In vitro Study on Role of Hsp70 Expression in DNA Damage of Human Embryonic Lung Cells Exposed to Benzo[a]pyrene[1]

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Objective Benzo[a]pyrene (B[a]P), a ubiquitous environmental pollutant, is a potent procarcinogen and mutagen that can elicit tumors, leading to malignancy. Heat shock proteins (Hsp) have been shown to protect cells against damages caused by various stresses including exposure to numerous chemicals. Whether Hsps, or more specifically Hsp70, are involved in repair of B[a]P-induced DNA damage is currently unknown. **Methods** We assessed the potential role of the inducible form of Hsp70 in B[a]P-induced DNA damage of human embryonic lung (HEL) cells using immunoblot and the comet assay (i.e., the single cell gel electrophoresis assay). **Results** Exposure to B[a]P induced a dose-dependent decrease in the level of Hsp70, but a dose-dependent increase in DNA damage both in untreated (control) HEL cells and in cells preconditioned by a heat treatment. Heat preconditioning prior to B[a]P exposure potentiated the effect of B[a]P at a low dose (10 μmol/L), but appeared to be protective at higher doses. There was a negative correlation between Hsp70 level and DNA damage in the non-preheated as well as in the preconditioned cells. **Conclusion** These data suggest that exposure of HEL cells to B[a]P may induce a dose-dependent reduction in the levels of the inducible Hsp70. The detailed mechanisms for the reduction of Hsp70 levels by B[a]P and the role of Hsp70 in DNA damage under different concentrations of B[a]P remains to be determined.

Key words: Hsp70; Benzo[a]pyrene; Heat; DNA damage

INTRODUCTION

Heat shock or stress proteins (Hsps) are highly conserved proteins, which are induced in cells upon exposure to supraoptimal temperatures or to many other forms of stresses such as exposure to heavy metals, oxidizing agents, and organic solvents, which are common in working and living environments. Many of these stresses cause an accumulation of unfolded and/or denatured proteins resulting in activation of the heat shock transcription factor (HSF)

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and synthesis of Hsps[1]. Hsps are classified into different families, including Hsp90-110, Hsp70/Hsc70, Hsp60, and small Hsps (Hsp10-30) on the basis of their apparent molecular masses in sodium dodecyl sulfate polyacrylamide gels. The most widely studied family of Hsps includes both stress-inducible (Hsp70) and constitutively expressed proteins (Hsc70: heat shock cognate). Members of the Hsp70 family have been shown to have the ability to act as molecular chaperones, protecting nascent proteins from aggregation and facilitating the synthesis, folding, assembly and, intracellular transport of many proteins. They have also been reported to be involved in the development of tolerance to many stresses such as heat and toxins[2]. In addition, some Hsps have been suggested to play important roles in the processes of growth, differentiation and development[3].

B[a]P is the prototype of a multitude of polycyclic aromatic hydrocarbons (PAHs), which are thought to be procarcinogens and toxicants generated from the combustion of fossil fuels and cigarette smoke[4]. Although B[a]P itself is not genotoxic, its biological effects can be mediated by binding to the aryl hydrocarbon receptor (AhR), and B[a]P can induce the expression of more than a dozen genes, including cytochrome p450[5]. Cytochrome p450 can oxidize B[a]P to the ultimate electrophilic metabolite BPDE[(±)-anti-7α, 8β-dihydroxy-9α,10α-epoxy-7, 8, 9, 10-tetrahydrobenzo(a)pyrene], which can irreversibly damage nucleic acids and proteins by covalent binding or oxidation of molecular targets within cells[6]. Epidemiological data have also shown an association between B[a]P exposure and an increased incidence of certain cancers in humans. B[a]P and its reactive metabolites have therefore been widely investigated and are considered to be prototype compounds that exhibit many of the cytotoxic, mutagenic and carcinogenic effects of PAHs.

