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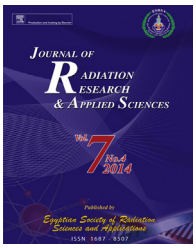

Study of electron spin resonance and viscosity for hemoglobin polymer after arsenic trioxide and gamma irradiation treatment

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ABSTRACT

The present work aimed to study the intensity of electron spin resonance (ESR) of hemoglobin rat's polymer and changes in rheological properties (viscosity). This study included five groups: control, irradiation with single dose of 5 Gy gamma irradiation, intraperitoneal injection with single dose of arsenic trioxide (As_2O_3) 10 mg/kg body weight, As_2O_3 followed by 5 Gy and 5 Gy followed by As_2O_3. The results of ESR spectroscopy indicated that the intensity increases significantly for As_2O_3 and non-significantly with 5 Gy of gamma radiation compared with control, which indicated an increase in the number of free radicals. Injection with As_2O_3 after and before 5 Gy resulted in non-significant decrease which given rise to a decrease in the number of free radicals. there was significant decrease in viscosity for 5 Gy at different shear rates (11.3–375 sec^{-1}), viscosity for As_2O_3 injection group showed non-significant decrease, and non-significant increase in case of 5 Gy followed by As_2O_3 injection, significant decrease at high shear rate in case of 5 Gy followed by As_2O_3. These results concluded that 5 Gy followed by As_2O_3 showed some sort of repair in the function of rats hemoglobin rather than injection with As_2O_3 and 5 Gy both individually.

1. Introduction

Arsenic agents have been used as anti-cancer agents in traditional Chinese medicine (Antman, 2001). Also, usage of As_2O_3 in therapy has been approved by the U.S. Food and Drug Administration (FDA) (Zhu, Chen, Lallemand-Breitenbach, & De Thé, 2002), it is reported to be an effective inducer of apoptosis in certain cancer cells including acute promyelocytic leukemia (APL), other myeloid

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leukemic cells, osephageal, prostate and ovarian carcinomas (Maeda et al., 2001; Shen, Shen, Cai, Hong, & Zheng, 2000; Uslu et al., 2000). Ionizing radiation is one of the most common radiotherapy techniques used for cancer patients. Since arsenic and radiation caused the production of free radicals (Nishigori, Hattori, & Toyokuni, 2004; Yamanaka, Hasegawa, Sawamura, & Okada, 1989; Yamanaka et al., 1990). Thus, one of the important points in the development of a new anti-cancer drug is to understand the effect of As_2O_3 and gamma irradiation and the combination of them on electron spin resonance and viscosity of hemoglobin molecule. One advantage of As_2O_3 is its capability to penetrate into the subcellular compartments of living cells (Bacquart, Deves, & Ortega, 2010). Also arsenic interact not only with the DNA molecules, but also with different proteins and important enzymes with the cell, the most reactive trivalent form of arsenic has a preference for SH groups present in various essential compounds. Trivalent arsenicals have a binding affinity for the cysteine residues present on cellular protein molecules (Zhou et al., 2006). Besides a number of mechanisms that have been proposed, oxidative stress has now become one of the major factors in arsenic induced toxic effects (Mishra, Mehta, & Flora, 2008). For most species, as much as 50–70% of absorbed arsenate As (V) is rapidly reduced to arsenite As (III) (Vahter, 1999), and this reduction happens primarily in the blood (Marafante, Vahter, & Envall, 1985; Vahter & Marafante, 1983). Arsenic may exert its toxic effects by generating radicals like superoxide anion, hydroxyl radicals, and hydrogen peroxides (Ito, Okamoto, & Kato, 1998). Free radicals have been suggested to be the most likely factor responsible for producing various toxic effects in chronic exposure (Flora, 1999; Flora, Bhadauria, Pant, & Dhaked, 2005). Arsenic induces oxidative stress by stimulating reactive oxygen species (ROS) (Liu, Athar, Lippal, Waldren, & Hei, 2001). Reactive oxygen species (ROS), in turn, is implicated in the development of carcinogenesis (Wang & Huang, 1994) and other cytotoxic effects. The formation of an As–Hb complex may occur and be involved in hemolysis (Fowler & Weissberg, 1974). Exposure to As_2O_3 has been widely studied the depletion of the glutathione GSH level in cells, which may lead to oxidative stress and has been linked to increases in radio-sensitivity (Jones & Douple, 1990; Monzen et al., 2004). The greatest regrowth delay when combining treatment with As_2O_3 and radiation every 3 days, at the time of maximal tumor oxygenation in their model, suggesting that the oxygen level is an important factor in terms of radio-sensitization by As_2O_3 (Griffin, Williams, Park, & Song, 2005). No work has been done on the effect of arsenic trioxide and gamma radiation on physical changes of rat's hemoglobin, However, several studies work in arsenic, Chun et al. (2002) studied the effect of As_2O_3 and ionizing radiation both in vitro and in vivo for human cervical cancer cells, which indicate that As_2O_3 can enhance radio-sensitivity of human cervix carcinoma cells in vitro and in vivo, suggesting a potential clinical applicability of combination treatment of As_2O_3 and ionizing radiation in cancer therapies. Modi, Mittal, and Flora (2007) showed the effect of arsenic (As) combined with selenium in influencing the arsenic induced changes in heme synthesis, hepatic, renal or brain oxidative stress with As concentration. Another has been studied the histological and hematological disturbance caused by arsenic containing water in mice (Rubina, Javaid, Shamim, & Qurbane, 2008). Finally, Gandhi, Panchal, & Patel (2012) studied the prenatal arsenic exposure through intraperitoneal of sodium arsenate of 2 or 4 mg/kg.

