Radioprotective Effects of Adenosine in Gamma Irradiated Rats

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A B S T R A C T

Adenosine is a non−toxic purine abundant in meat and sugar beets, was reported to provide health benefits including antioxidant, anti-inflammatory effects as well as vascular protective properties. This study was dedicated to determine the cardio-protective efficacy of adenosine against oxidative stress due to radiation exposure in male albino rats. Rats were divided into four groups viz. Control group: rats not subject to any treatment; Rats receiving adenosine (150ml/Kg body weight) by gavages for 14 days. Radiation groups in which rats were whole body gamma irradiated at 7Gy. Rats receiving adenosine for 14 days before whole body gamma irradiation at 7Gy. Animals were sacrificed 24 h post irradiation. Irradiated rats revealed a significant increase of heart thiobarbituric acid reactive substance (TBARS), superoxide dismutase (SOD) and catalase (CAT) activities as well as xanthine oxidase activity (XO) in parallel to a significant decrease of reduced glutathione (GSH) content, xanthine dehydrogenase (XDH) and uric acid. Blood showed elevation of TBAR, XO and uric acid accompanied by decrease of antioxidants and XDH. Radiation exposure induced a significant rise in the activities of creatine phosphokinase (CPK), lactic dehydrogenase (LDH) and aspartate aminotransferase (AST), markers of heart damage, both in the heart and blood indicating acute cardiac toxicity. The results revealed that administration of adenosine before irradiation induced significant protection to the parameters of oxidative stress, amelioration of xanthine oxidoreductase (XOR) system and uric acid, besides significant improvement of markers of heart damage. In addition, radiation induced myocardial degenerative changes, interstitial oedema between muscle fibres, necrosis and inflammatory cells infiltration, fibrotic and cellular damage to the heart. Administration of adenosine ameliorates the histological changes induced by gamma irradiation in the heart. It is concluded that the use of adenosine as an antioxidant is safe and may provide some beneficial effects, and could exhibit modulator effects on γ−radiation-induced oxidative damage in rats.

INTRODUCTION

Exposure to ionizing radiation initiates a cascade of events including oxidative damage that leads to alteration of tissue physiological function (Zhao et al., 2007). Lipid peroxidation is considered to be a critical event of ionizing radiation effect (Agrawal and Kale, 2001). Most of the toxic effects of ionizing radiation are due to generation of reactive oxygen species (ROS) by radiolysis of water which triggers formation of several reactive intermediates (Adaramoye et al., 2011). Therefore, to overcome this oxidant stress, the body is equipped with defense system including enzymatic and non-enzymatic radical scavengers that can either directly detoxify ROS or indirectly regulate their levels (Sandeep and Nair, 2012). Hence, an over production of ROS leads to uncontrolled chain reactions and lipid peroxidation, resulting in various pathological conditions that may include liver injury (Kotzampassi et al. 2009) testicular tissues injury (Adaramoye et al., 2012) in addition to lung and kidney damage (Sener et al., 2006).

Ionizing radiation is known to generate ROS in irradiated tissue. Because most tissues contain 80% water, the major radiation damage is due to the aqueous free radicals, generated by the action of radiation on water. Hydroxyl radicals (OH), are considered the most damaging of all free radicals generated in organisms (Spitz and Gius, 2004). These free radicals react with cellular macromolecules, such as DNA, RNA, proteins and cause cells dysfunction and mortality (Tominaga et al., 2004). Attention has been given to the roles of free radicals generated through the oxidative stress, especially induced by ionizing radiation (Riabchenko et al., 2011). Radiation induces an inflammatory response in target and surrounding tissues which is characterized by accumulation of plasma proteins and leukocytes (Johnson et al., 2004). The inflammatory reaction is a classical feature of radiation exposure and appears to be a key event in the development of the acute radiation syndrome (Van der Meeren et al., 2005). Oxidative stress occurs due to excessive free radical production and/or low...
antioxidant defence, and results in chemical alterations of bio-molecules causing structural and functional modifications (Robbins et al., 2002). ROS in turn are capable of initiating and promoting oxidative damage in the form of lipid peroxidation (Ozkan and Fiskin, 2004).

One of the major reasons for cellular injury after radiation exposure is the generation of free radicals and the possible increased levels of lipid peroxides in tissues. Efficient defense and repair mechanisms exist in living cells to protect against oxidant species. Superoxide dismutase (SOD) catalyzes the reduction of $O_2^-$ to $H_2O_2$, the majority of which is broken down to oxygen and water by catalase (CAT). In addition to CAT, glutathione peroxidase in presence of adequate amount of reduced glutathione (GSH) can also break down $H_2O_2$ (Sun et al., 1998).

