

TIME-DEPENDENT EFFECT OF SEVERE HYPOXIA/REOXYGENATION ON OXIDATIVE STRESS LEVEL, ANTIOXIDANT CAPACITY AND p53 ACCUMULATION IN MITOCHONDRIA OF RAT HEART

O. A. GONCHAR, I. N. MANKOVSKA

*Bogomoletz Institute of Physiology, National Academy of Sciences of Ukraine, Kyiv;
e-mail: olga.gonchar@i.ua*

The intensity of oxidative stress, protein expression of antiapoptotic Bcl-2 as well as antioxidant enzymes manganese superoxide dismutase (MnSOD) and glutathione peroxidase (GPx) and their regulator p53 were studied in the mitochondria of rat heart. Sessions of repeated hypoxia/reoxygenation ((H/R), 5 cycles of 10 min hypoxia (5.5% O₂ in N₂) alternated with 10 min normoxia, daily) were performed in our study. It was shown that short-term sessions of H/R (during 1-3 days) caused a significant increase in the oxidative stress markers (ROS formation and lipid peroxidation), mitochondrial p53 translocation, a decrease in MnSOD protein expression/activity and Bcl-2 protein content, but up-regulated GPx. We have demonstrated that prolonged H/R (7-14 days) induced myocardial tolerance to fluctuation in oxygen levels that was associated with the reduction in mitochondrial p53 protein content, elevation of mitochondrial Bcl-2 protein level, and increase in antioxidant capacity. A close correlation between the mitochondrial p53 accumulation and ROS formation as well as the activity and protein content of MnSOD and GPx allowed us to assume that p53 took an active part in the regulation of prooxidant/antioxidant balance in mitochondria of rat heart during repeated H/R.

Key words: hypoxia/reoxygenation, p53, Bcl-2, MnSOD, glutathione peroxidase, mitochondria of rat heart.

A large body of experimental data indicates that reactive oxygen species (ROS) play a fundamental role in hypoxia/reoxygenation injury of cells and subcellular structures [1]. Mitochondria are considered an important locus of ROS production mainly at the level of complexes I and III of the respiratory chain and hence a potential contributor to cells damage during hypoxia [2]. At the same time, mitochondria could be major targets of ROS attack. These events result in mitochondrial dysfunction with superoxide leakage, the formation of other aggressive ROS which attack lipids, proteins and other cell constituents, lead to energy and metabolic disorders, deplete of cellular antioxidant defense, and induce the apoptotic cascade [1, 2].

In general, mitochondrial oxidative stress is determined by the balance between ROS generation and their elimination by antioxidants [3]. The

antioxidants and free radical scavenging enzymes, including MnSOD, peroxiredoxin 3 and 5, thioredoxin, glutathione, and glutathione peroxidase, not only constitute the first line of defense against oxidative damage within the mitochondria but also are essential for maintaining the critical cellular redox balance and play key role in modulating cellular responses to external stimuli [4]. MnSOD and GPx – the key antioxidant defense enzymes that function in concert to prevent ROS reactions in response to oxidative stress [5]. There are plenty of reports concerning the role of MnSOD and GPx in the cellular redox homeostasis involved in the adaptive responses against oxidative stress [6-9], although the mechanisms associated with protein expression and specific activity of MnSOD as well as GPx during H/R of different duration are not fully understood.

In the past years, a number of transcription factors and signaling pathways have been identified and outlined to mediate critical transcriptional responses to oxidative stress [10]. The tumor suppressor protein p53 is an important integrator of cellular stresses which triggers cell cycle arrest, senescence, DNA repair, or apoptosis [11, 12]. However, recent studies have also shown that the p53 target genes are potentially involved in the control of other cell functions, including oxidative stress response [11, 13]. The interactions between the p53 protein and ROS production are complex and contradictory, since p53 can either increase or decrease ROS generation and, at the same time, these changes can modulate selective transactivation of p53 target gene [11]. Current data showed that hyper-physiological and physiological levels of p53 exert different effects on cellular redox status either through directly regulating the expression of pro-oxidant and antioxidant genes or through modulating the cellular metabolic pathways [6, 10]. P53 can upregulate the expression of various antioxidant enzymes such as aldehyde dehydrogenase 4 and mammalian sestrin homologues [13]. Recently, it was reported that GPX gene is subjected to p53 regulation and is a novel p53 target gene [14]. The relationship between p53 protein level and MnSOD expression is observed in many cell lines suggesting that p53 may regulate MnSOD expression [15-18], but whether p53 has a positive or negative effect on MnSOD expression remains ambiguous.

A number of stimuli can trigger p53 activation [11]. Although hypoxia has been reported to raise p53 protein levels in a variety of cell types [12, 19], however, the p53 response to sessions of short- as well as long-term H/R is largely unknown.

The effect of hypoxia/reoxygenation seems to be cell type specific [5, 8, 20], therefore, the organ that was considered was the heart as a site with the largest density/volume of mitochondria and an elevated rate of oxygen consumption under the nonstandard situation. H/R-induced tissues injury is of significance in cardiovascular pathophysiology because it occurs in a wide variety of clinical conditions, such as myocardial infarction, stroke, shock, cancer, and organ transplantation [1].

Based on previous research, the present study has been focused on the subject how the oxidative stress level, mitochondrial antioxidant defense system, antiapoptotic marker as well as oxidative stress-inducible proteins such as p53 produced by heart can be modulated by short- and prolonged sessions of H/R.

