Tissue-Specific Changes in Heme Oxygenase Activity and Level of Nonprotein Thiols in C57Bl/6 Mice after Whole-Body γ-Irradiation

Ensu Han, A. G. Platonov*, M. Ya. Akhalaya*, Ji Yon Son, Ji Yon An, and Yon Suk Yun

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 140, No. 9, pp. 328-331, September, 2005
Original article submitted February 4, 2005

Heme oxygenase catalyzes heme degradation and is an important component of the antioxidant defense. Nonprotein thiols participate in redox regulation of heme oxygenase gene expression. Changes in heme oxygenase activity and levels of nonprotein thiols in the liver, lungs, and brain of C57Bl/6 mice were studied on days 1-7 after whole-body γ-irradiation in a dose of 10 Gy. The maximum increase in heme oxygenase activity was observed in the liver (to 196% in females and to 250% in males) and was associated with an 8-fold increase in the level of heme oxygenase-1 (inducible form of the enzyme) mRNA. The increase in heme oxygenase activity was less pronounced in the lungs, while in the brain this parameter slightly decreased. Changes in the levels of nonprotein thiols were sex-dependent: in the liver and lungs this parameter increased in females and decreased in males.

Key Words: *heme oxygenase; nonprotein thiols; liver; lungs; γ-irradiation*

Microsomal enzyme heme oxygenase (HO; EC 1.14. 99.3) is an important component of intracellular antioxidant defense, realizing oxidative degradation of the heme molecule with the formation of carbon monoxide, Fe^{2+}, and biliverdin, which, in turn, is rapidly reduced to bilirubin by biliverdin reductase [5,8,11]. In mammals HO is presented by 3 isoforms: inducible isoform HO-1 and two constitutive isoforms HO-2 and HO-3 [6]. HO-1 belongs to heat shock proteins and is also known as HSP32 [4]. Induction of HO-1 by physical and chemical agents inducing ROS generation in the body is an adaptive defense response to oxidative stress [2,4]. The protective role of HO is attributed to removal of free heme (prooxidant) and formation of bioactive products [8,11]. HO ensures the only known biologically significant route of elimination of cytotoxic free heme forming during degradation of hemoproteins. Bilirubin is the main end-product of heme degradation and a potent antioxidant [3], while carbon monoxide actively participates in the signal transduction and exhibits potent antiinflammatory properties [6]. Bivalent iron released during heme degradation is characterized (similarly as free heme) by prooxidant effects, but increased level of iron is rapidly compensated by induction of ferritin synthesis [15]. Ferritin is an iron-binding protein preventing involvement of these ions in oxidative processes. The important role of HO in the regulation of cell homeostasis is also due to the fact that its substrate (heme) is a prosthetic group for many vital enzymes, including cytochrome P-450, NO-synthase, soluble guanylate cyclase, catalase, glutathione peroxidase, and other hemoproteins. Hence, HO can modify the antioxidant status by different ways, in contrast to other antioxidant enzymes realizing only one route of ROS elimination.

The key component of redox regulation of signal transduction and expression of antioxidant enzymes is

Laboratory of Immunology, Korean Institute of Radiological and Medical Sciences, Seoul; *Laboratory of Radiation Biophysics, Department of Biophysics, Biological Faculty, M. V. Lomonosov Moscow State University. ***Address for correspondence:*** imm@kcch.re.kr. Yon Suk Yun

0007-4888/05/1403-0341

reduced glutathione, which, as a direct capturer of free radicals and a component of the glutathione peroxidase system, possesses intrinsic antioxidant activity [7,9]. Reduced glutathione constitutes the greater (up to 90%) part of free intracellular nonprotein thiol (NPT) pool.

Here we studied changes in HO activity and NPT levels in the liver, lungs, and brain of C57Bl/6 mice after whole-body γ-irradiation in a lethal dose.

MATERIALS AND METHODS

Experiments were carried out on 7-8-week-old male and female C57Bl/6 mice (Daehan Biolink). The animals were kept under common vivarium conditions (KIRAMS: Korea Institute of Radiological & Medical Sciences): 12:12 h light:dark regimen, 22±2°C, relative humidity 50%, standard granulated fodder, and water *ad libitum*. Care of and experimental procedures on animals were carried out in accordance with the KIRAMS regulations. Whole-body γ-irradiation was carried out on a Theratron 780 device (Atomic Energy of Canada, Ltd.) with a ^{60}Co source in a single dose of 10 Gy (0.5 Gy/min dose power).

Activity of HO was determined by chloroform extraction of bilirubin formed in the incubation mixture, its measurement by spectrophotometry [10], and was expressed in picomoles of bilirubin formed per mg protein over 1 h. Protein concentration was measured by the standard method [12].

The content of NPT was measured as described previously [13] and expressed in μmol/g tissue.