Adenosine, an endogenous nucleoside, has potent effects on the immune, neural and cardiovascular systems (Guinzberg et al., 2006). It has been shown to be an immunomodulator and anti-inflammatory agents (Schneider and Klein, 2005).

Adenosine is classified as a non–toxic purine. The adenosine extract contains high amounts of purine (Gudkov et al., 2006). The extract protects mouse bone marrow cells against physical and chemical mutagenic agents. Antioxidants in natural products, meat and sugar beets, prevent oxidation reactions associated with cancer and heart diseases with minimum side effects (Gudkov et al., 2006a). Adenosine may have antioxidant, anti-inflammatory, antiallergic, antiviral, hypolipidimic and vasoprotective effects (Guinzberg et al., 2006), as well as powerful protective effects on the radiation-induced DNA damage (Hou et al., 2007) and was proven to be highly effective for protection (Schneider and Klein, 2005). The possible health benefits of adenosine is partly attributed to their potent antioxidant and free–radical scavenging activities (Schneider and Klein, 2005).

This study has been conducted to investigate the possible protective role of adenosine against the toxic effects of exposure to whole body gamma-irradiation in albino rats.

**MATERIALS AND METHODS**

**Experimental animals**

The animal care and handling was done according to the guidelines set by the World Health Organization, Geneva, Switzerland and according to approval from the Ethics Committee for Animals Care at the National Research Centre, (ethic No.10-230).

Adult male Sprague-Dawley rats (180±20 g, body weight), were purchased from the Egyptian Holding Company for Biological Products and Vaccines (Cairo, Egypt). The animals were housed under standard laboratory conditions (constant temperature 25-27°C, with 12 h light /dark cycle) during the experimental period. The rats were provided with tap water and commercial diets. The rats were acclimatized to laboratory conditions for 10 days before commencement of the experiment.

**Radiation facility**

Irradiation of rats was carried out using a Canadian Gamma Cell-40 (137Cs), manufactured by Atomic Energy of Canada Ltd., located at the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. The animal’s whole body was exposed to gamma rays at a dose of 7 Gy administered at a dose rate of 1 Gy/2.10 min.

**Adenosine treatment**

Adenosine was purchased from Sigma chemical Co., St. Louis, MO, USA. The product is provided as concentrated exudates, dissolved in distilled water. Adenosine was administered to rats by gavages at a dose of 150 mg/kg body weight, according to Mabley et al. (2003) for 14 days, prior to irradiation. Animals were sacrificed 24 h post irradiation.

**Animal groups**

Animals were divided into 4 groups, each of 6 rats.
2. Adenosine: received adenosine via gavages (150 mg/kg body weight/day) for 14 days.
3. Radiation: were whole body gamma irradiated at 7 Gy.
4. Adenosine+Radiation: received adenosine via gavages (150 mg/kg body weight/day) before whole body gamma irradiation at 7 Gy.

**Biochemical analysis**

The animals were sacrificed twenty four hours post irradiation and blood was collected and plasma samples were obtained by centrifugation at 3000 rpm for 10 min. Immediately after sacrifice, the heart was rapidly excised from the body of each animal, accurately weighted and tissues were homogenized in normal saline. The freshly prepared homogenate were then used for determination of thiobarbituric acid reactive substances (TBARS), CAT, GSH, SOD, creatine phosphokinase (CPK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), xanthine oxidase (XO), xanthine dehydrogenase (XDH), and uric acid contents.

Lipid peroxide content was determined by quantifying thiobarbituric acid TBARS content in blood and tissue homogenates according to the method.
RADIOPROTECTIVE EFFECT OF ADENOSINE

The activity of SOD was determined in blood erythrocytes according to the method of Minami and Yoshikawa (1979). The CAT activity was determined following the procedure described by Johansson and Hakan Borg (1988). Blood glutathione was determined according to Beutler et al. (1963). XO and XDH were assayed in serum following the procedure described by Kaminski and Jezewska (1979). LDH, CPK and AST activities were determined according to the methods of Henderson and Moss (2001), Rec (1977) and Reitman and Frankel (1957), respectively. Uric acid was determined according to Haismann and Muller (1977).

Histological methods

Hearts were removed and fixed in Bouin’s solution for 24 h according to Hartz (1972). Samples were serially sectioned at a thickness of 4-5 µm and stained applying the technique of Conn and Darrow (1960) using hematoxylin and eosin. Tissue sections were examined under a research light microscope.

