Original Article

Protective Effect of Ozone against *Hemiscorpius lepturus* Envenomation in Mice*

Parvaneh Naserzadeh[1], Farshad Shahi[2,3], Delavar Shahbazzadeh[4], Mostafa Ghanei[5], Khadijeh Ashtari[6], Yoones Panahi[5], Mir-Jamal Hosseini[7,8,#], and Morteza Izadi[3,9,#]

1. Department of Pharmacology and Toxicology, Faculty of Pharmacy, ShahidBeheshti University of Medical Sciences, Tehran 14155-6153, Iran; 2. Ozone Complementary Research Center, Baqiyatallah University of Medical Sciences, Tehran 14359-16471, Iran; 3. Young Researchers and Elite Club, Islamic Azad University, Tehran Medical Sciences Branch, Tehran 19168-93814, Iran; 4. Biotechnology Research Center, Pasteur Institute of Iran, Medical Biotechnology Group, Venom and Toxin Lab, Tehran 13169-43551, Iran; 5. Chemical Injuries Research Center, Baqiyatallah University of Medical Sciences, Tehran 14359-16471, Iran; 6. Department of medical nanotechnology Faculty of Advanced technology in medicine, Iran university of Medical Sciences, Tehran 14496-14535, Iran; 7. Department of Pharmacology and Toxicology, School of Pharmacy, Zanjan University of Medical Sciences, Zanjan 45139-56184, Iran; 8. Zanjan Applied Pharmacology Research Center, Zanjan University of Medical sciences, Zanjan 45139-56184, Iran; 9. Molecular Biology Research Center, Baqiyatallah University of Medical Sciences, Tehran 14359-16471, Iran

Abstract

Objective Scorpion (*Hemiscorpius lepturus*) stings are a public health concern in Iran, particularly in south and southwestern regions of Iran. The gold standard for the treatment of a scorpion sting is anti-venom therapy. However, immunotherapy can have serious side effects, such as anaphylactic shock (which can sometimes even lead to death). The aim of the current study was to demonstrate the protective effect of ozone against toxicity induced by Hemiscorpius lepturus (*H. lepturus*) venom in mice.

Methods Eight hours after the injection of ozone to the experimental design groups, the male mice were decapitated and mitochondria were isolated from five different tissues (liver, kidney, heart, brain, and spinal cord) using differential ultracentrifugation. Then, assessment of mitochondrial parameters including mitochondrial reactive oxidative species (ROS) production, mitochondrial membrane potential (MMP), ATP level, and the release of cytochrome c from the mitochondria was performed.

Results Our results showed that *H. lepturus* venom-induced oxidative stress is related to ROS production and MMP collapse, which is correlated with cytochrome c release and ATP depletion, indicating the predisposition to the cell death signaling.

Conclusion In general, ozone therapy in moderate dose can be considered as clinically effective for the treatment of *H. lepturus* sting as a protective and antioxidant agent.

Key words: *Hemiscorpius lepturus*; Venom; O_3/O_2 mixture (ozone); Oxidative stress

Biomed Environ Sci, 2017; 30(8): 581-590 *doi: 10.3967/bes2017.077* *ISSN: 0895-3988*

www.besjournal.com (full text) *CN: 11-2816/Q*

*This work was supported by Molecular Biology Research Center, Baqiyatallah University of Medical Sciences (NO.340-5-5771.Sin).

#Correspondence should be addressed to Mir-Jamal Hosseini, Assistant Professor, E-mail: jamal_hossini@yahoo.com, Tel: 98-912-5030167, Fax: 98-2433473639; Morteza Izadi, E-mail: Morteza_izadi@yahoo.com, Tel: 98-051-82483250, Fax: 98-051-88040106.

Biographical note of the first author: Parvaneh Naserzadeh, born in 1988, PhD candidate, majoring in mechanistic toxicology.

