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Role of retinoic acid in the modulation of benzo(a)pyrene-DNA adducts in human hepatoma cells: implications for cancer prevention

Guo-Dong Zhou, Molly Richardson, Inayat S Fazili, Jianbo Wang, Kirby C Donnelly, and Bhagavatula Moorthy

Abstract

Carcinogen-DNA adducts could lead to mutations in critical genes, eventually resulting in cancer. Many studies have shown that retinoic acid (RA) plays an important role in inducing cell apoptosis. In this study we have tested the hypothesis that levels of carcinogen-DNA adducts can be diminished by DNA repair or/and by eliminating damaged cells through apoptosis. Our results showed that the levels of total DNA adducts in HepG2 cells treated with benzo(a)pyrene (BP, 2 μ M) + RA (1 μ M) were significantly reduced compared to those treated with BP only ($P=0.038$). In order to understand the mechanism of attenuation of DNA adducts, further experiments were performed. Cells were treated with BP (4 μ M) for 24 h to initiate DNA adduct formation, following which the medium containing BP was removed, and fresh medium containing 1 μ M RA was added. The cells were harvested 24 h after RA treatment. Interestingly, the levels of total DNA adducts were lower in the BP/RA group (390.46 ± 34.19) than those in the BP/DMSO group (543.70 ± 32.82), $P=0.032$. Analysis of cell apoptosis showed an increase in BP+RA group, compared to BP or RA only group. Our results also indicated that attenuation of BP-DNA adducts by RA was not primarily due to its effects on CYP1A1 expression. In conclusion, our results suggest a mechanistic link between cellular apoptosis and DNA adduct formation, phenomena that play important roles in BP-mediated carcinogenesis. Furthermore, these results help in the understanding of the mechanisms of carcinogenesis, especially in relation to the chemopreventive properties of nutritional apoptosis inducers.

Keywords

DNA adducts; HepG2; Benzo(a)pyrene; 32 P-Postlabeling assay; DNA repair; Apoptosis

Introduction

Complex polycyclic aromatic hydrocarbons (PAH) mixtures may contain hundreds to thousands of compounds with different aromatic ring numbers. Numerous PAHs have been classified by the U.S. Environmental Protection Agency (EPA) as probable human carcinogens including model carcinogen benzo(a)pyrene (BP) (U.S., 2003). BP exhibits its biological effects through metabolic activation by cytochrome P450 enzymes to electrophilic species that are capable of reacting with nucleophilic sites of DNA to form adducts. DNA adducts represent premutagenic lesions, which play an essential role in the

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initiation phase of carcinogenesis through mutations of essential genes, ultimately leading to cancer. Covalent DNA adducts (modifications) are very sensitive and useful biomarkers for carcinogen exposure (Randerath *et al.*, 1986; Randerath *et al.*, 1989; Randerath *et al.*, 1996; Randerath *et al.*, 1997b; Randerath *et al.*, 1999) and oxidative stress (Randerath *et al.*, 1997a; Zhou *et al.*, 2000; Zhou *et al.*, 2001; Zhou *et al.*, 2004). DNA adducts are formed directly by the covalent binding of carcinogens and mutagens or their metabolites to nitrogen bases in DNA, representing the initiation stage of carcinogenesis. The induction of mutations is due primarily to chemical alterations in the structure of DNA that results in inaccurate replication of a particular region of the genome (Parkinson, 2001). DNA adducts can be removed by DNA repair processes (Hang, 2004) and damaged cells can be eliminated by cell death including apoptosis (Green and Martin, 1995; Vermeulen *et al.*, 2005), but during chronic exposures or high dose exposure to carcinogens such as smoking they often reach steady-state levels in carcinogen target tissues. Therefore, reduction in DNA adduct quantities by chemopreventive agents reduces tumor yield and it is very important measurement in cancer prevention.

