Cell Stress & Chaperones (1998) **3** (3), 161–167

Presence of antibodies to heat stress proteins in workers exposed to benzene and in patients with benzene poisoning

Tangchun Wu[1,2], Ye Yuan[1], Yang Wu[1,2], Hanzhen He[1], Guogao Zhang[1] and Robert M. Tanguay[2]

[1]Institute of Occupational Medicine, Tongji Medical University, Wuhan 430030, China
[2]CHUL Research Center (CHUQ) and Laboratory of Cell and Developmental Genetics, Pav. C.E. Marchand, Université Laval, Quebec, Canada G1K 7P4

Abstract Heat shock or stress proteins (Hsps) are a group of proteins induced by a large number of xenobiotics, many of which are common in the working and living environment. The biological significance of the presence of antibodies against Hsps in humans is presently unknown. In the present study, 112 workers were selected and divided into four groups on the basis of their level of occupational exposure to benzene: a control group, two groups of workers exposed to either low (< 300 mg/m^3) or high concentrations of benzene (> 300 mg/m^3) and a group of workers who had experienced benzene poisoning. Blood samples from these workers were assayed for the number of peripheral white blood cells, concentration of hemoglobin, activities of serum superoxide dismutase (SOD), lymphocyte DNA damage and finally for the presence of antibodies to different human heat-shock proteins (Hsp27, Hsp60, Hsp71 and Hsp90). Benzene-poisoned workers showed a high incidence of antibodies against Hsp71 (~ 40%) which was associated with a decrease in white blood cells ($3.84 \pm 1.13 \times 10^9$ versus $7.68 \pm 1.84 \times 10^9$ in controls) and with an increase in activities of serum SOD (138.43 ± 23.15 μ/ml) and lymphocyte DNA damage (18.7%). These data suggest that antibodies against Hsps can potentially be useful biomonitors to assess if workers are experiencing or have experienced abnormal xenobiotic-induced stress within their living and working environment.

INTRODUCTION

All organisms, plants and animals, react to exposure to supra-optimal temperatures by inducing the synthesis of a group of proteins known as heat shock or stress proteins (Hsps). This cellular response to heat is highly conserved and has been observed in virtually every organism in which it has been investigated. Synthesis of Hsps is not only induced by heat but also by a variety of noxious stimuli including abnormal physiological stresses such as ischemia, fever, viral infection, low pH and environmental xenobiotics or chemical stressors such as heavy metals, free radicals, carbon monoxide, many of which are very common in the working or living environment (Craig 1985; Lindquist 1986; Lindquist and Craig 1988; Morimoto et al 1994).

Hsps are usually identified in four general families – Hsp90–110, Hsp/Hsc70, Hsp60 and the small Hsps (Hsp10–30) – on the basis of their apparent molecular masses in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The precise function(s) of these different Hsps is still not entirely clear, but many studies have shown Hsps to be involved in many important basal biological processes. This is consistent with the highly conserved nature of many Hsps in different species. Hsps of the Hsp/Hsc70, Hsp60 and Hsp90 families have been shown to function as molecular chaperones, facilitating the synthesis, folding, assembly and intracellular transport of many proteins (Gething 1992; Bohen et al 1995). In addition, some Hsps can confer cells, organs and organisms a higher ability to recover from a stress treatment and thus protect them against

Received 28 January 1998; Revised 21 April 1998; Accepted 22 April 1998

Correspondence to: Robert M. Tanguay, Tel: +1 418 656 3339; Fax: +1 418 656 7176; E-mail: Robert.Tanguay@rsvs.ulaval.ca

damage by heat or by other harmful factors (reviewed in Parsell and Lindquist 1994). Induction and synthesis of Hsps has been correlated with the acquisition of thermotolerance, a property of cells and organisms to show transient protection from the adverse effects of subsequent heat or chemical stresses (Landry et al 1982, 1989; Li and Werb 1982; Laszlo 1988; Angelidis et al 1991; Li et al 1991; Rollet et al 1992; Currie et al 1993; Marber et al 1995; Mehlen et al 1995; Plumier et al 1995). Hsps may also play important roles in the processes of growth, differentiation and development (Arrigo and Tanguay 1991; Tanguay et al 1993; Michaud et al 1997). Some Hsps have also been shown to associate with important active molecules such as steroid hormone receptors and oncogenes (reviewed in Morimoto et al 1994).