An understanding of the roles of Hsps in the pathogenesis of cancer is a topic of great interest. Recently, Hsps have been shown to contribute to the stability of several proteins like tumor suppressor genes (i.e., p53 and Rb)[7,8] and to participate in the development of resistance to various cytotoxic drugs[9]. Hsps have also been reported to play important roles in the promotion and suppression (apoptosis) or differentiation of cancer cells[10]. Hsp70 is thought to be involved in chaperoning the *c-myc* oncogene and p53 tumor-suppressor gene products so that its aberrant expression could affect tumor cell transformation and progression[11].

Whether Hsp70 expression has any role in primary DNA damage induced by B[a]P or in its prevention is unknown. In the present study we therefore examined Hsp70 levels, DNA damage and the effects of Hsp pre-induction on DNA damage induced by B[a]P in human embryonic lung cells (HEL) using immunoblot and the comet assay *in vitro*.

MATERIALS AND METHODS

General Chemicals

Benzo(a)pyrene (B[a]P) was obtained from Sigma Chemical Co (St. Louis, MO, USA) and dissolved in dimethyl sulfoxide (DMSO) in a 1000×stock solution. The final concentration of DMSO in the incubation mixture was 0.1%. Low melting agarose was obtained from Gibco(Life Technologies, Breda, Netherlands). Na_2EDTA (disodium ethylenediamine-tetra-acetate), Tris [tris (hydroxymethyl sulfoxide) aminomethane hydrochloride], EB (ethidium bromide) and Triton X-100 were from Sigma Chemical Co (St.Louis, MO, USA).

Mammalian Liver S9 Mixture Preparation

Adult male Sprague-Daley rats weighing ~200 g were injected with Aroclor 1254 (500 mg/kg) 5 days before sacrifice. Postmitochondrial supernatants from homogenized rat livers (S9) were prepared as described by Maron and Ames[12] and stored at -80℃. The protein concentration of S9 mixtures was determined according to Lowry *et al*.[13]. The S9 mix was added to 10 mmol/L glucose-6-phosphate and 5 mmol/L NADP. The final ratio of S9 mix in the incubation mixture was about 3%[14].

Cell Culture and Treatments

HEL cells (China Type Culture Collection, Wuhan, China) were cultivated in Dulbecco's modified Eagle's Medium (high glucose; Life Technologies, Breda, Netherlands) supplemented with 10% v/v heat-inactivated fetal calf serum (Gibco; Life Technologies, Breda, Netherlands), 2 mmol/L L-glutamine and antibiotic supplement (penicillin 100 U/mL and streptomycin 0.1 mg/mL) at 37℃ in a humidified atmosphere containing 5% carbon dioxide at pH 7.2. The cells were replated every 3-4 days to prevent confluence. Exponentially growing cells were divided into two groups: control unheated cells and a group of cells preconditioned by heat treatment (41℃, 1 h followed by 2 h recovery at 37℃ and refered to as preheated group). The cells were synchronized into the G_0 state by density inhibition and mitogen deprivation. In both groups, HEL cells were treated with 0.1% DMSO alone as control or with B[a]P in DMSO at 10, 50, 100, 200 μmol/L for 3 h in the presence of rat-S9 mixture.

Determination of Hsp70 in HEL Cells

Treated cells were collected, washed with ice-cold PBS and total cell proteins were mixed with 2×SDS sample buffer, boiled at 94˚C for 10 min, separated on sodium dodecyl sulfate (SDS)-polyacrylamide gel and transferred electrophoretically to nitrocellulose membranes (NC) as described previously[15,16]. Protein transfer was monitored by staining with Ponceau Rouge. Membranes were saturated with blocking buffer (PBS containing 5% skim milk powder) for 1 h at 37˚C with gentle agitation and washed with PBS-0.05% Tween 80 for 5 min. The membrane was then incubated at 37˚C for 1 h with gentle agitation with a rabbit anti-human Hsp70 antibody diluted 1:1000 in the PBS blocking buffer[3]. After washing the membranes six times (10 min each) with 200 mL PBS-0.05% Tween 80, HRP labelled goat anti-rabbit IgG in blocking buffer (1:1000) was added and the membranes were incubated at 37˚C for another 1 h. The membranes were washed four times with 200 mL PBS-0.05% Tween 80. The presence of Hsp70 was revealed using DAB (3,3-diaminobenzidine tetrahydrochloride) buffer for 3-5 min as described previously[16]. Hsp70 was quantified using an imaging densitometer (CS-90, 460 nm Japan), and the value of the control non-preheated cell group without B[a]P was standardized to 1. All results in this study were average values of three experiments.