2. Materials and methods

2.1. Chemical

Arsenic trioxide (powder) was obtained from Sigma Aldrich code A1010, p code 1001268737, reagent plus®, ≥99% have molecular weight of 197.84 g/mol, appearance of white solid. As_2O_3 was dissolved in H_2O by continues stirring and kept at 4 °C as a stock solution.

2.2. Animals

Rats weighting 130 ± 20 g were kept in cages under routine light and dark system and freely provided with fresh water, they were divided into five equal groups of ten rats each: group (1): control group, group (2): irradiated group, group (3): injected group with As_2O_3, group (4): irradiated group and injected group and group (5): injected and irradiated group. Group (3) taken intraperitoneally (ip) single dose of 0.1% As_2O_3 10 mg/kg body weight since the intraperitoneal acute dose of As_2O_3, LD_{50} rat ip 871 mg/kg (Lewis, 1996), group (4, 5) taken single dose of 5 Gy after/before 2 h from injection, rats were scarified after 24 h.

2.3. Irradiation

Rats were irradiated whole body by cesium-137 source cell with dose rate: 0.758 cGy/s at the National Center for Radiation Research and Technology, Cairo, Egypt using single acute dose of 5 Gy.

2.4. Extraction of samples

Heparinized blood samples taken from rats were centrifuged at 3000 rpm for 20 min, then the supernatant plasma were removed and packed cells were washed three times with two volumes of physiological saline (0.9% NaCl) and the washing saline were removed after each washing. Packed cells were lysed with de-ionized water and then the mixture was centrifuged at 6000 rpm at 4 °C for 45 min in order to obtain hemoglobin polymer (Trivelli, Ronney, & Lai, 1971).

2.5. Electron spin resonance (ESR)

Hemoglobin samples were lyophilized in a freeze drier lyophilize, model (EDWARDS) high vacuum int. at −50 °C and 70 mBar. Powder hemoglobin samples measured at the National Center for Radiation Research and Technology (Cairo, Egypt) recorded at model BRUKER EMX at liquid nitrogen temperature (77 K) in a JES FE-1XG (X-band).