Materials and Methods

Animals and study design. Wistar rats weighing 220-260 g were used. They were housed in Plexiglas cages (4 rats per cage) and kept in an air-filtered and temperature controlled (20-22 °C) room. Rats received a standard pellet diet and water ad libitum and were kept under the artificial light-dark cycle of 12 h. The present study was approved by the Institutional Animal Ethics Committee, Bogomoletz Institute of Physiology, Kyiv and performed in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986). The rats were randomly divided into 5 groups. Group 1 – control (C): rats were sedentary and under normoxic condition. Animals from others groups were subjected to sessions of hypoxia/reoxygenation. Hypoxic episodes were created by breathing hypoxic gas mixture (5.5% O₂ in N₂) in normobaric conditions in a special chamber. We used experimentally repeated short-term hypoxia (10 min) with normoxic intervals (10 min). The rats had such five sessions daily. Animals, which had sessions of H/R were sacrificed after the 1st day (Group 2), 3rd day (Group 3), 7th day (Group 4) and 14th day (Group 5) of experimental exposure (eight rats from each time point). Ambient O₂ levels in the chamber were continuously monitored by the use of a Beckman O₂ analyzer (model OM-11) by sampling the air in the chamber. The duration of the gas flows during each hypoxic and normoxic episode was regulated by timed solenoid valves. Animals of each group were decapitated 24 h after the last hypoxic session. At the time of sacrifice, the animals were lightly anesthetized with ether.

Mitochondrial fraction preparation. All reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). In mitochondria of rat heart were isolated by differential centrifugation. Tissues were collected in isolation medium A (250 mM sucrose, 10 mM Tris/HCl (pH 7.6), 1 mM EGTA and 0.5% defatted bovine serum albumin) and homogenized. After centrifugation of the homogenate at 1000 g for 5 min, the supernatant was strained on gauze and recentrifuged at 12 000 g for 15 min. The resulting pellet was resuspended in ice-cold isolation medium B (250 mM sucrose, 10 mM Tris/HCl (pH 7.6) and 0.1 mM EGTA) and a new series centrifugation was performed. The final washing and resuspension of mitochondria were performed in the medium B without EGTA. Protein concentration was

determined by the Bradford method [21], using bovine serum albumin as a standard.

Analysis of ROS generation. ROS level was measured using the dichlorofluorescein (DCF) fluorescence assay as described previously [22], with minor modification. Isolated mitochondria (0.5 mg protein/ml) were incubated for 20 min at 37 °C in the respective buffer (130 mM KCl, 20 mM KH₂PO₄, 5 mM MgCl₂, 3 mM HEPES, 1 mM EGTA, pH 7.4 and 5 mM sodium succinate) containing membrane permeable non-fluorescent probe 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA). The final concentration of DCFH-DA was 10 µM. The solution was then centrifuged at 12 000 g for 5 min, and the supernatant containing excess DCFH-DA not crossing the mitochondrial membrane was discarded. DCF formation was followed at the excitation wavelength of 488 nm and the emission wavelength of 525 nm for 30 min by using a Hitachi F-2000 fluorescence spectrometer. The rate of DCFH-DA conversion to DCF was linear for at least 60 min, corrected with the autooxidation rate of DCFH-DA without protein. All assays were carried out in duplicates. Fluorescence was expressed as arbitrary fluorescence units.

Lipid peroxidation assay. Lipid peroxidation in isolated mitochondria was measured from the formation of thiobarbituric acid reactive substances (TBARS) using the Buege and Aust method [23]. TBARS were isolated by boiling tissue homogenates for 15 min at 100 °C with thiobarbituric acid reagent (0.5% 2-thiobarbituric acid/10% trichloroacetic acid/0.63 mM hydrochloric acid) and measuring the absorbance at 532 nm. The results were expressed as nM/mg of protein.

Enzymatic assays. Enzymatic activity in the mitochondrial preparations was determined upon solubilization in 0.5% deoxycholate. The activity of selenium-dependent GPx was determined according to the method of Flohe and Gunzler [24]. Briefly, the reaction mixtures consisted of 50 mM potassium phosphate buffer (pH 7.0) 1 mM EDTA, 1 mM NaN₃, 0.2 mM NADPH, 1 mM GSH, 0.25 mM H₂O₂, 226 U/ml glutathione reductase, and rates of NADPH oxidation followed at 340 nm. The activity was expressed as nM/min/mg of protein.

MnSOD activity was measured by the method of Misra and Fridovich [25], which is based on the inhibition of autooxidation of adrenaline to adrenochrome by SOD contained in the examined samples. The samples were preincubated at 0 °C for 60 min

with 6 mM KCN, which produces total inhibition of Cu, Zn-SOD activity [26]. The results were expressed as specific activity of the enzyme in units per mg protein. One unit of SOD activity was defined as the amount of protein causing 50% inhibition of the rate of adrenaline conversion to adrenochrome under specified conditions.