Many investigations have indicated that some of the Hsps can present as self-antigens to the immune system resulting in the production of autoantibodies to Hsps in patients with inflammatory diseases, autoimmune disorders, or after various infections caused by viruses, bacteria, mycobacteria and parasites (reviewed in Burdon 1993; Kaufmann and Schoel 1994). It has been suggested that antibodies against Hsps might be of significance in the pathogenesis of some pathophysiological processes, especially in autoimmune diseases (Jarjour et al 1991; Shingai et al 1995).

The presence of antibodies to Hsps in workers with long-term exposure to high temperature and carbon monoxide has been reported (Wu et al 1995, 1996). Benzene is used on a large scale in industry as a starting material in the synthesis of a wide variety of organic compounds, and it appears to be an ubiquitous pollutant (WHO 1993). There are many cases of human occupational exposure to benzene in the world, especially in developing countries. Heavy and chronic occupational exposure to benzene results in degeneration of the bone marrow, aplastic anemia, leukemia and dysfunctions of the immune system (Rinsky et al 1987; Mullin et al 1995). The present study investigated the presence of antibodies to various Hsps (Hsp27, Hsp60, Hsp71 and Hsp90β) in workers exposed to different concentrations of benzene and workers with benzene poisoning. These data were correlated with lymphocyte DNA damage, serum superoxide dismutase (SOD) levels, and peripheral white blood cell counts. The results suggest that the presence of antibodies to Hsps may be useful biomarkers of xenobiotic exposure.

MATERIALS AND METHODS

Workers groups

One hundred and twelve age-matched workers were selected and divided into four groups based on the environmental monitoring of their workplaces and certain general criteria such as age and employment period. The workers from the four groups were similar in their use of alcohol and tobacco. The control group consisted of 29 individuals working in an environment where neither benzene nor other known harmful factors were present. The group exposes to a low concentration of benzene (low benzene) and the group exposed to a high concentration of benzene (high benzene) were each composed of 29 workers working in an environment where the concentration of benzene was either lower (low benzene) or higher (high benzene) than 300 mg/m^3. The benzene-poisoned group (benzene poisoning) consisted of 25 workers who were diagnosed for benzene poisoning by the Occupational Diseases Diagnosis Group in Yichang, China. Benzene poisoning was diagnosed according to the National Criteria of the PRC (GB: 3230–82) for diagnostic criteria and principles of management of occupational benzene poisoning. Briefly, the diagnostic principles are: 1) occupational exposure to high concentration of benzene for half year or low concentrations for a prolonged period, 2) presence of clinical manifestation (inhibition of central nervous system or damage to the blood-producing system) and 3) monitoring of the working environment. This group included the chronic mild and chronic middle benzene poisoning classes. The diagnosis was based on counts of white blood cells ($< 4 \times 10^9$/l). After diagnosis of benzene poisoning, the workers were relocalized to working places where there was no exposure to benzene. Blood was drawn at 0–5 years after diagnosis.

The ages and years of employment of workers in the four groups were matched as shown in Table 1.

Detection of hemoglobin, white blood cells and serum SOD activity

Hemoglobin and white blood cell counts were determined in blood obtained by finger puncture. Venous blood was also collected and divided into two aliquots, one of which was heparinized to separate plasma and lymphocytes. The other aliquot was used to prepare sera. The activity of SOD in sera of all workers was determined with a reagent kit (Jiancheng Bioengineering Company, Nanjing, China).

Table 1 Age and employment years of workers in the four groups (mean ± SD)

Groups	*n*	Age	Employment years
Control	29	35.0 ± 9.0	14.0 ± 9.9
Low benzene	29	34.5 ± 7.4	13.7 ± 7.1
High benzene	29	36.4 ± 9.2	14.2 ± 8.5
Benzene poisoning	25	37.2 ± 7.3	10.8 ± 7.8