Apoptosis is a physiological process characteristic of pluricellular organisms leading to self-destruction of the cell (Mohamad *et al.*, 2005). Cell death is an essential strategy for the control of the dynamic balance in living system. Apoptosis involves the activation of an energy-requiring intracellular machinery, which is tightly regulated and conserved throughout evolution (Yuan, 1996). Single cell undergo apoptosis independent of the fates of their neighbors (Joseph, 2006). Undesirable cells are eliminated without leakage of the intracellular contents to the surrounding medium and no immune response is therefore triggered. As a defense system, apoptosis exists in living organisms. Infected cells or cells with damaged DNA are removed by apoptosis (Roos and Kaina, 2006). Apoptosis is an important response to toxicity, but also a pivotal process in differentiation and organogenesis in multicellular organisms. Many chemical compounds such as retinoids (Lu *et al.*, 1997; Sun *et al.*, 2002; Arce *et al.*, 2005), ω -3 fatty acids (Thompson, 1995; Chang *et al.*, 1998; Hong *et al.*, 2000; Hong *et al.*, 2002), and polychlorinated biphenyls (PCBs) (Lee *et al.*, 2003; Sanchez-Alonso *et al.*, 2004) induce apoptosis in cells and are therefore known as apoptosis inducers. DNA repair, especially, the nucleotide excision repair (NER), is a major cellular response to damage induced by environmental carcinogens (Braithwaite *et al.*, 1998). Benzo(a)pyrene diol epoxide (BPDE), which is converted from BP by phase I enzyme, can irreversibly damage DNA by the formation of DNA adducts. NER plays an important role to remove such adducts and restore DNA to normal structure (Sturgis and Wei, 2002).

Chemoprevention is defined as the use of specific natural and/or synthetic chemical agents to reverse or suppress carcinogenesis and prevent the development of invasive cancer (Hong and Lippman, 1995). The chemopreventive approach depends on the ability of certain chemical agents to block mutagenesis and control cellular differentiation and proliferation in epithelial tissues (Hong and Lippman, 1995). Retinoids are a group of compounds that regulate cell growth and differentiation. Retinoids have been used as chemopreventive and anticancer agents because of their pleiotropic regulator function in cell differentiation, growth, proliferation, DNA repair and apoptosis (Noy,; Mrass *et al.*, 2004; Bogos *et al.*, 2008; Gottschling *et al.*, 2008). The effects of retinoids are thought to be mediated through the metabolite retinoic acid, which binds to nuclear retinoic acid receptors that then interact with specific retinoic acid response elements (Sankaranarayanan R, 1996). However, chronic toxicities from long term therapy with retinoids may result in some side-effects such as skeletal abnormalities and growth inhibition if used in children (David *et al.*, 1988).

HepG2 cells, a metabolically competent human hepatoma cells, retain activities of phase I and phase II enzymes that are involved in the bioactivation (toxication) and detoxication of

chemicals (Natarajan and Darroudi, 1991; Knasmuller *et al.*, 1998). BP is a pro-carcinogen that needs to be biotransformed by cytochrom P450 1A/1B1 and epoxide hydroxylase to benzo(a)pyrene diol epoxide (BPDE), which is capable of forming DNA adducts. Therefore, this cell line is suitable to study DNA adduct formation induced by BP (Zhou et al., 2007). Here we have tested the hypothesis that levels of carcinogen-DNA adducts induced by BP can be diminished by eliminating damaged cells via augmentation of apoptosis.

Materials and methods

Chemicals

Benzo(a)pyrene (BP) and retinoic acid were obtained from Sigma-Aldrich (St. Louis, MO).