Western blot analysis. For immunoblotting analysis the isolated mitochondria were incubated with RIPA buffer containing 50 mM Tris-HCl pH 8.0, 150 mM NaCl, 1.0% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulphate, 1mM phenylmethylsulfonyl fluoride, 1 µg/ml Protease and Phosphatase inhibitor Cocktail (78440, ThermoScientific Inc, USA). The lysate was centrifuged at 14 000 g for 15 min. This fraction was labeled as the mitochondrial fraction and kept at -80 °C. The cytosol fraction was performed as follows. The tissues was homogenized in ice-cold lysis buffer containing 10 mM HEPES (pH 7.9), 1.5 mM MgCl₂, 10 mM KCl, 1 mM dithiothreitol, 0.1 mM EDTA, and 0.2 mM phenylmethylsulfonyl fluoride plus 1 µg/ml Protease and Phosphatase inhibitor Cocktail (78440, ThermoScientific Inc, USA). This suspension was incubated on ice for 15 min. Then 12.5 µl of 10% Nonidet P-40 was added and the mixture was vigorously vortexed for 15 s. The cytoplasmic and nuclear fractions were separated by centrifugation at 15 000 g at 4 °C for 2 min.

Equal amounts of protein (100 µg) were mixed with Laemmli buffer (S3401, Sigma), heated (99 °C, 5 min), and then loaded onto 10-12% SDS polyacrylamide gels. Separated proteins were transferred onto polyvinylidene difluoride (PVDF) membranes which were blocked in 5% non-fat milk in Tris-buffered saline-Tween (TBS-T) for 1 hour at room temperature. Primary antibodies were applied overnight at 4 °C. After washing in 1% non-fat milk in TBS-T (3×10 min) the membranes were incubated with a secondary antibody conjugated to horseradish peroxidase (HRP) for 1 h at room temperature. Each antigen-antibody complex was visualized by the amino-ethylcarbazol reaction. The band intensities were quantified by densitometry with a computerized image processing system (GelPro Analyzer). Results were expressed as percentages of control values. β-Actin was used as a loading control. Antibodies and dilutions: p53 1:250 (Thermo Scientific Inc, USA); MnSOD 1:500 (Sigma-Aldrich Co); GPx 1/2 (B-6) 1:500 (Santa Cruz Biotechnology, Inc); Bcl-2 1:250 (Santa Cruz Biotechnology, Inc); β-Actin

1:1000 (Santa Cruz Biotechnology, Inc); mouse anti-rabbit IgG HRP 1:1000 (Sigma-Aldrich Co).

Statistical analysis. Data are expressed as a mean \pm SD for each group. The differences among multiple experimental groups were detected by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. The correlation between pairs of variables was analyzed using the bivariate Pearson method. A *P* value of less than 0.05 was considered as significant.

Results and Discussion

In the present study, the use of severe hypoxia in sessions of intermittent H/R caused intensification of the oxidative process in mitochondria of rat heart. Our data confirmed that mitochondrial oxidative stress was involved already on the first day of H/R exposure and continued for the whole study period of 2 weeks, although the intensity of the oxidative processes was reduced gradually to the 14th day. Thus, in mitochondria of rat heart we registered a significant increase of ROS formation (by 75, 53, 24%, *P* < 0.05 and 13% on the 1st, 3rd, 7th and 14th days) as well as in TBARS content (by 28, 22, 20%, *P* < 0.05 and 10% on the 1st, 3rd, 7th and 14th days of IH exposure, respectively) as compared to control (normoxia) (Fig. 1).

Generally, an increased production of ROS triggers an array of signal transduction pathways, resulting in stimulatory or inhibitory output signals [1, 10]. In the present study, we have investigated whether H/R alters antioxidant capacity in the heart, regarding both expression and activity of antioxidant enzymes MnSOD and GPx, and whether these alterations could be related to their transcriptional regulators p53.

Simultaneously with an increase in ROS production, we registered changes in the protein content of p53 in both cell compartments (cytosol and mitochondria). We can declare that these changes were time-dependent ones. The data (not shown) suggest that a decrease in the p53 protein content in the cytoplasm can be caused by the movement of the p53 protein in the mitochondria and maybe to the nucleus as an active form, because it seems that p53 translocation to mitochondria precedes its nuclear translocation [17]. Thus, the increase in the p53 protein content in mitochondria of rat heart occurred during a brief period of H/R influence (for 1-3 days by 26-32%, *P* < 0.05), completely abolished after 1 week of H/R and slight rise to the end of the 2nd week (Fig. 2). It should be noted that in our study the ROS formation positively correlated with the mitochondrial p53 protein content (*r* = 0.78). Our findings are in agreement with previous reports that hypoxia causes an accumulation of p53 in the mitochondrial outer membrane [1, 19] and ROS level is the main modulator of p53 stability and function [11]. One of the factors that may lead to the stabilization of p53 under severe hypoxia is the decrease in Mdm2, an E3 ubiquitin ligase, which modifies and controls p53 protein level [19].

Recent studies indicate that p53 protein translocation to the mitochondria can induce transcription-independent apoptosis through direct interaction with Bcl-2 family proteins which are located in the outer membrane of mitochondria [27]. Anti-apoptotic Bcl-2 proteins serve as a known sensor of apoptotic signaling that prevents apoptosis and is an important factor determining the fate of a DNA-damaged cell. Indeed, upregulation of pro-apoptotic Bax and Bak and downregulation of antiapoptotic