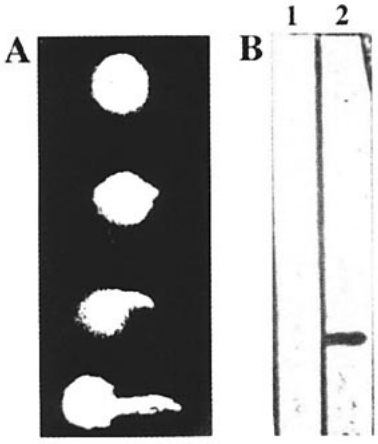

Fig. 1 Example of the Comet assay for DNA damage and of the Western blot assay. (A) Example of Comet assay for DNA damage. From top to bottom, normal (< 5 μm), low-level damage (5–25 μm), medium-level damage (25–45 μm) and high-level damage (> 45 μm). (B) Purified recombinant Hsps were electrophoresed in SDS-PAGE, transferred to nitrocellulose membranes and cut into 2–3 mm wide strips. These were incubated with the plasma (dilution 1:20) and the presence of antibodies to Hsps was detected as described in Materials and Methods. Lane 1, plasma from a worker showing no immunoreactivity against Hsps; lane 2, plasma from a worker showing immunoreactivity.

Determination of lymphocyte DNA damage

Plasma from heparinized whole blood was collected and diluted with 2.0 ml of normal saline and lymphocytes were isolated with a separation medium (Biochemical Reagent Co., Shanghai, China). DNA damage to lymphocytes was assayed by the Comet assay by the alkaline single cell gel technique (SCG) (Singh et al 1989; McKelvey-Martin et al 1993). Images of 25 randomly selected lymphocytes were analyzed for each sample. In this test, the damage to cells was graded visually into four categories corresponding to the following distance of DNA in the tail: no damage, < 5 μm (0); low-level damage, 5–25 μm (+); medium-level damage, 25–45 μm (++) and high-level damage, > 45 μm (+++) (see Fig. 1A for an example).

Determination of antibodies to Hsps in plasma

Recombinant human Hsp27 was a gift from Dr Lee A. Weber (Reno, Nevada, USA). The other recombinant human Hsps (Hsp60, Hsp71 and Hsp90β) were obtained through the expression of the corresponding cDNA in *Escherichia coli* BL2 (DE3) cells using a pET vector as described earlier (Tanguay et al 1993). Approximately 0.3 μg of each recombinant human Hsp was loaded on SDS-PAGE gels and transferred electrophoretically to nitrocellulose membranes (Gelman). The transfer was monitored by staining with rouge ponceau S (Carbajal et al 1986). The collected plasma was divided into two parts, one of which was preadsorbed to an *E. coli* BL2 (DE3) protein extract coupled to CNBr-activated Sepharose 4B (Pharmacia). Plasma diluted 1:20 in 5% skim milk powder in PBS was incubated with thin nitrocellulose strips containing Hsps, washed with PBS-0.05% Tween 80 and the presence of antibodies to the purified Hsps revealed with a horseradish peroxidase-conjugated anti-human IgG (Sigma) and DAB (3,3-diaminobenzidine tetrahydrochloride). An example of immunoblotting of the strips is shown in Figure 1B.

Statistical analyses

Analysis was carried out with the statistical analysis software (SAS/STAT release 6.03) package (SAS Institute Inc, Carry, NC, USA). Other analyses were carried out based on the χ^2 test. Results are given as mean and standard deviation. Statistical inferences are based on the different levels of significance ($P < 0.05$ or $P < 0.01$).

RESULTS

White blood cells, hemoglobin and serum SOD activities

The number of white blood cells, concentration of hemoglobin and the activity of blood SOD for the four occupational groups studied are given in Table 2. The number of white blood cells showed a significant decrease in the benzene-poisoned group as compared to the other three groups. The benzene-poisoned workers showed a slightly

Table 2 White blood cells, hemoglobin and serum SOD activities in blood of workers (mean ± SD)

Groups	Number of workers	White blood cells (× 10^9/l)	Hemoglobin (g/l)	SOD (μ/ml)
Control	29	7.68 ± 1.84	129.0 ± 30.2	102.53 ± 24.78
Low benzene	29	6.89 ± 1.58	134.1 ± 27.6	107.82 ± 21.77
High benzene	29	7.83 ± 1.79	135,1 ± 28.8	112.36 ± 20.41
Benzene poisoning	25	3.84 ± 1.13*	112.5 ± 25.7	138.43 ± 23.15†

*$P < 0.01$, compared with other three groups.
†$P < 0.05$, compared with control.