Cell Culture and Treatments

HepG2, an adherent type human hepatoma cell line (Knasmuller *et al.*, 1998), was obtained from American Type Culture Collection (Manassas, VA, USA). HepG2 cells were maintained in Dulbecco's minimal essential medium (DMEM) supplemented with 10% fetal calf serum, vitamins, essential and non-essential amino acids, 100 units/ml penicillin, and 100 µg/ml streptomycin. The stock culture was grown at 37°C in an atmosphere of 5% CO₂ and 95% air under high humidity conditions. All chemicals were dissolved in DMSO. Cells were treated in duplicate or triplicate for all groups. Experiment 1: Cells incubated with 2 µM BP only or BP + 1 µM retinoic acid (RA) for 24 h. Then the medium was removed and the cells were washed twice with fresh medium. The cells were harvested by trypsinization and centrifugation (3,000 rpm for 5 min) and the pellets kept at -80°C until DNA extraction. Experiment 2: Cells were incubated with 4 µM BP and BP+RA for 24 h or 48 h. Experiment 3: HepG2 cells were challenged with 4 µM BP for 24 h. After which the medium containing BP was removed and washed twice. The cells were treated with RA for 24 h, and then harvested and processed as described above.

Protein Isolation and CYP1A1 Analysis

Ethoxyresorufin-O-deethylase (EROD), which predominantly reflects CYP1A1 activity was assayed in whole cell lysates essentially as described previously (Moorthy, 2000). Each experiment was run in triplicate.

Apoptosis Detection Assay

The cells were harvested and staining was carried out using the annexin V-FITC apoptosis detection kit (BioVision, Mountain View, CA). Briefly, 2×10^5 cells were resuspended in 1 × binding buffer and incubated with annexin V-FITC and propidium iodide (PI) for 5 min in darkness at room temperature. Annexin V FITC binding was analyzed by FACS Vantage SE DiVa flow cytometer (BD Biosciences, Franklin Lakes, NJ) equipped with FL1 detector (530/30 bandpass filter). Propidium iodide staining was detected on FL3 detector (610/20 bandpass filter). The results are based on a percentage of apoptotic cells of total gated cells (10^4 cells).

Western blot analysis

Cell lysates were obtained and equal amounts of protein from each sample were diluted with loading buffer, denatured, and separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by protein transfer to polyvinylidene fluoride membranes (Amersham, Little Chalfont, UK). Proteins were detected by incubation with corresponding antibodies followed by blotting with secondary antibody. The blots were then exposed to chemiluminescent substrate (Amersham) for detection. This analysis was performed three times.

DNA Adduct Analyses

DNA was isolated by solvent extraction combined with enzymatic digestion of protein and RNA (Gupta, 1984) and stored at -80°C until use. The nuclease P1-enhanced bisphosphate version of the ^{32}P -postlabeling assay was performed as described by Reddy (Reddy and Randerath, 1986) and Randerath (Randerath et al., 1999). Briefly, DNA (10 μg) was enzymatically degraded to normal (Np) and modified (Xp) deoxyribonucleoside 3'-monophosphates and their base-modified derivatives with a mixture of micrococcal endonuclease and spleen phosphodiesterase. After 3'-dephosphorylation of the normal nucleotides with nuclease P1, the enriched nuclease P1-resistant modified 3'-nucleotides were converted to 5'- ^{32}P -labeled deoxyribonucleoside 3',5'-bisphosphate derivatives by incubation with carrier-free (γ - ^{32}P)ATP and T4 polynucleotide kinase. The radioactively labeled modified nucleotides were separated by multidirectional anion-exchange thin-layer chromatography (TLC) on polyethyleneimine (PEI)-cellulose sheets (Mabon et al., 1996). After removal of orthophosphate and traces of radioactive impurities by one-dimensional development (D1) with solvent 1 (Table 1), bulky labeled DNA adducts retained in the lower (2.8×1.0 cm) part of D1 chromatogram. The cut-out containing adducts were excised from the D1 chromatogram, then were contact-transferred to fresh thin-layer sheets and resolved by two-dimensional TLC with solvents 2 and 3 as outlined in Table 1. Radioactive spots were located by imaging using an Instant Imager from Packard Instrument Co. (800 Research Parkway, Meriden, CT 06450) and by screen-enhanced autoradiography on Kodak XAR-5 X-ray film at -80°C for ~ 5 h. Radioactivities of TLC fractions from individual cell culture sample were determined with the aid of an Instant Imager (Zhou et al., 1999). Appropriate blank rates were automatically subtracted by the instrument from sample values. The extent of DNA adducts was calculated from corrected sample count rates (Reddy and Randerath, 1986). Quantitative data represented minimum estimates because 100% recovery presumably was not achieved. Statistical analysis of the data was performed by using the unpaired Student's *t*-test (Zar, 2009).