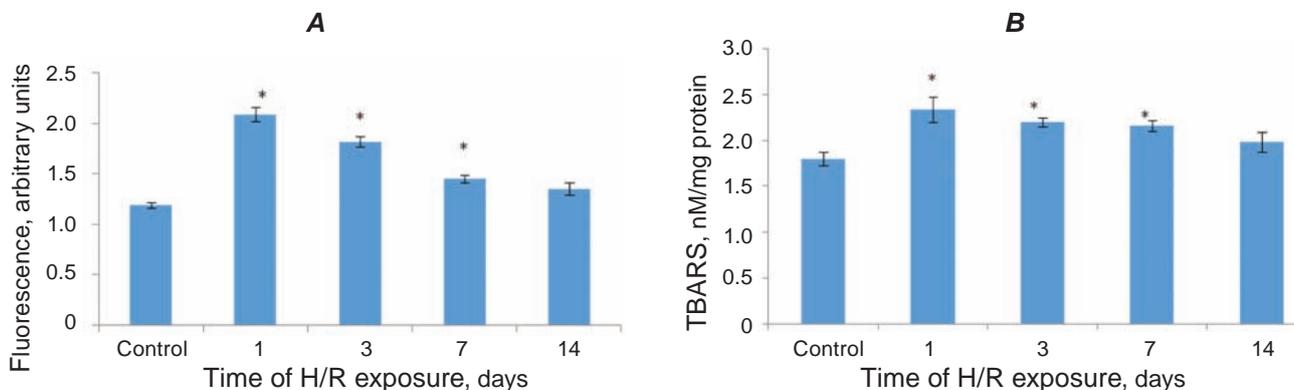


Fig. 1. Effect of hypoxia/reoxygenation (H/R) on mitochondrial ROS formation (A) and TBARS content (B). Values are mean \pm SD, *n* = 8 in each group. **P* < 0.05 vs control

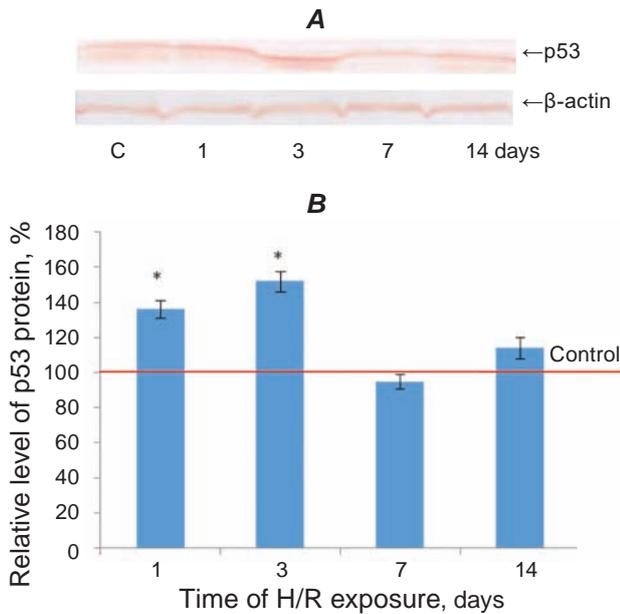


Fig. 2. Time-dependent changes of p53 protein expression in heart mitochondria under hypoxia/reoxygenation (H/R) conditions. (A) Representative Western blot and (B) densitometric analysis of p53 protein content. Isolated mitochondrial protein extracts were separated by performing SDS-PAGE and subsequently electroblotted onto PVDF membranes. Final Western blot figured as the histogram is expressed as mean percentages (\pm SD) over control values from three independent experiments. The control values are taken as 100%. Statistically significant differences are indicated as * $P < 0.05$ vs. control

Bcl-2 and Bcl-x1 result in marked disruption of mitochondrial membranes and subsequent cytochrome *c* release and procaspase-3 activation [28]. In our study, short-term H/R resulted in a remarkable rise in ROS formation as well as in the induction of p53 protein content that was associated with a decrease in Bcl-2 protein level in mitochondria (Fig. 3). These changes we registered after the 1st and 3rd days of H/R exposure (by 27 and 17%, $P < 0.05$, respectively), while during long-term H/R Bcl-2 protein content verges towards control level that is evidence of the reduced apoptotic process. We found a negative correlation between Bcl-2 protein content and ROS level ($r = -0.97$) and p53 ($r = -0.66$).

Although the fact of apoptosis induction during hypoxia was confirmed in several independent studies [19, 28], the exact mechanisms of this effect remain unknown. It was reported that the Hif-1 gene overexpression might play a trigger role in this pro-

cess because Hif-1 α can bind to the p53 and promote p53-dependent apoptosis [29]. In addition, it was found that prolonged intermittent hypoxic exposure induces myocardial tolerance against H/R injury in association with an elevation of Bcl-2 protein level through NF- κ B activation. This possibility is supported by recent studies that indicated that Bcl-2 overexpression limits apoptosis in I/R injury of heart and liver [28].

It is known that the relationship between p53 and ROS is multifactorial [11, 13, 18]. On the one hand, excessive ROS may cause p53 translocation to mitochondria and enhance mitochondrial oxidative stress leading to apoptosis. On the other hand, p53 can also affect ROS production and pro-/antioxidative balance in mitochondria by the impact on its targets MnSOD and GPx [9, 13-16]. We found that the repetitive situation of severe hypoxia followed by reoxygenation causes a disturbance of the mitochondrial pro-oxidant/antioxidant homeostasis, which appeared in the alteration of MnSOD and GPx protein