Detection of DNA Damage

Complete details of the single cell gel electrophoretic assay (also known as Comet assay) were previously described[17]. Cell viability was assessed by trypan blue exclusion and was found to be >90% in all groups. The cells cultured in this study did not exceed the 15th passage. The cells were kept at 4℃ before being loaded on the gel. DNA was allowed to unwind and alkaline-labile-damage expression was allowed for 25 min in the electrophoresis

running buffer solution (300 mmol/L NaOH and 1 mmol/L Na_2EDTA, pH>13.0). All steps were conducted under red light to prevent additional DNA damage. Electrophoresis was performed for 30 minutes at 25 V and 300 mA. The slides were neutralized by rinsing 3 times with Tris buffer (0.4 mol/L Tris, pH7.5 with HCl) for 5 min and 75 μL of ethidium bromide solution (20 μg/L) was added to each slide. After stained with a coverslip for 5 min, slides were rinsed in distilled water and covered again for microscopic examination.

Image analysis was performed with 200× magnification using a fluorescence microscope (Olympus B-60F5) equipped with an excitation filter of 549 nm and a 590 nm barrier filter, coupled to a CCD camera (Kodak, USA). The length of DNA migration was determined with an optical micrometer. At least 25 cells per group from a field of vision randomly chosen were scored. In this test, DNA damage of the cells was evaluated using the ratio of DNA tail length/total cell length[18,19].

Statistical Analysis

Analysis was performed using STATA (3.1) software package. A difference at $P<0.05$ was considered statistically significant.

RESULTS

Expression of Hsp70 in HEL Cells Exposed to Different Concentrations of B[a]P

The level of Hsp70 in HEL cells exposed to B[a]P was determined by Western blot using an antibody specific for the inducible member of this family. The changes of Hsp70 levels induced by B[a]P with or without a preheat treatment are shown in Fig. 1. Exposure of cells to increasing B[a]P concentrations for 3 h in the presence of the S9-mixture resulted in a decrease of the amount of Hsp70 in the non-preheated cells (Fig. 1A). The decrease in Hsp70 was particularly significant (~40%) in the cells exposed to 200 μmol/L B[a]P when compared with cells not exposed to B[a]P. In preheated cells, the basal level of Hsp70 was significantly higher (3.5B×) as expected. There was a very minor decreasing trend in Hsp70 levels (2%-3%) observed upon increasing concentrations of B[a]P (Figs.1B and 1C).

DNA Damage in HEL Cells Induced by B[a]P

Treatment of HEL cells with B[a]P for 3 h led to a dose-dependent increase in DNA damage as revealed by the comet assay. This trend was observed both in the non-preheated cells and in the preheated ones (Table 1 and Fig. 2). Comparison between the two cell groups indicated that there was generally less DNA damage in the preheated cells with the exception of the group of cells exposed to 200 μmol/L B[a]P in which case there was a significant increase of DNA damage in preheated cells as compared to the untreated group. At the maximum dose of B[a]P (200 μmol/L) there was significantly less DNA damage in preheated cells when compared with the corresponding group of non-preheated cells ($P<0.05$).