Results

HepG2 cells treated with BP or BP+RA exhibited qualitatively similar profiles of DNA adducts (Fig. 1). BP is a pro-carcinogen that is metabolically activated by CYP1A/1B1 enzymes and epoxide hydrolase to BPDE which binds to N^2 of guanosine, and represents the major DNA adduct, BPDE-deoxyguanosine (BPDE-dG) dduct (Jeffrey et al., 1977; Randerath et al., 1998) (Fig. 1). In addition, BP also induces the formation of other minor adducts (Fig. 1).

Treatment of HepG2 cells with BP (2 μM) for 24 h led to marked induction of DNA adducts, the levels of BPDE-dG and total DNA adducts (spots 1 to 4) being 516.63 ± 55.08 and 632.48 ± 69.77 adducts in 10^9 nucleotides, respectively (Fig. 2). Co-treatment of RA (1 μM) with BP (2 μM) significantly decreased BPDE-dG and total adducts after a 24 h incubation (Fig. 2). RAL values of BPDE-dG and total adducts in BP + RA group were 362.06 ± 49.00 and 427.70 ± 46.26 adducts in 10^9 nucleotides, respectively. Comparisons of levels of BPDE-dG or total adducts between BP only and BP+RA groups obtained P values 0.046 and 0.038, respectively. (Fig. 2).

Dose-response was observed when cells treated with 4 μM BP for 24 h (Fig. 3) compared to 2 μM BP group (Fig. 2). Total adduct levels of 4 μM BP group were 1042.29 ± 61.36 in 10^9 nucleotides (Fig. 3). Adduct levels in 4 μM BP group was significant higher than those in 2 μM BP group ($P=0.02$). Time-response results displayed that 24 h cell incubation with BP obtained higher yields of DNA adducts compared to 48 h incubation. After 48 h incubation, adduct levels (773.05 ± 37.64) were diminished by about 26% compared to the 24 h time point ($P=0.06$). At 48 h, RA (1 μM) also diminished the levels of DNA adducts by about

30%. The difference of total DNA adduct levels between BP only (773.05 ± 37.64) and BP + RA groups (555.38 ± 11.47) was very significant ($P=0.006$).

In order to study the mechanisms of modulation of BP-DNA adducts by RA, we carried out the following experiment. Since adduct levels were decreased with incubation time by about 30%, high dose of BP ($4 \mu\text{M}$) was selected in this experiment. Cells were treated with $4 \mu\text{M}$ BP for 24h. Then the medium with BP was removed and cells were washed twice with fresh medium. Then cells were treated with DMSO (control) or $1 \mu\text{M}$ RA for additional 24 h. Interestingly, the results showed that RA decreased carcinogen-DNA adduct levels by 28% through removal of damaged cells or DNA repair (Fig. 4). The levels of total DNA adducts in cells treated with BP (24 h), followed by DMSO (24 h), and treated with BP (24 h), followed by RA (24 h) were 543.70 ± 32.82 and 390.46 ± 34.19 in 10^9 nucleotides, respectively. P value was 0.032. These results indicated that RA mainly affected on the adducted nucleotides or damaged cells through inducing DNA repair and apoptosis, but not BP through detoxification.