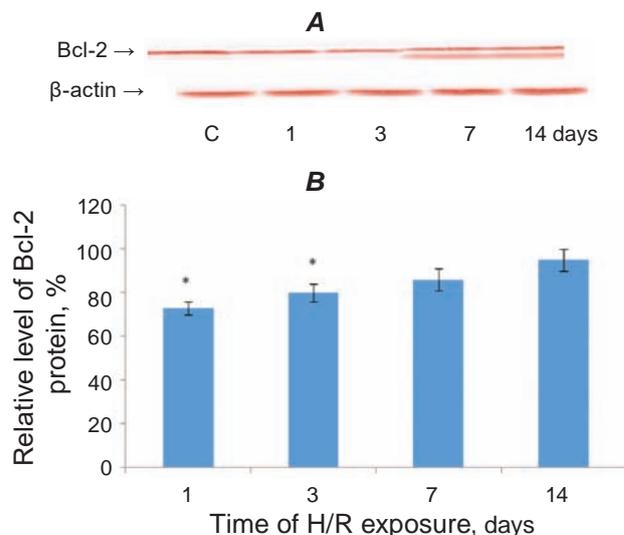


Fig. 3. Time-dependent changes of Bcl-2 protein expression in heart mitochondria under hypoxia/reoxygenation (H/R) conditions. (A) Representative Western blot and (B) densitometric analysis of Bcl-2 protein content. Isolated mitochondrial protein extracts were separated by performing SDS-PAGE and subsequently electroblotted onto PVDF membranes. Final Western blot figured as the histogram is expressed as mean percentages (\pm SD) over control values from three independent experiments. The control values are taken as 100%. Statistically significant differences are indicated as * $P < 0.05$ vs. control

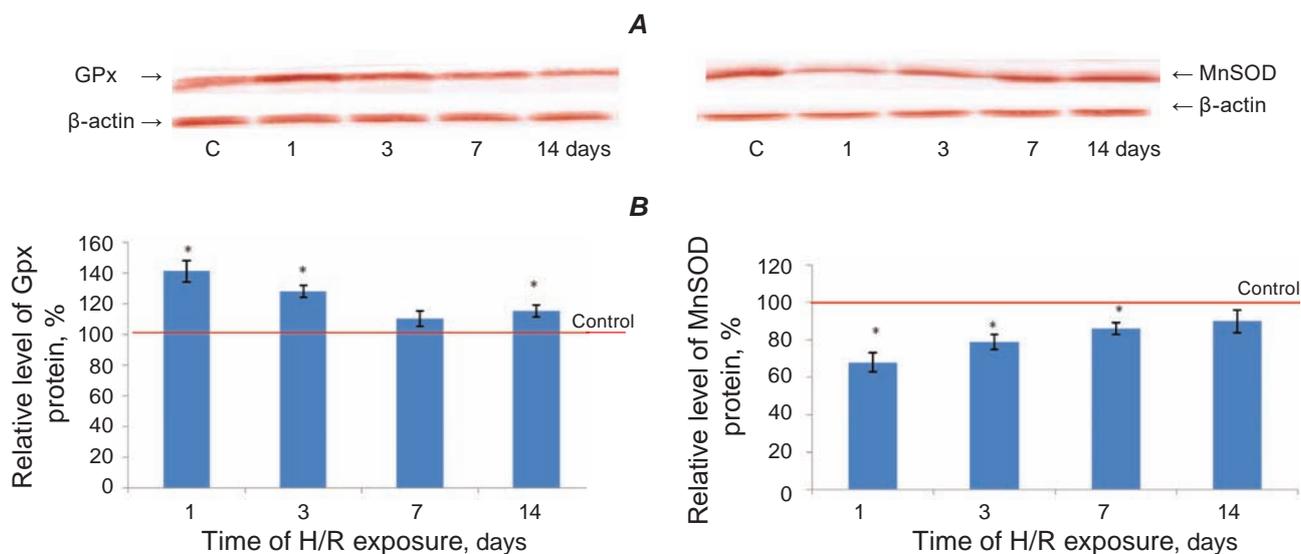


Fig. 4. Effect of short- and long-term hypoxia/reoxygenation on glutathione peroxidase (GPx) and MnSOD protein expression in heart mitochondria. (A) Representative Western blot and (B) densitometric analysis of GPx and MnSOD protein content. Isolated mitochondrial protein extracts were separated by performing SDS-PAGE and subsequently electroblotted onto PVDF membranes. Final Western blot figured as the histogram is expressed as mean percentages (\pm SD) over control values from three independent experiments. The control values are taken as 100%. Statistically significant differences are indicated as * $P < 0.05$ vs. control

expression and activity (Fig. 4-5). In mitochondria of rat heart at the early period of H/R exposure (1-3 days) we observed the reduction in MnSOD activity (by 18 and 27%, respectively, $P < 0.05$) but on 7th and 14th days this index was approximated to the basal level (Fig. 5).

MnSOD protein level dropped down below the control level by 32, 21, 14% ($P < 0.05$) and 10% after the 1st, 3rd, 7th and 14th days of H/R exposure (Fig. 4).

This study showed that MnSOD activity and protein levels in mitochondria of rat heart were decreased when high levels of the p53 expression were recorded (Fig. 2, 4-5). Thus, mitochondrial p53 protein content negatively correlated with MnSOD activity ($r = -0.63$) and MnSOD protein content ($r = -0.62$) which suggests the effect of p53 on the MnSOD functional activity/protein content under H/R condition in mitochondria of rat heart. Our findings are in agreement with other studies. Drane et al. reported that overexpression of p53 suppresses MnSOD transcription and that the level of MnSOD increased in the absence of p53 [15]. Pani G. et al. [16] demonstrated that E1ARas-transformed fibroblasts lacking p53 showed increased MnSOD expression in comparison to wild-type controls. Furthermore, transfection of HeLa cells with wt p53 led to a drop in MnSOD mRNA level and activity [18].

