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Hepatoprotective effects of capping protein gelsolin against hyperoxia-induced hepatotoxicity, oxidative stress and DNA damage in neonatal rats

Sedat Per[a,*], Mehmet Kose[b], Ahmet Ozdemir[c], Dilek Pandir[a]

[a] Department of Biology, Bozok University, Yozgat, Turkey
[b] Department of Pediatrics, Division of Pediatric Pulmonology Unit, Erciyes University, Kayseri, Turkey
[c] Department of Pediatrics, Division of Neonatology, Erciyes University, Kayseri, Turkey

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ABSTRACT

Tissues and organs get exposed to high oxygen (O_2) supply in hyperoxia conditions. The goal of this research was to investigate the protective effect of actin binding protein gelsolin on hyperoxia-induced hepatotoxicity through histopathology and measurement of oxidative stress parameters and DNA damage in a neonatal Wistar albino rats. The pups were randomly separated to four equal groups such as: normoxia control group (NC), normoxia plus gelsolin group (NG, 10 ng/kg bw/day gelsolin), hyperoxia (≥85% O_2) group (HC), hyperoxia plus gelsolin group (HG, ≥85% O_2; 10 ng/kg bw/day gelsolin). Histopathological changes of pups in hyperoxia condition were revealed in the form of severe leukocyte infiltration, vascular congestion, necrosis, vacuolar degeneration, binucleated hepatocytes and hemorrhage in the liver tissue. SOD, CAT, GPx and GST activities decreased and MDA level increased in the hyperoxia-induced group in liver tissue ($P < 0.05$). Tail DNA%, tail length and moment indicating DNA damage statistically increased in hyperoxia treatment groups when compared to controls. Treatment of rats with hyperoxia plus gelsolin prevented hyperoxia-induced changes in tissue structure, antioxidant enzyme activities and MDA level, mean tail DNA% and length. Based on these findings, gelsolin restored these changing to near normal levels but it does not protect completely in the hyperoxia conditions.

1. Introduction

Premature babies can be inside a well-ventilated room and exposed to extra oxygen for a long time and some bodies of infants may be damaged for survival (Askie et al., 2011). Resistance to hyperoxia, above the normal physiological levels of O_2 (0.21), can be used as an indicator of the longevity potential of an organism. Hyperoxia has been shown to reduce the lifespan and induce a similar pattern of gene expression with aging and oxidative damage (Landis et al., 2004). In accordance with these findings, there are positive correlation between mammalian longevity and reduction of oxidative stress (Kapahi et al., 1999).

Production of various types of reactive nitrogen and oxygen species can directly harm biomolecules and can lead to cell death, especially under prolonged oxygen supplementation (Potteti et al., 2013). The major antioxidant enzymes may alleviate effect of toxic reactive species. Oxidative stress occurs when the production of reactive oxygen species exceeds the capacity of the antioxidative mechanisms to neutralize them (Sobočanec et al., 2016)

Gelsolin (GSN) is an important protein because of arranging actin assembly and disassembly and the structure of the actin network for cell motility. GSN is found both plasma and cytoplasm (Kwiatkowski et al., 1986; Sun et al., 1999) from lower eukaryotes to higher mammals (Napolitanoa et al., 2014). It has an important role as actin filament-divided proteins identified up to date (Spinardi and Witke, 2007). It bind to the actin filament and does not allow the rapidly growing of filament and then many of short actin filaments are generated as an unwanted structure. Finally, the actin network is disassembled. Therefore, cell morphology, growth and apoptosis can be controlled by GSN (Kwiatkowski, 1999). It is involved in signaling pathways of apoptosis in normal human cells (Kankaya et al., 2015).

Protein expression is changing during carcinogenesis so recent advancement in proteomics is using as a powerful tool for the identification of potential biomarker in cancers (Zhang et al., 2015). Tumors cell synthesize GSN protein at a higher level. It has been shown that it is frequently down regulated in various cancers, such as lung, breast and prostate cancer (Asch et al., 1999; Lee et al., 1999; Sagawa et al., 2003). However, there are differences in various types tumors that have similar stage and grade with regard to biological behavior and clinical outcome (Kankaya et al., 2015)

* Corresponding author at: Bozok University, Faculty of Arts and Science Department of Biology, Yozgat, 66200, Turkey.
E-mail address: sedat.per@bozok.edu.tr (S. Per).

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Malondialdehyde (MDA) is an indicator of lipid peroxide (LPO) and is produced during peroxidation of polyunsaturated fatty acids. Intracellular antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) exterminate reactive oxygene species under physiological conditions, hereby they protect the cell against the antioxidative stress (Bukowska, 2004). Administration of any stress on human causes an increase in the MDA levels and decrease in the activities of enzymes that play an integral role in cell defence (Nazıroğlu et al., 2004). Gelsolin has been investigated for its beneficial effect on human health, especially its anti-carcinogenic, anti-apoptotic, anti-oxidant and anti-amyloidogenic properties (Chauhan et al., 2008). Ji et al. (2008) shown that lipid peroxidation and oxidative stress increased in PC12 cells (differentiated and undifferentiated) upon treatment with H_2O_2 as concentration-dependent manner. Intracellular gelsolin may increase in the various reaction especially redox (McDonagh and Sheehan, 2007) and oxidative stress in the cell because of antioxidant properties (Chauhan et al., 2008).