Table 3 Lymphocyte DNA damage in the workers

Group	n	Total number of cells	Number of damaged cells	0		+		++		+++	
				N	%	N	%	N	%	N	%
Control	29	725	18	707	97.52	17	2.34	1	0.14	0	0
Low benzene	29	725	28	697	96.14	28	3.86	0	0	0	0
High benzene	29	725	59*†	666	91.86	55	7.59*†	4	0.55*†	0	0
Benzene poisoning	25	625	117*†‡	508	81.28	104	16.64*†‡	13	3.08*†‡	0	0

n, number of workers; N, cell number in different state.
*$P < 0.01$ compared with control. †$P < 0.01$ compared with low benzene.
‡$P < 0.01$ compared with high benzene.

Table 4 Presence of antibodies to Hsps in benzene-exposed workers

Group	n	Hsp90β*		Hsp90β†		Hsp71*		Hsp71†		Hsp60*		Hsp60†		Hsp27*		Hsp27†	
		NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%
Control	29	2	6.9	0	0	5	17.2	5	17.2	7	24.1	4	13.8	1	3.5	0	0
Low benzene	29	0	0	0	0	7	24.1	6	20.7	3	10.3	3	10.3	0	0	0	0
High benzene	29	2	6.9	1	3.5	6	20.7	5	17.2	5	17.2	3	10.3	2	6.9	1	3.5
Benzene poisoning	25	2	8.0	2	8.0	11	44.0†	10	40.0	5	20.0	3	12.0	2	8.0	2	8.0

n, number of workers in each group; NO, number of positive antibodies to Hsps; ND, not detected.
*Before pre-adsorbtion to *E. coli* protein extract.
†After pre-adsorbtion to *E. coli* protein extract.
‡$P < 0.05$ compared with control.

lower hemoglobin concentration (112 g/l) than the three other groups, but this value was within the range of the normal values (male 120–160 g/l; female 110–150 g/l). The activities of SOD in sera of workers in the three benzene-exposed groups were higher than in the controls, but only those from the benzene-poisoned group showed a statistically significant increase ($P < 0.05$).

Lymphocyte DNA damage and presence of antibodies to Hsps

Table 3 summarizes the data on measurement of lymphocyte DNA damage in the four groups of workers. Lymphocyte DNA damage was significantly higher in the group exposed to high concentrations of benzene and in the benzene-poisoned group ($P < 0.01$) as compared with the control group. In the latter group, up to 18% of the cells examined showed damaged cells in this assay. The benzene-poisoned workers also had a significantly higher level of DNA damage ($P < 0.01$) when compared with the group exposed to high levels of benzene.

In view of earlier observations on the presence of antibodies to some stress proteins in workers exposed to high temperatures and carbon monoxide (Wu et al 1996) the present study also tested for such antibodies in the plasma of the benzene-exposed workers. Table 4 summarizes the data on the presence of antibodies to Hsps in plasma of workers in the four groups. Very few sera had antibodies to either Hsp90 or Hsp27. About 10% of workers in each group showed reactivity against Hsp60. Hsp71 was clearly recognized in 17% (control) to 40% (benzene-poisoned) of workers depending on the group. Pre-adsorption of sera to a bacterial extract to remove non-specific antibodies reacting with bacterial proteins prior to reacting with recombinant Hsp71 gave identical results, showing that the reaction was specific to human Hsp71. As can be seen from the data, there was a significant increase in the percentage of workers with antibodies to Hsp71 in the benzene-poisoned group (40% versus 17% in the other three groups).

DISCUSSION

Benzene is a common industrial chemical widely used in many industries. Occupational exposure to benzene is especially high in developing countries. The most sensitive targets of benzene toxicity appear to be hematopoietic tissues, and heavy exposure has been linked to the development of a variety of blood disorders in humans ranging from mild reduction in peripheral blood cell concentration to aplastic anemia, acute myelogenous leukemia and lymphoma (Rinsky et al 1987). The oxidized metabolites of benzene are thought to play an important role in the hematotoxicity and carcinogenicity

associated with benzene exposure (Eastmond et al 1987). It has been proposed that tumorigenicity and hematotoxicity may arise from adduct formation with DNA and proteins (Smith et al 1989). The detailed mechanism(s) and the possible factors involved in inducing these disorders are not completely clear.