Taken together, these results describe MnSOD as a downstream target of p53, which can be specifically downregulated [13, 15].

The present data demonstrating the decrease in MnSOD activity indicate that mitochondrial superoxide radical is a potentially critical effector of oxidative processes in mitochondria of rat heart exposed to H/R. In our previous study, we demonstrated a decline in the MnSOD activity after severe hypoxia that is in accordance with an enhancement of superoxide production in mitochondria [8]. Consistent with the decreased MnSOD protein expression/activity found in our study, some authors [30] also observed that severe hypoxia in sessions of intermittent hypoxia causes a decrease in MnSOD protein expression *in vivo* with a marked diminution in the dismutating capacity in the liver, heart and brain tissues of animals subjected to intense intermittent hypoxia.

Conceivably, an inadequate low MnSOD activity could result in a rise in the level of $O_2^{\cdot-}$ and subsequent OH^{\cdot} formation via a Haber-Weise reaction, as well as in peroxynitrite formation due to mitochondrial NO production by iNOS. It was reported that severe hypoxia increases mitochondrial peroxynitrite formation and will ultimately lead to nitration and inactivation of MnSOD [31]. Moreover, p53 can

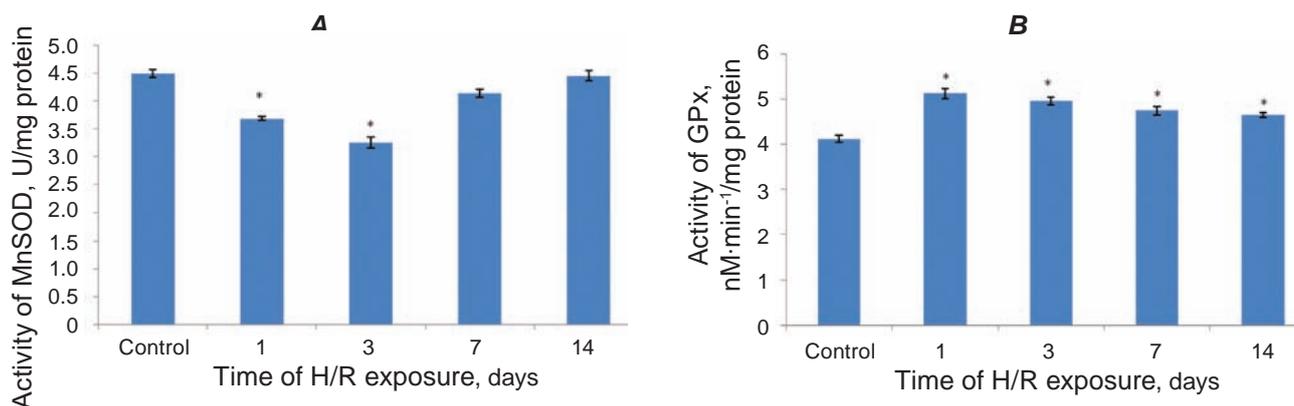


Fig. 5. Effect of short- and long-term hypoxia/reoxygenation on activities of MnSOD (A) and glutathioneperoxidase (B) in heart mitochondria. Values are mean \pm SD, $n = 8$ in each group. The data were analyzed for statistical significance using ANOVA followed by Bonferroni posthoc test. * $P < 0.05$ vs control

directly inhibit MnSOD catalytic activity by physically interacting with the enzyme in mitochondria [11]. However, the actual mechanism can be more complicated with other players possibly being involved. It is widely known that MnSOD regulation is complex and occurs at both pre- and post-translational levels. Moreover, MnSOD mRNA levels can be up- or downregulated by several factors including VEGF, AP-2, Egr-1, Sp-1, p53, HIF-2 α , PKC-NF- κ B, PI3K-Akt-Forkhead signaling pathways [10, 31] depending on the stress levels.

As shown in Fig. 2 and 4, in mitochondria of rat heart in parallel with the p53 induction we registered the increase in GPx protein synthesis in a time-dependent manner, reaching a maximum at the 1st day (~141%) followed by the reduction of GPx protein expression to 128% on the 3rd day and to 110-115% on the 7th and 14th days ($P < 0.05$) of H/R exposure. A close positive correlation was identified between p53 and GPx protein content ($r = 0.69$). The GPx activity remained higher in comparison with control during the study period (Fig. 5). Our findings are consistent with previous studies showing that the high level of oxidative and nitrosative stresses promoted to induction of GPx mRNA transcription as well as protein expression in various cell lines [7].

In conclusion, we have found that severe hypoxia in sessions of H/R differentially influenced on mitochondrial oxidative stress level, mitochondrial p53 protein accumulation, antioxidant capacity as well as Bcl-2 protein content and these effects were time-dependent ones. Our data indicate that short-term H/R activates the p53 pathway as an oxidative stress response leading to the elevation in mitochon-

drial protein levels of p53 in parallel with its target GPx. At the same time, a decrease in MnSOD protein expression/activity and anti-apoptotic Bcl-2 protein content induce mitochondrial prooxidant/antioxidant disbalance. In contrast, prolonged H/R promotes cell survival through upregulation of proteins that prevent apoptosis and promotes adaptation process forming.

