducted in duplicate.
FIG. 3. COX-2 induction by 2,2′,4,6,6′-PeCB is mediated by ERK1/2 MAPK and p53. (A) Rat-1 cells stimulated with 10 μM 2,2′,4,6,6′- or 2,3,3′,4,4′-PeCB for the indicated times. Western blots were probed with antibodies to phosphorylated forms of ERK1/2 MAPK. (B) Rat-1 cells were pretreated with 20 μM PD98059 for 1 h and then stimulated with 2,2′,4,6,6′-PeCB for 6 h. Western blots were probed with antibody specific for COX-2 or actin. (C) Rat-1 cells were transiently transfected with luciferase reporter gene under control of p53. Transfected cells were treated with DMSO, 10 μM 2,2′,4,6,6′- or 2,3,3′,4,4′-PeCB for 6 h. Luciferase activity represents data that have been normalized into cotransfected renilla luciferase activity. Data represent the means ± SE of three separate experiments, each conducted in duplicate. (D) p53+/+ or p53−/− MEFs stimulated with 10 μM 2,2′,4,6,6′-PeCB for the indicated times. Western blots were probed with antibodies to phosphorylated forms of ERK1/2. (E) p53+/+ or p53−/− MEFs were treated with DMSO, 10 μM 2,2′,4,6,6′- or 2,3,3′,4,4′-PeCB for 6 h. Western blot analysis with specific antibodies against COX-2 or actin was performed. (F) p53+/+ or p53−/− MEFs were treated with 1 μM sphingosin-1 phosphate (SIP) or 100 ng/ml EGF for 6 h. Total cell extracts were isolated for western blot analysis with specific antibodies against COX-2 or actin. Data presented are from one of three experiments with similar findings.
In contrast, in p53−/− MEFs, no detectable upregulation of COX-2 expression was observed in response to 2,2',4,6,6'-PeCB (Fig. 3E). These findings indicate that p53 mediates 2,2',4,6,6'-PeCB-stimulated COX-2 induction. On the other hand, COX-2 induction by EGF or sphingosin-1-phosphate (S1P), proliferative stimuli, was detected in both p53+/+ and p53−/− MEFs, implying that PCB-induced COX-2 expression is mediated through a different pathway from those initiated by EGF or S1P (Fig. 3F).

COX-2 Reduces Apoptotic Susceptibility

We previously reported that among the congeners examined, 2,2',4,6,6'-PeCB induces apoptosis in human monocytic cells (Shin et al., 2000). Furthermore, COX-2 has been shown to be associated with cell survival by many groups (Adderley and Fitzgerald, 1999; Dowd et al., 2001; Shinmura et al., 2000). Therefore, we investigated the effect of COX-2 induction on 2,2',4,6,6'-PeCB-induced apoptosis using the selective COX-2 inhibitor, NS-398. Co-treatment with 2,2',4,6,6'-PeCB and NS-398 further increased cell death and caspase 3 activity, as compared with 2,2',4,6,6'-PeCB treatment alone (Figs. 4A and 4B). To examine how COX-2 reduces apoptosis by 2,2',4,6,6'-PeCB, we analyzed key steps in the mitochondrial apoptotic pathway. NS-398 potentiated the 2,2',4,6,6'-PeCB-stimulated attenuation of Bcl-xL mRNA, an anti-apoptotic gene, and increased mitochondrial cytochrome c release into the cytosol (data not shown), suggesting that COX-2 induction restricts cell death by blocking the mitochondrial apoptotic pathway. Furthermore, the cell death caused by 2,2',4,6,6'-PeCB was reduced about 30 % by PEG 2 treatment (Fig. 4C). These data imply that 2,2',4,6,6'-PeCB-induced apoptosis is limited by an increase in prostaglandin resulting from COX-2 induction.