Moreover, our results also showed that BP elicited significant induction of CYP1A1 enzyme activities after 24 h incubation (Table 2). However, co-treatment of the cells with BP and RA did not significantly alter the inducibility of CYP1A1 compared with BP treatment only. These results suggested that attenuation of BP-DNA adducts by RA was not primarily due to its effects on CYP1A1 expression. Expression of glutathione S-transferase Ya (GST Ya) was analyzed by Western blot for DMSO, BP, RA, and BP+RA groups. There was no induction of GST Ya activity among these groups.

To address the issues whether cell apoptosis and DNA repair play important roles in attenuation of levels of BP-DNA adducts, apoptosis detection by flow cytometer and gene expression of DNA repair were analyzed. The samples were obtained from the first experiment (BP, $2 \mu\text{M}$; RA, $1 \mu\text{M}$; 24 h incubation). The results showed that apoptosis activity existed spontaneously in HepG2 cells. However, RA enhanced apoptosis in HepG2 cells. The rates of apoptosis were 3.2%, 3.3%, 6.6%, and 9.1% respectively for DMSO, BP, RA, and BP+RA groups. When DNA were injured by adducts, apoptosis was extensively enhanced by RA, resulting in loss of damaged cells. Fig. 5 demonstrated that BP+RA group increased cell apoptosis compared to BP or RA only groups. Two typical DNA repair genes, XRCC1 and KU80, were determined by Western blotting. Our results showed that the expression of XRCC1 in HepG2 cells was not affected by RA at doses of 0.5, 1.0, and $2.0 \mu\text{M}$. However, the expression of KU80 was slightly enhanced by $1 \mu\text{M}$ RA after 24 h incubation compared to DMSO, BP, and RA groups (Fig. 6). Further enhancement of KU80 expression was observed at 48 h incubation (Fig. 6). These results indicate that DNA repair induced by RA may be also involved in the decrease of DNA adducts in HepG2 cells treated with BP.

Discussion

In this study we have tested the hypothesis that levels of carcinogen-DNA adducts will be diminished by eliminating damaged cells through apoptotic pathways or/and by DNA repair. DNA is the main target of environmental genotoxins such as PAHs. DNA adducts, induced by chemical carcinogens, are very sensitive biomarkers in chemical carcinogenesis. The formation of covalent DNA adducts is a key event in the initiation of neoplasia and the adducts also contribute to the progression of tumors (McCarty et al., 2009). BPDE-dG adducts can be eliminated by apoptosis and DNA repair. If the DNA adducts are repaired or adducted cells are removed, DNA and cells are returned to their normal state. But if the adducts persist during DNA replication, miscoding can occur, which leads to a permanent mutation in the DNA sequence (Hecht, 2003). Reduction in quantities of DNA adducts by

chemopreventive agents could significantly reduce tumor incidence (Tang *et al.*, 2001; Wang *et al.*, 2010). Our results demonstrate that DNA adducts are useful biomarkers in cancer prevention studies, especially during the initiation phase of carcinogenesis.

DNA repair system, especially NER which is the primary repair system for most bulky DNA adducts (Reardon and Sancar, 2005), plays an important role in the removal of mutated nucleotides including DNA adducts. KU80 protein, which is encoded by the XRCC5 gene, may play a role in attenuation of DNA adducts (Guggenheim *et al.*, 2009). Our results displayed that KU80 was enhanced by RA at 24 h. It got further enhanced at 48 h. These results indicated that DNA repair may contribute to the reduction of carcinogen DNA adducts in HepG2 cells. However, repair is inevitably less than perfect, and carcinogen-DNA adducts accumulate over time, especially during exposure to high concentrations of environmental carcinogens or chronic exposure to carcinogens such as cigarette smoking. Therefore, apoptosis (programmed cell death) may also play important roles as a subsequent defense tool in removing damaged cells with bulky DNA adducts or mutated DNA (Hecht, 2003).