The level of HO-1 mRNA expression was evaluated by reverse transcription PCR (RT-PCR). RNA was extracted from mouse liver samples using RNAzol (Tel-Test). cDNA was obtained is a reaction mixture (total volume 50 μl) containing 50 mM Tris-HCl buffer (pH 8.3), 3 mM $MgCl_2$, 75 mM KCl, 2.5 μg/ml $pd(N)_6$-primer, 0.5 mM each dNTP, 10 U AMV reverse transcriptase (Amersham Pharmacia Biotech), and 1 μg RNA extracted from liver samples. The resultant cDNA was amplified by PCR in a reaction mixture (total volume 40 μl) containing 10 mM Tris-HCl buffer (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 0.5 mM each dNTP, 1.0 μM 5'- and 3'-primers, 0.5 U Taq-DNA-polymerase (Takara), and 10 μl cDNA preparation. Primers for HO-1: 5'-primer 5'-AACAAGCAG AACCCAGTC-3' and 3'-primer 5'-TGTCATCTCCAG AGTGTTC-3'; for β-actin: 5'-primer 5'-TGGAATC CTGTGGCATCCATGAAA-3' and 3'-primer 5'-TA AAACGCAGCTCAGTAACAGTCCG-3'. PCR was carried out in a thermocycler (Hybaid), 30 sec at 94, 56, and 72°C, a total of 32 cycles. Amplification products were visualized by electrophoresis in 1% agarose gel in the presence of ethidium bromide (0.5 μg/ml). The density of bands was evaluated on a Flour-S Multimager analyzer (Bio-Rad). The levels of HO-1 mRNA expression were evaluated in comparison with β-actin mRNA expression.

The results were statistically processed using GraphPad Prism 3.0 software. The data were presented as M±SEM; 4-8 mice of experimental and respective control groups were examined per point. Changes in HO activity and NPT levels were evaluated in groups of irradiated animals in comparison with the corresponding controls for all periods after γ-irradiation. The significance of differences between irradiated and control animals was evaluated using Student's *t* test. The significance of differences in the levels of HO-1 mRNA expression was analyzed using the Kruskal—

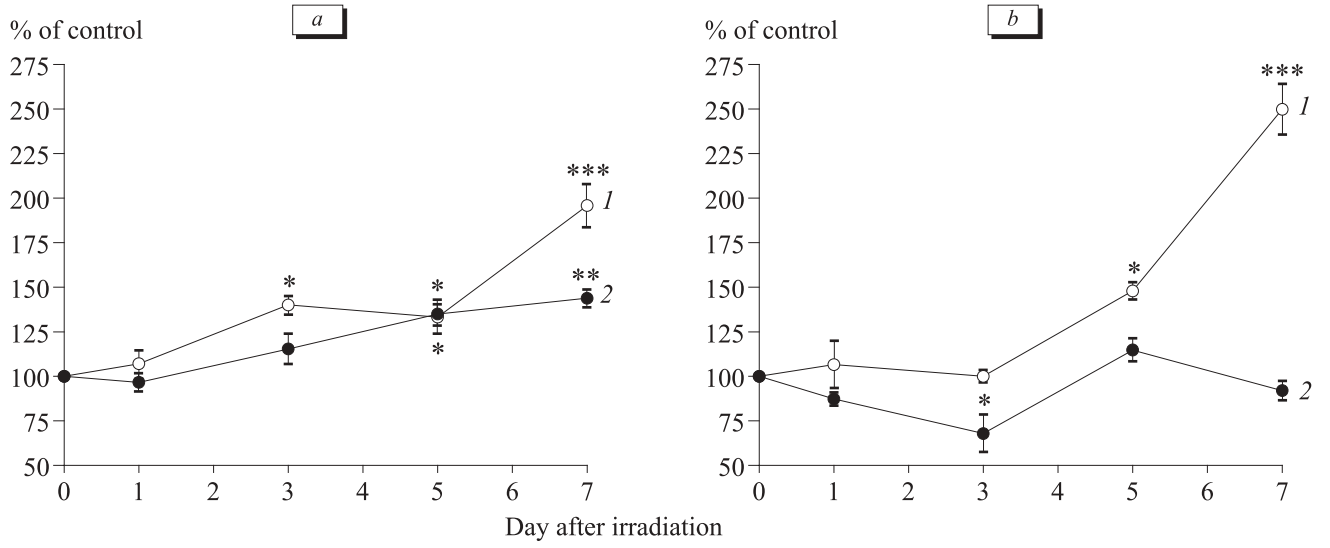

Fig. 1. Heme oxygenase activity (HO; *1*) and level of nonprotein thiols (NPT; *2*) in the liver of female (*a*) and male C57BL/6 mice after whole-body γ-irradiation (*b*). *p<0.05, **p<0.01, ***p<0.001 compared to the control group.