DISCUSSION

The toxic effects of PCBs range from immunotoxicity and carcinogenesis to apoptosis, and PCB congeners with different structures are considered to have distinct mechanisms of action, which result in different cellular toxicities (Koopman-Esseboom et al., 1994; Safe, 1993). In the present study, we investigated the effects of PCBs on the induction of COX-2 in Rat-1 cells. Of the congeners examined, only 2,2',4,6,6'-PeCB, one of the highly ortho-substituted PCB congeners, specifically activated COX-2 promoter (Fig. 1A). Also, treatment with 2,2',4,6,6'-PeCB increased COX-2 mRNA and protein levels, and enhanced PGE2 synthesis (Figs. 1B, 1C, and 1F). Such an increase in COX-2 expression is regulated by a transcriptional mechanism. Mutating the CRE site of COX-2 promoter caused a loss of responsiveness to 2,2',4,6,6'-PeCB (Fig. 2A). Furthermore, 2,2',4,6,6'-PeCB-mediated induction of COX-2 promoter activity was suppressed by dominant negative c-Jun (Fig. 2C). These findings indicates that the CRE site and c-Jun are important
for the transcriptional control of COX-2 induction in response to 2,2′,4,6,6′-PeCB. These results are consistent with the findings of Xie and Herschman (1995, 1996), who showed that, in response to v-src expression or treatment with platelet-derived growth factor, c-Jun induced murine COX-2 via the CRE site. However, another report found that 2,2′,4,4′,5,5′-PCB induces COX-2 expression through NF-kB activation in mast cells, which is involved in inflammation (Kwon et al., 2002). In contrast, in our study, mutation of NF-kB site had no effect on 2,2′,4,6,6′-PeCB-induced COX-2 promoter activity in Rat-1 cells (Fig. 2A). These results imply that PCB congeners with different structures have distinct mechanism of action.

We also found that COX-2 expression by 2,2′,4,6,6′-PeCB is mediated through the activation of ERK1/2 MAPK and p53 (Fig. 3). Recently, it was reported that a biochemical link between p53 signaling and Ras/Raf/MAPK cascade results in COX-2 expression (Han et al., 2002). Our results also showed that MAPK activation required the p53 transcription function (Fig. 3D). Furthermore, HB-EGF, which activates the EGF receptor and other members of the erbB family, has been shown to be induced in a p53-dependent manner in response to DNA damage and to account, at least in part, for the activation of MAPK in response to p53 (Fang et al., 2001). In our study, 2,2′,4,6,6′-PeCB, but not 2,3,3′,4,4′-PeCB increased HB-EGF mRNA in Rat-1 cells (data not shown), suggesting the possibility that 2,2′,4,6,6′-PeCB-stimulated COX-2 induction may be mediated by p53-dependent HB-EGF, which activates the Ras/Raf/MAPK pathway.

Several groups have reported that COX-2 and its major final product, PGE2, protect cells against apoptosis (Hoshino et al., 2002; Munkarah et al., 2002; Sheng et al., 1998). Rat intestinal epithelial cells overexpressing COX-2 were resistant to butyrate-induced apoptosis and had elevated Bcl2 protein expression, which were reversed by a COX inhibitor (Tsujii and Dubois, 1995). Also, co-treatment with NS-398 and doxorubicin increased apoptosis by downregulating the anti-apoptotic protein, Bcl-xL, and the cell cycle inhibitor, p27, as compared with cells treated with doxorubicin alone (Han et al., 2002). Oxidative damage of cardiomyocytes is limited by an increase in prostaglandin formation (Adderley and Fitzgerald, 1999). In addition, Sheng et al. reported that PGE2 inhibits the apoptosis caused by a COX-2 inhibitor and induces Bcl-2 expression in human colon cancer cells (Sheng et al., 1998). These findings suggest that COX-2 is a cellular component of a program that favors cell survival rather than apoptosis in response to stresses, and that its induction represents an example of an adaptive response to protect the cell from stress. In the present study, co-treatment with 2,2′,4,6,6′-PeCB and NS-398 increased cell death and caspase-3 activity, versus cells treated with 2,2′,4,6,6′-PeCB alone. Moreover, the inhibition of COX-2 function by NS-398 enhanced the 2,2′,4,6,6′-PeCB-induced Bcl-xL mRNA downregulation, and resulted in increased mitochondrial cytochrome c release (data not shown). Figure 4C shows that treatment with PGE2 reduced the cell death caused by 2,2′,4,6,6′-PeCB, thus demonstrating that PGE2 produced by COX-2 activates anti-apoptotic genes such as Bcl-xL, which may result in reduced apoptosis through the mitochondrial pathway.

In conclusion, our study show that COX-2 is induced by 2,2′,4,6,6′-PeCB via its CRE site, and by c-Jun, ERK1/2 MAPK and p53, and that the inhibition of COX-2 activity enhances 2,2′,4,6,6′-PeCB-induced apoptosis through the mitochondrial pathway. These results suggest that COX-2 expression by 2,2′,4,6,6′-PeCB may be a compensatory mechanism designed to abate its toxicity by reducing apoptotic susceptibility.

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