Association of Hsp70 Expression With DNA Damage

Since the relation between Hsp70 expression and DNA damage caused by exposure of HEL cells to B[a]P under different conditions of treatment seemed to be very complex, we further analyzed the possible association of Hsp70 expression levels with DNA damage by statistical methods in preheated and non-preheated cells. The non-significant association

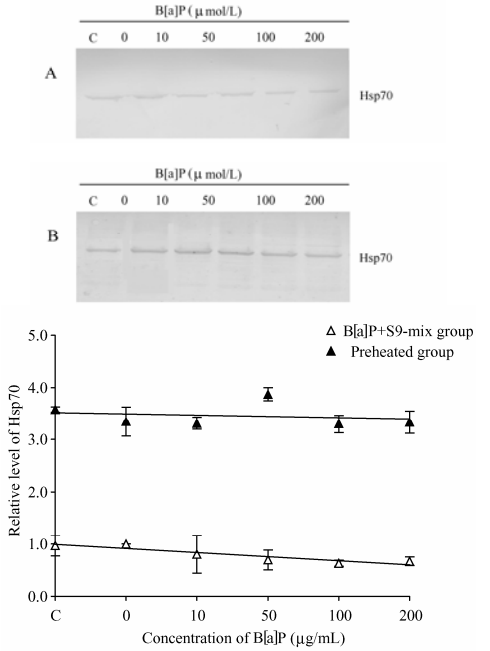

FIG. 1. Detection of Hsp70 in HEL cells by Western blot. In non-preheated groups, HEL cells were treated with DMSO only as control and other cell groups were treated with B[a]P at 0, 10, 50, 100, 200 μmol/L for 3 h in the presence of rat-S9 mixture respectively (Fig. 1A). In preheated groups, all cells were preheated at 41℃ for 1 h, recovered at 37℃ for 2 h, then treated with B[a]P under the same conditions as the non-preheated groups (Fig. 1B). Graphic representation of the densitometric scans from immunostaining of Hsp70 in non-preheated and preheated groups of cells (Fig. 1C). The protein levels of cells without exposure to B[a]P were standardized to 1 and the data in Fig. 1C are mean of these experiments for three times. N: non-preheated cells, C: control cells without exposure to B[a]P. **P*<0.05, compared with cells without exposure to B[a]P.

TABLE 1

Effect of B[a]P on DNA Tail Length/ Total Length Ratio in HEL Cells (*n*=25)

B[a]P (μmol/L)	Tail Length/Total Length	
	Non-preheated Group	Preheated Group
0	0.000±0.000	0.000±0.000
10	0.005±0.005	0.137±0.052[a,b]
50	0.622±0.064[a]	0.385±0.071[a]
100	0.428±0.064[a]	0.406±0.068[a]
200	0.733±0.038[a]	0.341±0.061[b]

Note. [a]*P*<0.05, vs control. [b]*P*<0.05, vs non-preheated groups.

was y=-1.5541X+1.5144 (P>0.05, r=-0.799) in the non-preheated groups and y=-0.2975X +0.5336 (P>0.05, r=-0.161) in the preheated groups. This indicated that the DNA damage was milder in both groups as the Hsp70 expression level increased. Moreover the same amount of Hsp70 in non-preheated cells seemed to contribute more protection against DNA damage than it did after a preheating treatment, although a high level of Hsp70 induced by heat could protect DNA from damage induced by B[a]P.

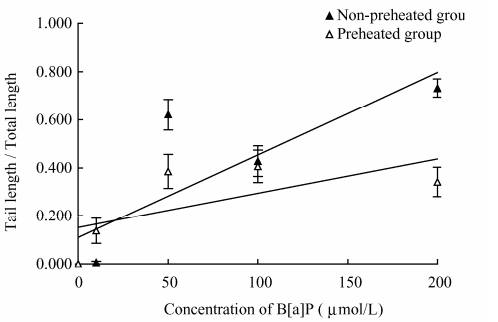

FIG. 2. Analysis of DNA migration in HEL cells treated with B[a]P.

DISCUSSION

Heat stress or heat shock proteins (Hsps) are highly conserved proteins induced by heat and a large variety of noxious stimuli including abnormal physiological stresses such as ischemia, fever, viral infection, and environmental xenobiotics or chemical stressors such as heavy metals, free radicals, carbon monoxide[1,18,19]. Many of these stimuli are common in working or living environments. Hsps have been shown to function as molecular chaperones, facilitating the synthesis, folding, assembly and intracellular transport of proteins and have also been reported to protect cells and organs from different stresses. B[a]P, a ubiquitous environmental pollutant, is a potent procarcinogen and mutagen that elicit tumors in normal cells and malignancy in experimental animals. Epidemiological data have also shown an association between B[a]P exposure and increased risk of certain cancers in humans. However, the detailed mechanisms of B[a]P-induced carcinogenesis remain unknown.