Statistical analysis

ANOVA (one-way classification F-test) followed by Duncan (Multiple Range-test) were carried out for the statistical analysis as described by Lind and Masson (1996) and data were represented in Tables as mean ± standard error.

RESULTS

The oxidative stress parameters viz. SOD and CAT activities and GSH content as well as TBARS concentration in heart and blood are presented in Table I. The results pointed to a significant increase in the activity of SOD and CAT both in the heart of rats subjected to 7 Gy gamma radiation whereas their activities decreased in the blood. However, 24 h post irradiation, there was a significant reduction of GSH accompanied by a significant increase of TBARS both in the heart and blood as compared to that of control group. The results showed significant protection to oxidative stress parameters in animals treated with adenosine compared to irradiated group.

Table also revealed a significant increase in XO associated with significant decrease in XDH activities in the heart and blood of animals exposed to 7 Gy gamma radiation as compared to control group. Uric acid was significantly decreased in the heart but was elevated in the blood. Administration of adenosine before irradiation ameliorated xanthine oxidoreductase system and protected uric acid values.

CPK, LDH and AST increase significantly after exposure to gamma rays compared to control both in the heart and blood. Administration of adenosine reduced the increase of the enzymes activities compared to irradiated groups.

Each value represents the mean ± SE (n=6) P<0.05: significant, a: Significantly different compared to control. b: Significantly different compared to radiation.

Histopathological observation

Heart section of the control group and group treated with adenosine (150 mg/kg body wt) showed normal architecture. The heart cells have normal amount of cytoplasm with one or two nuclei and defined cell boundaries. Cardiac tissues, cardiac muscle fibers, appeared as short branching and Anastomosing cylinders with moderately stabilized eosinophilic sacroplasm and centrally located oval nuclei (Fig. 1 A,B). Exposure to gamma rays induced changes manifested as slight disruption of the striated appearances and disorganization of the myofilamentous arrangement in many cardiomyocytes, discontinued, fragmented and lysis. Structural changes in the cardiac muscle fibers, deformation of the striated appearance and areas of vacuolation (Fig. 1C), were also detected, in addition to patches, necrosis of muscle fibres, pyknotic myocardial cells and myocardial damage. In the groups treated with adenosine (150 mg/kg body wt) prior to whole body gamma irradiation, amelioration of many of the radiation induced changes in the histological structure was observed. The pyknotic cells were not observed, the degree of myocardial damage was less than that of irradiated groups, the interstitial odemas as well as inflammation were less than irradiated group, myonecrosis was also not remarkable in this group (Fig. 1D).

DISCUSSION

Several evidences had indicated that accumulation of ROS led to the alteration in wide range of gene expression such as antioxidant enzymes, stress response genes and cytokines (Zhang et al., 2002). During oxidative stress, the endogenous antioxidant defenses are likely to be weakened because of overproduction of oxygen radicals, consumption of antioxidant and failure to adequately replenish these antioxidant enzymes in tissues (Droge, 2002). Exposure of mammals to ionizing radiations, leads to the development of a complex, dose-dependent series of changes, including injury to different organs, which cause changes in the structure and function of cellular components. Oxidative stress with the subsequent production of ROS was postulated as one of the mechanisms of radiation toxicity.
In the present study, whole body exposure of male albino rats to gamma radiation (7Gy) has provoked an imbalance between oxidant and antioxidant species. Significant increase in the level of TBARS, accompanied by significant decreases of SOD and CAT activities were recorded in the blood. The increase of TBARS level is probably due to the interaction of OH resulting as a by-product of water radiolysis, upon exposure to ionizing radiation, with the polyunsaturated fatty acids present in the phospholipids portion of cellular membranes (Spitz et al., 2004; Oktem et al., 2004). CAT directly neutralizes the H$_2$O$_2$ produced from the superoxide dismutation reaction into water and molecular oxygen. The significant decrease in the activity of SOD and CAT might also be attributed to the excess of ROS, which interacts with the enzyme molecules causing their denaturation and partial inactivation (Kregel and Zhang, 2007). The ROS as chemically reactive molecules can modify most cell components such as lipids, nucleic acid, carbohydrates and proteins (Stadtman and Levine, 2003).

GSH is the most abundant non-protein sulfhydryl-containing compound and constitutes the largest component of the endogenous thiol buffer (Holmgren et al., 2005). Assessment of GSH in biological samples is essential for evaluation of the redox homeostasis and detoxification status of cells in relation to its protective role against oxidative and free radical-mediated cell injury (Rossi et al., 2005). Depletion of GSH may be partly attributed to inflammation (Meister, 1991). Excessive lipid peroxidation can increase GSH consumption (Gudkov et al., 2006a). Elevation of MDA by irradiation which could be attributed to enhanced utilization of the antioxidant system in an attempt to detoxify radiation-generated free radicals (Krishna and Kumar, 2005) which probably also accounts for the decrease of SOD and CAT activities.