However, currently there is no available information about hepatotoxicity in hyperoxia condition. As stated before, liver toxicity can occur when O_2 is administered in high levels. The goal of this work is to search the effect of hyperoxia exposure on the liver tissue of neonatal rats and to investigate whether administration of gelsolin may alleviate these changes. For this reason, neonatal rats were applied hyperoxia and/or gelsolin for seven days and then histopathological changes, malondialdehyde (MDA) levels, SOD, CAT, GPx and GST activities and DNA damage in pups' liver tissue were determined.

2. Material and methods

2.1. Materials

Gelsolin and RPMI-1640 were bought from Sigma–Aldrich. Other chemicals were took from Sigma.

2.2. Animal treatment protocol

The experiments were approved by the state authorities and followed ethical guidelines on the care and use of animals. The protocol used in this study was endorsed by the Erciyes University Animal Experiments Local Ethics Committee. Sexually mature female Wistar albino rats bred in laboratory with pellet food and ad libitum. Housing was at 22–24 °C with provided 12 h sunlight and after 12–24 h of delivery pups were included in the study. The rat pups (10 days old) were randomly divided into four groups and each containing ten rats pups were housed in stainless-steel cages.

The pups were distributed into four experimental groups. Each group consisted of 10 rats in the beginning of the study. Normoxia plus gelsolin group (n = 10, treated with 10 ng/kg bw/day gelsolin), hyperoxia group (n = 10), hyperoxia plus gelsolin group (n = 10, treated with 10 ng/kg bw/day gelsolin). Normoxia and gelsolin groups were kept at room air. Hyperoxia and hyperoxia plus gelsolin groups were put in plexiglass chambers which could allow maintaining oxygen levels ≥85% together with their mothers and oxygen level was monitored in the chambers. CO_2 in the environment was absorbed using soda lime. Mothers were rotated between hyperoxia and normoxia groups every 24 h.

During the study, all groups were given equal volumes of intraperitoneal (i.p.) injections. The gelsolin preparation was diluted with in water at a concentration of about 5 μg/ml. The NC group was given serum physiological at ambient air. The normoxia plus gelsolin groups were given 10 ng/kg bw/day gelsolin at ambient air. The Hyperoxia group was given ≥85% O_2 as well as i.p. serum physiological. The hyperoxia plus gelsolin groups were given ≥85% O_2 and 10 ng/kg bw/day. Experimental first day was accepted beginning of the application to animal. At the end of the 7 days of treatment, the pups were sacrificed under general anesthesia by an intraperitoneal injection of ketamine hydrochloride (60 mg/kg, Ketalar) and xylazine hydrochloride (10 mg/kg) and dissected and liver tissue samples in each group were obtained to determine histological changes *via* light microscope examination, antioxidative defence activities and MDA level and DNA damage of liver tissue.

2.3. Histopathology

Liver tissues of pups were dissected and fixed in 10% neutral formalin for 24 h for histopathological analysing. The samples were then kept the graded ethanol series and embedded in paraffin. Paraffin block were cut into 5 μm using a microtome (Leica RM2255). Slides were stained with hematoxylin and eosin dye. Light microscope (Olympus BX53, Tokyo, Japan) was used for obtaining image and its attached camera (Olympus DP72 Olympus Optical Co., Ltd., Japan) was used for photographing. From each tissue, 10 slides were prepared. Histopathological changes in all of the groups were scored and determined for intensity of pathologic changes [none (-), moderate (+) and severe (++) damage].

2.4. Biochemical analyses

After dissection, liver tissue was washed in sodium phosphate buffer (pH 7.2), then stored at −80 °C until the analysis. A teflon homogenizer (IKA T18) was used for homogenization of the tissues and the homogenate were centrifuged at 10,000*g* for 15 min at 4 °C. A spectrophotometer (Shimadzu UV 1800, Kyoto, Japan) was used for measuring absorbance changing of MDA level and antioxidant enzyme activities of the liver tissue.

2.5. Assessment of biochemical structure

2.5.1. Determine of malondialdehyde (MDA) level

The thiobarbituric acid (TBA) test was used for determining MDA level in cell. (Ohkawa et al., 1979). Formed MDA and TBA complex given colored structure and these stuctures were measured spectrophotometrically at 532 nm to define MDA levels. The level was determined as nmol/mg protein.

2.5.2. Determine of superoxide dismutase (SOD)

Obtained inhibition of autoxidation of pyrogallol demonstrate SOD activity according to Marklund and Marklund's method (Marklund and Marklund, 1974). Activity of SOD was measured at 440 nm for 180 s. This activity was stated as USOD/mg protein.

2.5.3. Determine of catalase (CAT)

The level of decomposition hydrogen peroxide (H_2O_2) in liver tissue was defined for CAT activity according to the method of Aebi (Aebi, 1984). Activity of CAT was explained as UCAT/mg protein.

2.5.4. Determine of glutathione peroxidase (GSH-Px)

GPx activity was analysed via hydrogen peroxide as substrate by the study of Paglia and Valentine's work (Paglia and Valentine, 1987). The oxidation of NADPH was monitored at 240 nm. Activity of GPx was pointed out as UGPx/mg protein.

2.5.5. Determine of glutathione- S-transferase (GST) activity

Enzyme activities of GST of livers were analysed by determination of the generation of glutathione and the 1-chloro 2,4-dinitrobenzene conjugate (Habig et al., 1974). Increments in absorbance were stated at 340 nm. The enzyme is represented as units of glutathione 1-chloro 2,4-dinitrobenzene conjugate formed per minute per milligram protein.

2.5.6. Determine of protein concentration

The protein concentration of samples was measured according to