ВПЛИВ ГІПОКСІЇ/РЕОКСИГЕНАЦІЇ РІЗНОЇ ТРИВАЛОСТІ НА РІВЕНЬ ОКИСНОГО СТРЕСУ, АНТИОКСИДАНТНИЙ СТАТУС І НАКОПИЧЕННЯ p53 В МІТОХОНДРІЯХ СЕРЦЯ

O. O. Гончар, I. M. Маньковська

Інститут фізіології ім. О. О. Богомольця
НАН України, Київ;
e-mail: olga.gonchar@i.ua

У мітохондріях серця щурів вивчали вплив періодичних сеансів гіпоксії та реоксигенації ((Г/Р), 10-хвилинне дихання газовою сумішшю з 5,5% O₂ в азоті, яке чергувалося з 10-хвилинними нормоксичними інтервалами, щодня) на інтенсивність окислювальних процесів, експресію антиапоптичного протеїну Bcl-2, а також антиоксидантних ензимів MnСОД і глутатіонпероксидази та їх регулятора – транскрипційного фактора p53. Показано, що нетривалі сеанси Г/Р (протягом 1–3 днів) збільшували утворення вільних радикалів, посилювали процеси пероксидного окислен-

ня ліпідів, спричинювали транслокацію p53 з цитозолю в мітохондрії. Відзначали зниження активності/експресії протеїну MnСОД, Bcl-2, а також збільшення активності та вмісту протеїну глутатіонпероксидази. Тривалі впливи Г/Р (7–14 днів) зумовлювали зниження рівня p53 в мітохондріях, збільшували вміст протеїну Bcl-2 і MnСОД, що сприяло формуванню толерантності міокарда до зміни рівня кисню. Наявність близьких кореляційних зв'язків між рівнем p53 і вмістом протеїну MnСОД і глутатіонпероксидази, а також інтенсивністю вільнорадикальних процесів, дозволяє припустити активну участь p53 в регуляції окисного стресу, індукованого сеансами Г/Р різної тривалості.

Ключові слова: гіпоксія/реоксигенація, Bcl-2, p53, MnСОД, глутатіонпероксидаза, мітохондрії серця.

ВЛИЯНИЕ ГИПОКСИИ/РЕОКСИГЕНАЦИИ РАЗЛИЧНОЙ ПРОДОЛЖИТЕЛЬНОСТИ НА УРОВЕНЬ ОКИСЛИТЕЛЬНОГО СТРЕССА, АНТИОКСИДАНТНЫЙ СТАТУС И НАКОПЛЕНИЕ p53 В МИТОХОНДРИЯХ СЕРДЦА

О. А. Гончар, И. Н. Маньковская

Институт физиологии им. А. А. Богомольца
НАН Украины, Киев;
e-mail: olga.gonchar@i.ua

В митохондриях сердца крыс изучали влияние периодических сеансов гипоксии и реоксигенации ((Г/Р), 10-минутное дыхание газовой смесью с 5,5% O₂ в азоте, которое чередовалось с 10-минутными нормоксическими интервалами, ежедневно) на интенсивность окислительных процессов, экспрессию антиапоптотического протеина Bcl-2, а также антиоксидантных энзимов MnСОД и глутатіонпероксидазы и их регулятора – транскрипционного фактора p53. Показано, что непродолжительные сеансы Г/Р (в течение 1–3 дней) увеличивали образование свободных радикалов, усиливали процессы пероксидного окисления липидов, вызывали транслокацию p53 из цитозоля в митохондрии. При этом отмечалось снижение активности/экспрессии протеина MnСОД, Bcl-2, а также увеличение активности и содержание протеина глутатіон-

пероксидазы. Продолжительные воздействия Г/Р (7–14 дней) вызывали снижение уровня p53 в митохондриях, увеличивали содержание Bcl-2 и MnСОД, что способствовало формированию толерантности миокарда к меняющемуся уровню кислорода. Наличие близких корреляционных связей между уровнем p53 и содержанием протеина MnСОД и глутатіонпероксидазы, а также интенсивностью свободнорадикальных процессов, позволяет предположить активное участие p53 в регуляции окислительного стресса, индуцированного сеансами Г/Р различной продолжительности.

Ключевые слова: гипоксия/реоксигенация, Bcl-2, p53, MnСОД, глутатіонпероксидаза, митохондрии сердца.

References

1. Li C, Jackson RM. Reactive species mechanisms of cellular hypoxia-reoxygenation injury. *Am J Physiol Cell Physiol.* 2002; 282(2): C227-C241.
2. Bartosz G. Reactive oxygen species: destroyers or messengers? *Biochem Pharmacol.* 2009; 77(8): 1303-1315.
3. Sies H. Oxidative stress: a concept in redox biology and medicine. *Redox Biol.* 2015; 4: 180-183.
4. Limón-Pacheco J, Gonsbatt ME. The role of antioxidants and antioxidant-related enzymes in protective responses to environmentally induced oxidative stress. *Mutat Res.* 2009; 674(1-2): 137-147.
5. Gonchar O, Mankovska I. Antioxidant system in adaptation to intermittent hypoxia. *J Biol Sci.* 2010; 10(6): 545-554.
6. Pardo M, Tirosh O. Protective signalling effect of manganese superoxide dismutase in hypoxia-reoxygenation of hepatocytes. *Free Radic Res.* 2009; 43(12): 1225-1239.
7. Arrigo AP. Gene expression and the thiol redox state. *Free Radic Biol Med.* 1999; 27(9-10): 936-944.
8. Gonchar O. Effect of intermittent hypoxia different regimes on mitochondrial lipid peroxidation and glutathione-redox balance in stressed rats. *Cent Europ J Biol.* 2008; 3(3): 233-242.
9. Hussain SP, Amstad P, He P, Robles A, Lupold S, Kaneko I, Ichimiya M, Sengupta S, Mechanic L, Okamura S, Hofseth LJ, Moake M, Nagashima M, Forrester KS, Harris CC. p53-