Wallis test. The differences between the groups were significant at $p<0.05$

RESULTS

Irradiation significantly elevated HO activity in the liver of females and males (Fig. 1). In females this increase was observed on day 3, in the males on day 5. On day 7 after irradiation HO activity in the liver increased to 195.8±12.0% in females and 249.9±14.6% in males (compared to the control). Changes in the content of NPT in the liver were sex-dependent: this parameter significantly increased in females (by 35.44% on days 5-7), but decreased in males (by 32% on day 3).

A significant increase (by 27-29%) in HO activity in the lungs was observed on day 5 after exposure in females and on day 7 in males (Fig. 2). Significant changes in NPT levels in the lungs were observed only on day 1 after irradiation: this parameter slightly increased in females and decreased in males.

No postradiation increase in HO activity was detected in the brain. In males HO activity slightly decreased on day 5 after irradiation (to 83.0±3.3% of control level, $p<0.05$). No changes in cerebral NPT levels were observed in mice of both sexes.

The level of HO-1 mRNA in the liver of females increased significantly after irradiation: 8.7 times on day 5 and 8-fold on day 7 compared to the control (Fig. 3).

Hence, the most pronounced and stable increase in HO activity after whole-body γ-irradiation was observed in the liver (in both females and males). It was paralleled by a significant increase in the expression of HO-1 gene (this isoform is also induced by other oxidative factors). A similar increase in HO activity in rat liver was observed during the same periods after X-ray exposure [1]. A transient increase in HO activity and HO-1 mRNA level in rat liver was also observed early (several hours) after X-ray exposure [14].

The effect of irradiation on HO activity in the lungs and brain was never studied before. These organs are frequent objects of exposure in radiotherapy of tumors, and hence, any components of intracellular antioxidant defense in these tissues can be regarded as factors modulating cell susceptibility to irradiation. Postradiation induction of HO was observed in the lungs, which, similarly as the liver, are liable to HO induction under the effect of oxidative exposure [8], though this induction is less pronounced than in the liver. The absence of elevation and even decrease in HO activity in the brain seems to be due to predominance of HO-2 in this tissue (this HO isoform is induced by adrenal glucocorticoids [6]), while in the liver and lungs the percentage of HO-1 is high. A certain decrease in HO activity in the brain of males can be caused by disorders in hormone status after exposure.

The liver is most prone to postradiation increase in HO activity due to massive effects of free heme forming from hemoglobin of damaged erythrocytes and other destabilized hemoprotein cells. Free heme, a prooxidant, stimulates oxidative processes and induces HO-1 [11]. It was previously shown that the increase in HO activity is preceded by the increase in free heme pool in the liver of irradiated rats [1].

The decrease in free intracellular NPT (reduced glutathione) pool leads to increased HO-1 expression [9]. We revealed no clear-cut correlation between postradiation changes in NPT level and HO activity in our experiments. Moreover, changes in NPT activity were opposite in males and females: in males NPT levels