INTRODUCTION

Ozone (O_3/O_2 mixture) therapy has been used in the treatment of ischemic disorders; however, there are no data from clinical trials yet, and it has not yet been accepted as standard medicine in all countries[1]. It has been suggested that ozone could stimulate antioxidant system, regulate inflammatory responses, improve vascular rheology, and increase blood flow in cerebral arteries and tissue oxygenation in hypoxic tissues[2]. It shows that the therapeutic efficacy of ozone is related to the controlled and moderate oxidative stress as a result of ozone reactions with biological components[2].

The recent published data found that scorpion (*Hemiscorpius lepturus*) toxin can lead to high toxicity in the kidney, heart, liver, lungs, stomach, and intestines, which is similar to massive histopathological alterations results in kidney and liver. In addition, it seems that the kidney and heart have a higher sensitivity to damage after subcutaneous (SC) envenoming in mice[3-4]. Moreover, it has been reported that the immunotoxicity of *H. lepturus* venom is related to the over-expression of IL-12, TNF-α, IL-6, and IL-10 in human monocytes[5-9].

An *in vivo* study in rats showed a significant increase in amino-alanine peptidase (AAP) and N-acetyl-β-D-glucosaminidase (NAG) enzyme activity, which are biomarkers of nephrotoxicity and acute renal failure (ARF), and this was confirmed by histopathological abnormalities. This was associated with severe anemia, thrombocytopenia, pyuria, hematuria, and considerable proteinuria[10-11]. In addition, inhibition of pyrimidine, histidine, and tyrosine metabolism and steroid hormone biosynthesis was observed[12].

H. lepturus venom toxicity is endemic in the southwestern province of Khuzestan and in other parts of western Iran, and this scorpion is responsible for 10% of the reported stings with high mortalities (approximately 19.5 deaths each year), mostly during spring and summer[13-15]. The toxic *H. lepturus* sting causes severe damage in high perfusion organelles, which are very sensitive to oxidative stress due to high oxygen consumption, high concentrations of polyunsaturated fatty acids, and low levels of some antioxidant enzymes[10,16].

Unfortunately, the lack of basic information on clinical manifestations and specific treatments has led to problems in designing clinical trials. Mitochondria are the important organelles during oxidative stress induction due to their poor antioxidant system and high lipid content[2]. Taking into consideration the limited data on the possible mechanisms of *H. lepturus* venom toxicity, we planned to study the protective effect of ozone against *H. lepturus* venom–induced oxidative stress in different tissues through precise measurements using various multi-parametric assays.

MATERIALS AND METHODS

Scorpion Venom

H. lepturus venom from Khuzestan (Iran) was collected by the veterinarian service of the RAZI Vaccine Development and Serum Research Institute of Iran and stored under refrigeration conditions (-20 °C) until use.

Medozone

Medozone (HAB company 2015, Ozone generator, UMDNS-Nr.12899, power supply = 230/115V-50/Hz, Power input = 240 VA) was used for the generation of the oxygen/ozone mixture (O_3/O_2 ratio = 99.95%/0.05%) for medical application. The ozone concentrations were adjusted to 5, 30, and 80 μg per mouse to determine the optimal effective dose. The ozone concentration was regulated by dosimeter with flow rate of 0.8 L/min, and the total ozone volume in the injection was 8 mL/kg.

Animals

Overall, 48 male *BaLb/c* mice (28 ± 2 g) were purchased from Pasteur Institute of Iran to be used in the current study. The mice were housed in an air-conditioned room with a controlled temperature of 25 ± 2 °C and maintained on a 12:12 h light cycle with free access to food and water. The experiments were performed in the same lab in accordance with the institutional guidelines for animal care and use. Each mouse, in all experimental groups, was injected a 100 μL volume of the venom solution. The approximate median lethal dose (LD50) of the crude venom was found to be 5.6 mg/kg after IP injection in *Balb/c* mice, which is equal to 140 μg per mouse. Then, the effective lethal dose, which can cause death in 3-5 min after a single IP injection of venom in the mice, was determined to be equal to 1 mg of venom per mouse.