- induced up-regulation of MnSOD and GPx but not catalase increases oxidative stress and apoptosis. *Cancer Res.* 2004; 64(7): 2350-2356.
10. Ma Q. Transcriptional responses to oxidative stress: pathological and toxicological implications. *Pharmacol Ther.* 2010; 125(3): 376-393.
 11. Liu B, Chen Y, St Clair DK. ROS and p53: a versatile partnership. *Free Radic Biol Med.* 2008; 44(8): 1529-1535.
 12. Minchenko DO, Danilovskyi SV, Kryvdiuk IV, Bakalets TV, Lypova NM, Karbovskyi LL, Minchenko OH. Inhibition of ERN1 modifies the hypoxic regulation of the expression of TP53-related genes in U87 glioma cells. *Endoplasm Reticul Stress Dis.* 2014; 1(1): 18-26.
 13. Borrás C, Gómez-Cabrera MC, Viña J. The dual role of p53: DNA protection and antioxidant. *Free Radic Res.* 2011; 45(6): 643-652.
 14. Tan M, Li S, Swaroop M, Guan K, Oberley LW, Sun Y. Transcriptional activation of the human glutathione peroxidase promoter by p53. *J Biol Chem.* 1999; 274(17): 12061-12066.
 15. Drane P, Bravard A, Bouvard V, May E. Reciprocal down-regulation of p53 and SOD2 gene expression-implication in p53 mediated apoptosis. *Oncogene.* 2001; 20(4): 430-439.
 16. Pani G, Bedogni B, Anzevino R, Colavitti R, Palazzotti B, Borrello S, Galeotti T. Deregulated manganese superoxide dismutase expression and resistance to oxidative injury in p53-deficient cells. *Cancer Res.* 2000; 60(16): 4654-4660.
 17. Zhao Y, Chaiswing L, Velez JM, Batinic-Haberle I, Colburn NH, Oberley TD, St Clair DK. p53 translocation to mitochondria precedes its nuclear translocation and targets mitochondrial oxidative defense protein-manganese superoxide dismutase. *Cancer Res.* 2005; 65(9): 3745-3750.
 18. Lebedeva MA, Eaton JS, Shadel GS. Loss of p53 causes mitochondrial DNA depletion and altered mitochondrial reactive oxygen species homeostasis. *Biochim Biophys Acta.* 2009; 1787(5): 328-334.
 19. Koumenis C, Alarcon R, Hammond E, Sutphin P, Hoffman W, Murphy M, Derr J, Taya Y, Lowe SW, Kastan M, Giaccia A. Regulation of p53 by hypoxia: dissociation of transcriptional repression and apoptosis from p53-dependent transactivation. *Mol Cell Biol.* 2001; 21(4): 1297-1310.
 20. Gonchar O, Mankovska I. Hypoxia/reoxygenation modulates oxidative stress level and antioxidative potential in lung mitochondria: possible participation of p53 and NF- κ B target proteins. *Arch Pulmonol Respir Care.* 2017; 3(2): 35-43.
 21. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* 1976; 72(1-2): 248-254.
 22. Young TA, Cunningham CC, Bailey SM. Reactive oxygen species production by the mitochondrial respiratory chain in isolated rat hepatocytes and liver mitochondria: studies using myxothiazol. *Arch Biochem Biophys.* 2002; 405(1): 65-72.
 23. Buege JA, Aust SD. Microsomal lipid peroxidation. *Methods Enzymol.* 1978; 52: 302-310.
 24. Flohé L, Günzler WA. Assays of glutathione peroxidase. *Methods Enzymol.* 1984; 105: 114-21.
 25. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem.* 1972; 247(10): 3170-3175.
 26. Iqbal J, Whitney P. Use of cyanide and diethyldithiocarbamate in the assay of superoxide dismutases. *Free Radic Biol Med.* 1991; 10(1): 69-77.
 27. Speidel D. Transcription-independent p53 apoptosis: an alternative route to death. *Trends Cell Biol.* 2010; 20(1): 14-24.
 28. Dong JW, Zhu HF, Zhu WZ, Ding HL, Ma TM, Zhou ZN. Intermittent hypoxia attenuates ischemia/reperfusion induced apoptosis in cardiac myocytes via regulating Bcl-2/Bax expression. *Cell Res.* 2003; 13(5): 385-391.
 29. Suzuki H, Tomida A, Tsuruo T. Dephosphorylation hypoxia-inducible factor 1 α as a mediator of p53-dependent apoptosis during hypoxia. *Oncogene.* 2001; 20(41): 5779-7588.
 30. Quintero M, Gonzalez-Martin Mdel C, Vega-Agapito V, Gonzalez C, Obeso A, Farré R, Agapito T, Yubero S. The effects of intermittent hypoxia on redox status, NF- κ B activation, and plasma lipid levels are dependent on the lowest oxygen saturation. *Free Radic Biol Med.* 2013; 65: 1143-1154.
 31. Yamakura F, Kawasaki H. Post-translational modifications of superoxide dismutase. *Biochim Biophys Acta.* 2010; 1804(2): 318-325.

Received 29.06.2017