A reduction in white blood cells is the commonest feature of occupational exposure to benzene. The data in the present study confirm that the most sensitive targets of benzene toxicity in human are hematopoietic tissues. Thus, a significant decrease in peripheral white blood cells ($P < 0.01$) and a mild reduction in hemoglobin were observed in workers with benzene poisoning (see Table 2). There was also an increase in the activity of SOD in the serum of workers exposed to benzene, with the highest values recorded in workers with benzene poisoning. SOD is one of the key enzymes in the clearance of free radicals. There are many free radicals produced during the metabolic activation of benzene, which are strong inducers of SOD (Greenlee et al 1981). Therefore an increase in SOD activities in sera of workers may reflect an increase in both the production of free radicals and cell damage. Evidence for cell damage was also obtained through the detection of lymphocyte DNA damage by the single cell gel electrophoresis assay (Comet assay) (McKelvey-Martin et al 1993). Lymphocyte DNA damage was observed in all groups exposed to benzene, but was statistically higher in the group exposed to high concentrations of benzene and in the benzene-poisoned group (Table 3). Intriguingly, the level and the length of exposure to benzene in benzene-poisoned workers were almost the same as those found in the workers exposed to high or low concentrations of benzene, as many of these individuals work in the same sections of the same industry. This is one of the reasons why the study investigated whether the presence of antibodies to heat stress proteins, which are highly induced by many toxic substances, might have some relationship to the toxicity of benzene.

Heat shock or stress proteins are a group of proteins which have been shown to have the ability to protect cells against various types of damage caused by harmful factors (Parsell and Lindquist 1994). It is also recognized that Hsps and Hsp peptides are antigens to the immune system, a situation which can lead to the production of autoantibodies (Kaufmann and Schoel 1994). Patients with inflammatory diseases, autoimmune disorders or who have had various infections caused by bacteria, mycobacteria and parasites have been reported to foster such antibodies. It has also been suggested that these antibodies might be of significance in the pathogenesis of some pathophysiological processes, especially in the case of autoimmune diseases (Burdon 1993; Minowada and Welch 1995). Alternatively, Hsps could be beneficial in promoting immune protection against various infection agents.

The results presented here show that there is a higher incidence of antibodies against human Hsp71 in workers with benzene poisoning. No other significant differences were observed in the incidence of antibodies against the other Hsps (Hsp27, Hsp60 or Hsp90) between workers in the four groups. Whether the presence of antibodies against Hsps might play a role in disorders such as those caused by benzene exposure remains to be established.

Some possible reasons for the production of autoantibodies against Hsps are: 1) genetic factors; 2) viral infection; 3) the denaturation and release of Hsps as a result of cell damage and 4) the presence of antigen-specific lymphocytes. No genetic diseases could be documented in the workers in the present study nor were there significant differences in the occurrence of infectious diseases among the four groups. These results suggest that neither the genetic background nor previous infections are directly responsible for the high occurrence of antibody to Hsp71. A reasonable alternative explanation is that benzene and/or its metabolites may induce the synthesis of Hsps (Hsp71 being the most frequently induced) and that prolonged exposure to these compounds can lead to cell damage with protein denaturation and release of Hsps. This is consistent with reports that benzene and its metabolites induce reactive oxygen species (ROS) such as H_2, O_2, O_2^-, HO^- (Lee et al 1996) and that these ROS are also strong inducers of Hsps (Polla et al 1995) in addition to causing DNA damage (Lu et al 1995). It is also consistent with the present data showing high activities of serum SOD and a high rate of lymphocyte DNA damage. Whether benzene exposure induces Hsps in workers is presently unknown.

Many investigations have also suggested that autoantibodies against Hsps might be of significance in the generation, formation and prognosis of diseases. For example, Jarjour et al (1991) suggested that the difference in the level of anti-stress protein antibodies seen in sera of patients with various rheumatoid and other inflammatory diseases relative to normal controls could merely reflect disease-associated polyclonal B cell activation. A recent report on the presence of antibodies against Hsps in patients with autoimmune liver diseases suggests that the presence of anti-Hsc70 antibodies is an indicator for the disease activity of primary biliary cirrhosis (Shingai et al 1995). The present study found no evidence for autoantibody syndromes such as polyarthritis syndrome in the benzene-poisoned group.

In summary, the results gathered in the present study show that the higher incidence of antibodies to Hsp71 in a group of workers who had benzene poisoning is associated with a reduction in white blood cells, a higher activity of serum SOD and a high occurrence of lymphocyte DNA

damage. Thus such antibodies can be potentially useful biomonitors to assess whether workers are experiencing abnormal xenobiotic-induced stress within their living and working environment. Whether such antibodies mediate the cytotoxicity of benzene on lymphocytes and bone marrow cells directly or indirectly remains to be determined.