In fact, the elimination of unwanted cells by apoptosis is an important developmental and homeostatic process in multicellular organisms (Ellis et al., 1991; Metzstein et al., 1998). Excessive DNA damage causes relatively insufficient amount of apoptosis that results in uncontrolled cell proliferation, which could lead to cancer. Induction of apoptosis by natural chemopreventive agents may increase elimination of more damaged cells. Our studies showed that RA enhanced cell apoptosis which may contribute to decrease of carcinogen-DNA adducts. Interestingly, bulky DNA adducts induced by BP after 24 h incubation in HepG2 cells were further diminished by incubation with RA only for additional 24 h incubation (Fig. 4), suggesting that RA eliminated DNA adducts through apoptosis or/and DNA repair pathways, but not through detoxification of BP. Selection of chemopreventive agents, time and dose regimens may be very important for the modulation of apoptosis. In this study, we selected RA, as an apoptosis inducer, to determine if it could diminish BP-DNA adducts *in vitro* in a human cell line.

The formation of DNA adducts is considered as a crucial step in the initiation phase of carcinogenesis (Lippman and Hawk, 2009). Recently, our laboratory observed a significant positive linear regression between levels of DNA adducts and tumor incidence in animal experiments (data not shown). Animals treated with different concentrations of reconstituted mixtures of PAHs (mimic actual concentrations of environmental PAHs). Levels of DNA adducts detected at 1, 3, and 7 days after treatment were significantly correlated with tumor incidence examined pathologically at 10 months. These results suggest if the formation of DNA adducts are suppressed in the phase of tumor initiation, the risk of tumors may be significantly diminished.

Chemopreventive agents decrease carcinogen-DNA adducts through several pathways: 1) inactivation of chemical carcinogens by detoxification enzymes, such as upregulated phase I and II enzymes, for example, CYP1A1 (Uno *et al.*, 2004; Uno *et al.*, 2006) and glutathione transferases; 2) enhancement of DNA repair systems; and 3) induction of cell apoptosis. Support for the chemopreventive approach is based on the biologic concepts of field cancerization and multistep carcinogenesis (Hong and Lippman, 1995). Currently, most of chemopreventive measurements (Dennis et al., 2009; Lippman and Hawk, 2009; Tsao et al., 2009) focus on reversing premalignant lesions and preventing second primary tumors, or slowing tumorigenesis.

In other words, these approaches affect cells in the last phase of carcinogenesis --- Progression. If the chemopreventive measurements can start from earlier phases of

carcinogenesis such as initiation and promotion for high risk populations such as smokers and others exposed to high concentrations of environmental or occupational carcinogens, significant consequence of cancer prevention may be observed in long term.

Recent years, natural chemopreventive agents have received great attention for cancer prevention because of their various health benefits, lack of toxicity and less side effects (Manson et al., 2005). Natural agents and their derivatives such as vitamin A, selenium, green tea, fish oil, curcumin, resveratrol, deguelin, myoinositol, aspirin, and probiotics have potential benefits in chemoprevention (Hong *et al.*, 2000; Dennis *et al.*, 2009; Lippman and Hawk, 2009). In our current studies, RA, a natural chemopreventive agent, was used as an apoptosis inducer. The results displayed that significantly reduced BP-DNA adducts in HepG2 cells after adducts formed/cells damaged (Fig. 4).

As previously reported (Couroucli et al., 2006), RA can modulate CYP1A1 activity in animals. However, our results displayed that RA did not induce CYP1A1 activity in HepG2 cells by itself (Table 2). Co-treatment of the cells with BP and RA did not significantly alter the inducibility of CYP1A1 compared to BP only group (Table 2), suggesting that attenuation of BP-DNA adducts by RA was not primarily due to its effects on CYP1A expression.

In conclusion, our results showed that RA significantly diminished levels of bulky DNA adducts in HepG2 cells induced by BP. Cell apoptosis and DNA repair were probably involved to remove damaged cells and adducted nucleotides. Future animal studies should help our understanding the roles of chemopreventive agents in cancer prevention through carcinogen detoxication, cell apoptosis and DNA repair.