The present data showed that exposure of HEL cells to B[a]P induced a dose-dependent reduction in the level of inducible Hsp70. This reduction was particularly important in non-preheated cells. Cells subjected to a prior heat treatment only showed a slight decrease of Hsp70 upon B[a]P treatment. Our results are consistent with studies in other cell types where B[a]P was unable to induce Hsp70 expression or activate the heat shock promoter[20]. They are also consistent with a recent report of Vayssier-Taussat on the cellular effects of tobacco smoke: while tobacco smoke filtrates with B[a]P induced Hsp70, exposure to B[a]P alone did not evoke a stress response but rather induced cell death by necrosis[21]. In HEL cells we did not see any evidence of cytotoxicity under our experimental conditions. So it is unlikely that the reduction of Hsp70 was due to necrosis. Interestingly B[a]P has also been reported to show very low cytotoxicity in HeLa cells[20].

To our knowledge this study is the first one to report that B[a]P could reduce the cellular level of Hsp70 in non-preheated cells. Hsp70 synthesis is under the control of the

HSF-1 transcription factor binding to the HSE of the heat shock protein promoter. Hsp70 has been reported to act as a molecular chaperone for HSF-1, behaving as a negative regulator of HSF-1 transcriptional activity[22]. An inhibition of transcription of Hsp genes by B[a]P was suggested by the results of Bartosiewicz *et al*. using DNA arrays containing 148 genes coding for xenobiotic metabolizing enzymes, DNA repair enzymes, heat shock proteins (including Hsp105, Hsp86 and Hsp25), cytokines, and housekeeping genes to examine gene expression patterns in the liver in response to cadmium chloride, B[a]P and trichloroethylene (TCE)[23]. B[a]P is converted to electrophilic metabolites such as BPDE, which is known to covalently bind to nucleophilic sites on various cellular macromolecules. Cell damage resulting from BPDE is generally thought to result from the formation of adducts with DNA[24]. Previous studies have shown that B[a]P affects cellular signal transduction pathways and induces G_1 cell cycle arrest in NIH 3T3 fibroblasts[25], which could also induce apoptosis in hepatoma cell lines[26].

B[a]P and its metabolites could induce DNA damage as reported by many investigations including the present data using the comet assay[14,24,27,28]. However, our results also showed that a heat shock pretreatment of cells could potentiate the effect of B[a]P at low dose (10 μmol/L) while being protective at higher doses. Heat shock pretreatment not only induces Hsp70 synthesis but all the other Hsps and can also affect cell apoptosis and damage. This may result in differences in the combined effects of temperature and different concentrations of B[a]P on DNA damage.

The association between Hsp70 levels and DNA damage was also analyzed in preheated and in non-preheated cells in order to examine the potential role of Hsp70 in B[a]P-induced DNA damage. There were negative correlations between the level of Hsp70 expression and DNA damage under both types of treatments. However, our results also suggested that the same amount of Hsp70 in non-preheated cells contributed more protection against DNA damage than it did after a preheat treatment, although higher levels of Hsp70 induced by heat could protect DNA from damage of B[a]P. Vayssier *et al*. reported that tobacco smoke- or heat shock-induced Hsp70 had no protective effects either on apoptosis or necrosis, but that Hsp70 over-expression prevented tobacco smoke-induced necrosis and consequently led to increased apoptosis[29].

Expression of Hsps is a conserved, adaptive response to numerous stresses and Hsps, in particular inducible Hsp70, acting as molecular chaperones and contributing to the folding of polypeptides and to protein transport and degradation. However, contradictory data about Hsp functions are emerging. An interesting alternative model in which Hsps act as a danger signal in stressed cells modulating the immune response has been recently presented by Moseley[30]. It will be very important to further investigate the detailed mechanisms responsible for the reduction of Hsp70 levels by B[a]P and the role of Hsp70 in DNA damage and carcinogenesis in cells exposed to different concentrations of B[a]P.