ACKNOWLEDGEMENTS

We are particularly grateful to the workers who voluntarily participated in the study and to the many members of the medical personnel of Yichang Health and Prevention Station for their availability and their generous help in the examination and sampling of workers. We acknowledge the excellent technical assistance of Dr Louis Nicole (Québec) and the gift of Hsp27 from Dr L. Weber (Reno, Nevada). This work was supported by the National Natural Science Foundation of China (NNSFC) and a joint exchange program grant between NNSFC and the Medical Research Council (MRC) of Canada to G.Z. and R.M.T. Work in R.M.T.'s laboratory was supported by the MRC of Canada.

REFERENCES

Angelidis CE, Lazaridis I and Pagoulatos GN (1991) Constitutive expression of heat shock protein 70 in mammalian cells confers thermoresistance. Eur. J. Biochem. **199**, 35–39.

Arrigo AP and Tanguay RM (1991) Expression of heat shock proteins during development in *Drosophila*. In: Heat Shock and Development, eds L Hightower and L Nover, Springer-Verlag, Berlin, 106–119.

Bohen SP, Kralli A, Keith R and Yamamoto Y (1995) Hold'em and fold'em: chaperones and signal transduction. Science **268**, 1303–1304.

Burdon RH (1993) Heat shock proteins in relation to medicine. Mol. Aspects Med. **14**, 83–165.

Carbajal ME, Duband JL, Lettre F, Valet JP and Tanguay RM (1986) Cellular localization of *Drosophila* 83-kilodalton heat shock protein in normal, heat-shocked, and recovering cultured cells with a specific antibody. Biochem. Cell Biol. **64**, 816–825.

Craig EA (1985) The heat shock response. Crit. Rev. Biochem. **18**, 239–280.

Currie RW, Tanguay RM and Kingma JG Jr (1993) Heat-response and limitation of tissue necrosis during occusion/reperfusion in rabbit hearts. Circulation **87**, 963–971.

Eastmond DA, Smith MT and Jrons RD (1987) An interaction of benzene metabolites reproduces the myelotoxicity observed with benzene exposure. Toxicol. Appl. Pharmacol. **91**, 85–95.

Gething MJ (1992) Protein folding in the cell. Nature **355**, 33–45.

Greenlee WF, Sun JD and Bus JS (1981) A proposed mechanism of benzene toxicity: formation of reactive intermediates from polyphenol metabolites. Toxicol. Appl. Pharmacol. **59**, 187–196.

Jarjour WN, Jeffries BD, Davis JS, Welch WJ, Mimura T and Winfield JD (1991) Autoantibodies to human stress proteins. Arthritis Rheum. **34**, 1133–1138.

Kauffmann SHE and Schoel B (1994) Heat shock proteins as antigens in immunity against infection and self. In: The Biology of Heat Shock Proteins and Molecular Chaperones, eds RI Morimoto, A Tissieres and C Georgopoulos, Cold Spring Harbor Laboratory Press, NY, 495–531.

Landry J, Bernier D, Chrétien P, Nicole LM, Tanguay RM and Marceau N (1982) Synthesis and degradation of heat shock proteins during development and decay of thermotolerance. Cancer Res. **42**, 2457–2461.

Landry J, Chrétien P, Lambert H, Hickey E and Weber LA (1989) Heat shock resistance conferred by expression of the human HSP27 gene in rodent cells. J. Cell Biol. **109**, 7–15.

Laszlo A (1988) Evidence for two states of thermotolerance in mammalian cells. Int. J. Hyperthermia **4**, 513–526.

Lee SF, Liang YC and Lin JK (1996) Inhibition of 1,2,4-benzenetriol-generated active oxygen species and induction of phase-II enzymes by green tea polyphenols. Chem. Biol. Interact. **98**, 283–301.

Li GC and Werb Z (1982) Correlation between synthesis of heat shock proteins and development of thermotolerance in Chinese hamster fibroblasts. Proc. Natl. Acad. Sci. USA **79**, 3218–3222.

Li GC, Li LY, Liu K, Mak JK, Chen L and Lee WMF (1991) Thermal response of rat fibroblasts stably transfected with the human 70 kDa heat shock protein encoding gene. Proc. Natl. Acad. Sci. USA **88**, 1681–1685.

Lindquist S (1986) The heat shock response. Annu. Rev. Biochem. **55**, 1151–1191.