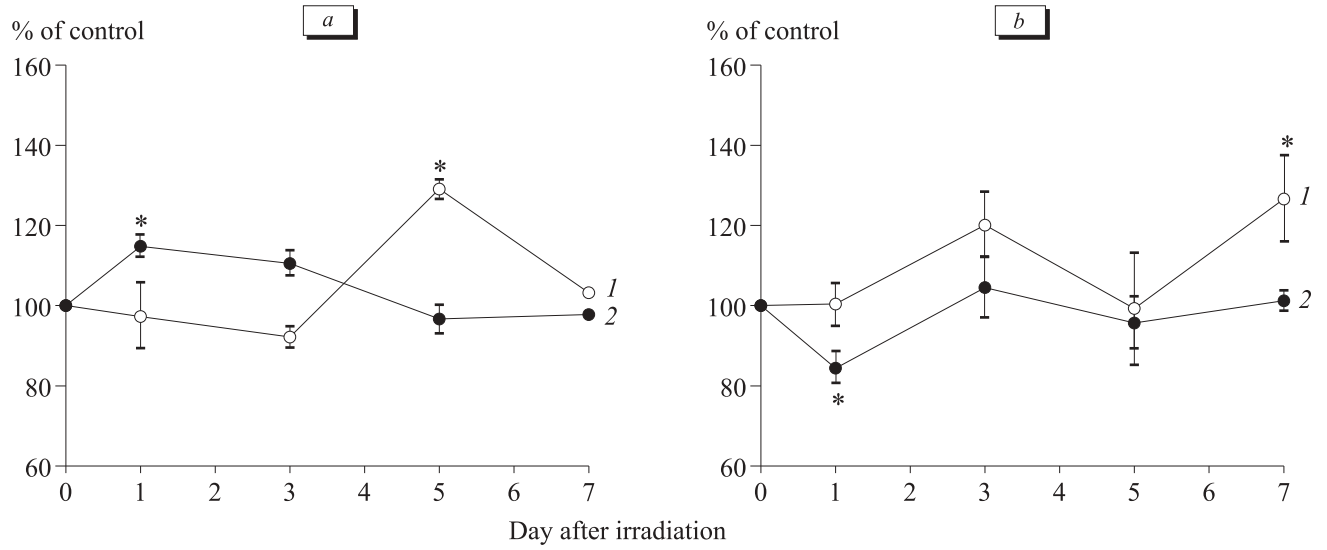

Fig. 2. HO activity (*1*) and NPT level (*2*) in the lungs of female (*a*) and male (*b*) C57Bl/6 mice after whole-body γ-irradiation. *$p<0.05$ compared to the control group.

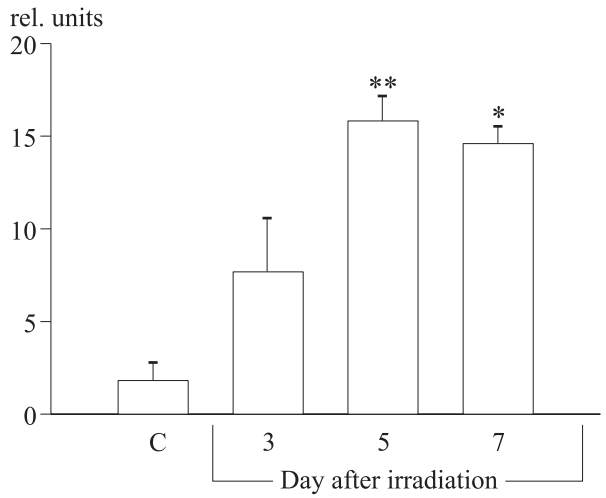

Fig. 3. Level of HO-1 mRNA expression in the liver of female C57Bl/6 mice after whole-body γ-irradiation. C: control. *$p<0.05$, **$p<0.01$ compared to the control.

in the liver and lungs decreased during some periods after exposure, while in females NPT level always increased (before or in parallel with the increase in HO activity). This fact can be explained by higher radiation resistance of females in comparison with males, which was shown in our experiments with whole-body γ-irradiation. Thus we demonstrate not only tissue-specific changes in some antioxidant systems of the body, but also sex-specific differences in these shifts, which can be important for evaluating body reactivity to irradiation.

The study was supported by the Ministry of Science and Technology of Republic of Korea.

REFERENCES

1. L. I. Deev, G. I. Topchishvili, M. Ya. Akhalaya, and A. G. Platonov, *Byull. Eksp. Biol. Med.*, **99**, No. 6, 681-683 (1985).
2. L. A. Applegate, P. Luscher, and R. M. Tyrrell, *Cancer Res.*, **51**, No. 3, 974-978 (1991).
3. J. E. Clark, R. Foresti, C. J. Green, and R. Motterlini, *Biochem. J.*, **348**, Pt. 3, 615-619 (2000).
4. S. M. Keyse and R. M. Tyrrell, *Proc. Natl. Acad. Sci. USA*, **86**, No. 1, 99-103 (1989).
5. M. D. Maines, *FASEB J.*, **2**, No. 10, 2557-2568 (1988).
6. M. D. Maines, *Annu. Rev. Pharmacol. Toxicol.*, **37**, 517-554 (1997).
7. L. K. Moran, J. M. Gutteridge, and G. J. Quinlan, *Curr. Med. Chem.*, **8**, No. 7, 763-772 (2001).
8. L. E. Otterbein and A. M. K. Choi, *Am. J. Physiol. Lung Cell. Mol. Physiol.*, **279**, No. 6, L1029-L1037 (2000).
9. I. Rahman and W. MacNee, *Free Radic. Biol. Med.*, **28**, No. 9, 1405-1420 (2000).
10. S. W. Ryter, E. Kvam, and R. M. Tyrrell, *Methods Mol. Biol.*, **99**, 369-391 (2000).
11. S. W. Ryter and R. M. Tyrrell, *Free Radic. Biol. Med.*, **28**, No. 2, 289-309 (2000).
12. G. R. Schacterle and R. L. Pollack, *Anal. Biochem.*, **51**, No. 2, 654-655 (1973).
13. J. Sedlak and R. H. Lindsay, *Ibid.*, **25**, No. 1, 192-205 (1968).
14. K. Suzuki, M. Mori, F. Kugawa, and H. Ishihara, *J. Radiat. Res.* (Tokyo), **43**, No. 2, 205-210 (2002).
15. G. F. Vile, S. Basu-Modak, C. Waltner, and R. M. Tyrrell, *Proc. Natl. Acad. Sci. USA*, **91**, No. 7, 2607-2610 (1994).