REFERENCES

1. Lindquist, S. and Craig, E. A. (1988). The heat shock Proteins. *Ann. Rev. Genet.* **22**, 631-677.
2. Hightower, L. E. (1991). Heat shock, stress protein, chaperones, and proteotoxicity. *Cell* **66**, 191-197.
3. Tanguay, R. M., Wu, Y., and Khandjian, E. W. (1993). Tissue-specific expression of heat shock stress proteins of the mouse in the absence of stress. *Dev. Genet.* **14**, 112-118.
4. IARC, IARC Monographs on the evaluation of carcinogenic risks to human. Overall evaluations of carcinogenicity: an updating of the IARC Monographs Volumes 1 to 42, Suppl7. Lyon, France: International Agency for Research on Cancer, 1987.
5. Conney, A. H. (1982). Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G. H. A. Clowes; Menorial Lecture. *Cancer Res.* **42**, 4875-4917.

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6. Buening, M. K., Wislocki, P. G., Levin, W., Yagi, H., Thakker, D. R., Akagi, H., Koreeda, M., Jerina, D. M., and Conney, A. H. (1978). Tumorigenicity of the optical enantiomers of the diasteriomeric benzo(a)pyrene 7,8-diol-9,10-epoxides in newborne mice: exceptional activity of (+) -7beta,8alpha-dihydroxy-9alpha, 10alpha-epoxy-7, 8, 9, 10- tetrahydrobenzo (a)pyrene. *Proc. Natl. Acad. Sci. USA* **75**, 5358-5361.
7. King, F. W., Wawrzynow, A., Hohfeld, J., and Zylicz, M. (2001). Co-chaperones Bag-1, Hop and Hsp40 regulate Hsc70 and Hsp90 interactions with wild-type or mutant p53. *E. M. B. O. J.* **20**, 6297-6305.
8. Sepehrnia, B., Paz, I. B., Dasgupta, G., and Momand, J. (1996). Heat shock protein 84 forms a complex with mutant p53 protein predominantly within a cytoplasmic compartment of the cell. *J. Biol. Chem.* **271**, 15084-15090.
9. Vargas-Roig, L. M., Gago, F. E., Tello, O., Aznar, J. C., and Ciocca, D. R. (1998). Heat shock protein expression and drug resistance in breast cancer patients treated with induction chemotherapy. *Intern. J. Cancer* **79**, 468-475.
10. Mehlen, P., Schulze-Osthoff, K., and Arrigo, A. P. (1996). Small stress proteins as novel regulators of apoptosis. Heat shock protein 27 blocks Fas/APO-1- and staurosporine-induced cell death. *J. Biol. Chem.* **271**, 16510-16514.
11. Pinhasi-Kimhi, O., Michalovitz, D., Ben-Zeev, A., and Oren, M. (1986). Specific interactions between the p53 cellular tumour antigen and major heat shock proteins. *Nature* **320**, 182-185.
12. Maron, D. and Ames, B. N. (1983). Revised method for the *Salmonella* mutagenicity test. *Mutat. Res.* **113**, 173-215.
13. Lowry, O. H, Rosebrought, N. J., Farr, A. L., and Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**, 265-275.
14. Hanelt, S., Helbig, R., Hartmann, A., Lang, M., Seidel, A., and Speit, G. (1997). A comparative investigation of DNA adducts, DNA strand breaks and gene mutations induced by benzo(a)pyrene and (±)-anti-benzo[a]pyrene -7,8 -diol 9,10-oxide in cultured human cells. *Mutat. Res.* **390**, 179-188.
15. Wu, T., Yuan, Y., Wu, Y., He, H., Zhang, G., and Tanguay, R. M. (1998). Presence of antibodies to heat stress proteins in workers exposed to benzene and in patients with benzene poisoning. *Cell Stress Chaperones* **3**, 161-167.