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Fig. 1. Typical pattern of ^{32}P -postlabeled benzo(a)pyrene DNA adducts with a major adduct benzo(a)pyrene-7,8-dihydrodiol-9,10-epoxide deoxy-guanosine (BPDE-dG) in HepG2 cells. Cells were treated with BP ($2\ \mu\text{M}$) for 24 hours. Screen-enhanced film exposure was performed with Kodak XAR-5 film at room temperature for 5 hours.

Fig. 2.

Comparison of the levels of BPDE-dG and total DNA adducts in HepG2 cells. Cells were treated with BP (2 μM) or BP + RA (1 μM). Cells were harvested 24 h after treatment. RA decreased the levels of BPDE-dG and total adducts significantly, * P=0.046; ** P=0.038. RAL, Relative Adduct Labeling (adducted nucleotides/total nucleotides).

Fig. 3.

Levels of BPDE-dG and total DNA adducts in HepG2 cells treated with 4 μM BP or 4 μM BP+1 μM RA for 24 h or 48 h. Compared to 24 h, total adduct levels in 48 h incubation were lower (* $p=0.06$). However, after 48 h incubation, BP+RA significantly decreased levels of total adducts compared to BP only (** $P=0.006$).

Fig. 4.

Effect of RA treatment on BPDE adduct levels in HepG2 cells. Levels of BPDE-dG and total DNA adducts in cells treated with $4 \mu\text{M}$ BP for 24 h then incubated another 24 h with DMSO were significantly lower compared to 48 h incubated with $4 \mu\text{M}$ BP ($*P=0.02$). After 24 h incubation with $4 \mu\text{M}$ BaP, the fresh medium was replaced with medium containing $1 \mu\text{M}$ RA for another 24 h. Total adduct levels were diminished significantly by RA compared to the medium with DMSO in second 24 h incubation ($** p=0.032$).

Fig. 5. Detection of apoptosis in HepG2 cells by flow cytometry. The apoptosis was determined by Annexin V Apoptosis Detection Kit.

Fig. 6.

Proteins isolated from HepG2 cells in different groups were probed with KU80 antibody by Western blot. **A**, DMSO; **B**, RA (1 μ M); **C**, BP (2 μ M); **D**, BP (2 μ M) + RA (0.5 μ M); **E**, BP (2 μ M) + RA (1.0 μ M); **F**, BP (2 μ M) + RA (2.0 μ M). Groups from A to F were incubated for 24 h. **G**, BP (2 μ M) + RA (1.0 μ M), incubated 48 h; **H**, BP (2 μ M) + RA (1.0 μ M) for 24 h, then RA (1.0 μ M) only for additional 24 h.

Fig. 7.

Proteins isolated from HepG2 cells in different groups were probed with KU80 antibody by Western blot. Samples **A** to **F** were incubated for 24 h. Sample **G**, BP (2 μM) + RA (1.0 μM), incubated for 48 h; **H**, BP (2 μM) + RA (1.0 μM) for 24 h, then incubated with RA (1.0 μM) only for additional 24 h.

Table 1

Solvents for thin-layer chromatography

Solvent	Composition
1	2.3 M sodium phosphate, pH 5.75
2	3.82 M lithium formate, 6.75 M urea, pH 3.35
3	0.72 M sodium phosphate, 0.40 M Tris-HCl, 7.65 M urea, pH 8.2

Table 2

Effect of BP and RA on EROD (CYP 1A1) activities

Group	EROD Activities (pmol/mg/min)			P		
	n	Mean	SD	DMSO	RA	BP
DMSO	3	4.08	0.26	-	-	-
RA (1 μ M)	3	3.71	0.49	0.3178	-	-
BP (2 μ M)	3	8.65	1.97	0.0161	-	-
BP + RA	3	8.49	0.43	0.0001	0.0002	0.8950