Lindquist S and Craig EA (1988) The heat shock proteins. Annu. Rev. Genet. **22**, 631–677.

Lu JX, Jiang C, Kaeck M, Ganther H, Ip C and Thompson H (1995) Cellular and metabolic effects of triphenylselenonium chloride in mammary cell-culture model. Carcinogenesis **16**, 513–517.

McKelvey-Martin VJ, Green MHL, Schmezer P, Pool-Zobel BL, De Méo MP and Collins A (1993) The single cell gel electrophoresis assay (comet assay): a European review. Mut. Res. **288**, 47–63.

Marber MS, Mestril R, Chi S, Sayen MR, Yellon DM and Dillmann WH (1995) Overexpression of the rat inducible 70-kD heat stress protein in a transgenic mouse increases the resistance of the heart to ischemic injury. J. Clin. Invest. **96**, 1446–1456.

Mehlen P, Preville X, Chareyron P, Briolay J, Klemenz R and Arrigo AP (1995) Constitutive expression of human hsp27, *Drosophila* hsp27 and human α-B crystallin confers resistance to tumor necrosis factor-α and oxidative stress-induced cytotoxicity in stably transfected murine L929 fibroblasts. J. Immunol. **154**, 364–374.

Michaud S, Marin R and Tanguay RM (1997) Regulation of heat shock gene induction and expression during *Drosophila* development. Cell. Mol. Life Sci. **53**, 104–113.

Minowada G and Welch WJ (1995) Clinical implications of the stress response. J. Clin. Invest. **95**, 3–12.

Morimoto RI, Tissières A and Georgopoulos C, eds (1994) The Biology of Heat Shock Proteins and Molecular Chaperones, Cold Spring Harbor Laboratory Press, NY.

Mullin AH, Rando R, Esmundo F and Mullin DA (1995) Inhalation of benzene leads to an increase in the mutant frequencies of a *lacI* transgene in lung and spleen tissues of mice. Mut. Res. **327**, 121–129.

Parsell DA and Lindquist S (1994) Heat shock proteins and stress tolerance. In: The Biology of Heat Shock Proteins and Molecular Chaperones, eds RI Morimoto, A Tissières and C Georgopoulos, Cold Spring Harbor Laboratory Press, NY, 457–494.

Plumier C, Ross BM, Currie RW, Angelidis CE, Kazlaris H, Kollias G and Pagoulatos GN (1995) Transgenic mice expression of the human heat shock protein 70 have improved post-ischemic myocardial recovery. J. Clin. Invest. **95**, 1854–1860.

Polla PS, Mariéthoz E, Hubert D and Barazzone C (1995) Heat-shock proteins in host–pathogen interactions: implication for cystic fibrosis. Trends Microbiol. **3**, 392–396.

Rinsky RA, Smith AB, Hornung R, Filloon TG, Young RJ, Okun AH and Landrigan PJ (1987) Benzene and leukemia: an epidemiologic risk assessment. N. Engl. J. Med. **316**, 1044–1050.

Rollet E, Landry J, Lavoie J and Tanguay RM (1992) Expression of *Drosophila* 27 kDa heat shock protein in rodent cells confers thermal resistance. Biochem. Biophys. Res. Commun. **185**, 116–120.

Shingai R, Maeda T, Onishi S and Yamamoto Y (1995) Autoantibody against 70 kD heat-shock protein in patients with autoimmune liver diseases. J. Hepatol. **23**, 382–390.

Singh NP, Banner DB, Tice RR, McCoy MT, Collins GD and Schneider EL (1989) Abundant alkali sensitive sites in DNA of human and mouse sperm. Exp. Cell Res. **184**, 461–470.

Smith MT, Yager JW, Steinmetz KL and Eastmond DA (1989) Peroxidase-dependent metabolism of benzene's phenolic metabolites and its potential role in benzene toxicity and carcinogenicity. Environ. Health Perspect. **82**, 23–29.

Tanguay RM, Wu Y and Khandjian EW (1993) Tissue-specific expression of heat shock stress proteins of the mouse in the absence of stress. Dev. Genet. **14**, 112–118.

WHO (1993) Environmental Health Criteria. World Health Organization, Geneva.

Wu T, He H, Tanguay RM, et al (1995) The combined effects of high temperature and carbon monoxide on heat stress response. J. Tongji Med. Univ. **15**, 178–183.

Wu T, Tanguay RM, Wu Y, et al (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.