16. Wu, T., Ma, J., Chen, S., Sun, Y., Xiao, C., Gao, Y., Wang, R., Poudrier, J., Dargis, M., Currie, R. W., and Tanguay, R. M. (2001). Association of plasma antibodies against heat stress protein Hsp70 with hypertension and harsh working conditions. *Cell Stress Chaperones* **6**, 394-401.
17. Singh, N. P., McCoy, M. T, and Tice, R. R. (1988). A simple technique for quantification of low levels of DNA damage in individual cells. *Exp. Cell Res.* **175**, 184-191.
18. Wu, T., Tanguay, R. M., Wu, Y., He, H., Xu, D., Feng, J., Shi, W., and Zhang, G. (1996). Presence of antibodies to heat stress proteins and its possible significance in workers exposed to high temperature and carbon monoxide. *Biomed. Environ. Sci*. **9**, 370-379.
19. Xiao, C., Chen, S., Li, J., Hai, T., Lu, Q., Sun, E., Wang, R., Tanguay, R. M., and Wu, T. (2002). Association of HSP70 and genotoxic damage in lymphocytes of workers exposed to coke-oven emission. *Cell Stress & Chaperones* **7**, 396-402.
20. Aït-Aïssa, S., Porcher, J. M., Arrigo, A. P., and Lambré, C. (2000). Activation of the *hsp70* promotor by environmental inorganic and organic chemicals: relationships with cytotoxicity and lipophilicity. *Toxicology* **145**, 147-157.
21. Vayssier-Taussat, M., Camilli, T., Aron, Y., Meplan, C., Hainaut, P., Polla, B. S., and Weksler, B. (2001). Effects of tobacco smoke and benzo[a]pyrene on human endothelial cell and monocyte stress responses. *Am. J. Physiol. Heart Circ. Physiol.* **280**, H1293-1300.
22. Shi, Y., Mosser, D. D., and Morimoto, R. I. (1998). Molecular chaperones as HSF1-specific transcriptional repressors. *Gene & Dev.* **12**, 654-666.
23. Bartosiewicz, M., Peen, S., and Buckpitt, A. (2001). Application of gene arrays in environmental toxicology: Fingerprints of gene regulation associated with cadmium chloride, benzo[a]pyrene, and trichloroethylene. *Environ. Health Perspect* **109**, 71-74.
24. Beland, F. A. and Poirier, M. C. (1994). DNA adducts and their consequences, in: Tardiff RG, Lohmann PHM and Wogan GN (Eds), *Methods to Assess DNA Damage and Repair: Interspecies Comparisons*, John Wiley and Sons, Chichester, pp. 29-55.
25. Vaziri, C. and Faller, D. V. (1997). A benzo(a)pyrene-induced cell cycle checkpoint resulting in p53-independent G1 arrest in 3T3 fibroblasts. *J. Biol. Chem.* **272**, 2762-2769.
26. Wei, L., Yu, R., Mandlekar, S., and Kong, T. (1998). Induction of apoptosis and activation of interleukin 1β -converting enzyme/ced-3 protease (caspase-3) and c-Jun NH_2-terminal kinase 1 by benzo(a)pyrene. *Cancer Res.* **58**, 2102-2106.
27. Drouin, E. E., Lech, J., and Loechler, E. L. (1995). The major N2-Gua adduct of the (+) –anti-benzo(a)pyrene diol epoxide can be unstable in double-stranded DNA. *Biochem.* **34**, 2251-2259.
28. Speit, G., Hanelt, S., Helbig, R., Seidel, A., and Hartmann, A. (1996). Detection of DNA effects in human cells with the comet assay and their relevance for mutagenesis. *Toxicol. Lett.* **88**, 91-98.
29. Vayssier, M., Banzet, N., François, D., Bellman, K., and Polla, B. S. (1998). Tabacco smoke induces both apoptosis and necrosis in mammalian cells: differential effects of HSP70. *Am. J. Physiol. Lung Cell Mol.*

Physiol. **275**, L771-L779.
30.Moseley, P. L. (2000). Exercise, stress, and the immune conversation. *Exerc. Sport Sci. Rev.* **28**, 128-132.

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