Adenosine treatment improved CAT, SOD and TBARS that may be due to adenosine free radical scavenging ability by redox active sulphhydryl group directly reacting with oxidants (Guinzberg et al., 2006). In the past few years, innovations in the management of poisoning have also been directed toward the use of antioxidants, since gamma rays induces its toxic effect via oxidative stress-mediated mechanisms (Hou et al., 2007).

CPK, LDH and AST are important enzymes used to confirm a myocardial infarction or heart injury. In the present study, the elevation in these enzyme activities after whole body gamma irradiation was attributed by Fahim (2008) to the alterations in dynamic permeability of membranes induced by ionizing radiation, allowing leaking of biologically active materials out of the injured cells. The amount of these markers in plasma is directly
Table I. Effect of adenosine on thiobarbituric acid reactive substance (TBARS), glutathione (GSH) content, superoxide dismutase (SOD) and catalase (CAT) activity xanthine oxidase (XO), xanthine dehydrogenase (XDH), uric acid, creatine phosphokinase (CPK), lactic dehydrogenase (LDH) and aspartate aminotransferase (AST) in blood and heart of rat groups.

<table>
<thead>
<tr>
<th></th>
<th>Control (mIU/mg protein)</th>
<th>Blood (U/g Hb)</th>
<th>Adenosine (mIU/mg protein)</th>
<th>Blood (U/g Hb)</th>
<th>Radiation (mIU/mg protein)</th>
<th>Blood (U/g Hb)</th>
<th>Adenosine+Radiation (mIU/mg protein)</th>
<th>Blood (U/g Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD</td>
<td>13.69±0.31</td>
<td>448.12±11.9</td>
<td>15.53±0.39</td>
<td>422.09±9.3</td>
<td>27.33±1.69</td>
<td>329.00±8.43</td>
<td>18.35±0.63</td>
<td>376.00±8.61</td>
</tr>
<tr>
<td>CAT</td>
<td>5.19±0.10</td>
<td>16.64±0.45</td>
<td>5.04±0.07</td>
<td>15.98±0.36</td>
<td>7.2±0.56</td>
<td>11.47±0.28</td>
<td>6.43±0.18</td>
<td>13.89±0.39</td>
</tr>
<tr>
<td>GSH</td>
<td>31.09±0.67</td>
<td>58.63±18.6</td>
<td>34.89±0.87</td>
<td>60.18±1.28</td>
<td>23.5±1.09</td>
<td>42.7±1.09</td>
<td>25.28±1.42</td>
<td>48.80±1.08</td>
</tr>
<tr>
<td>TBARS</td>
<td>188.00±4.93</td>
<td>17.38±0.49</td>
<td>182.70±4.09</td>
<td>15.6±4.30</td>
<td>298.13±5.06</td>
<td>8.19±0.90</td>
<td>310.18±5.42</td>
<td>20.58±0.85</td>
</tr>
<tr>
<td>XO</td>
<td>5.06±0.23</td>
<td>1.28±0.09</td>
<td>4.63±0.28</td>
<td>1.14±0.07</td>
<td>6.64±0.30</td>
<td>2.48±0.13</td>
<td>6.08±0.36</td>
<td>2.07±0.11</td>
</tr>
<tr>
<td>XDH</td>
<td>13.73±0.39</td>
<td>1.18±0.06</td>
<td>13.59±0.08</td>
<td>1.09±0.08</td>
<td>9.34±0.59</td>
<td>0.81±0.05</td>
<td>8.79±0.46</td>
<td>0.97±0.10</td>
</tr>
<tr>
<td>Uric acid</td>
<td>50.46±1.36</td>
<td>2.43±0.09</td>
<td>41.65±1.24</td>
<td>2.39±0.05</td>
<td>32.44±1.10</td>
<td>4.38±0.21</td>
<td>58.13±1.10</td>
<td>3.37±0.14</td>
</tr>
<tr>
<td>CPK (U/ml)</td>
<td>28.6±0.18</td>
<td>14.8±0.46</td>
<td>27.8±0.21</td>
<td>14.5±0.60</td>
<td>38.5±4.50</td>
<td>23.1±1.28</td>
<td>31.49±0.46</td>
<td>173.80±7.0</td>
</tr>
<tr>
<td>LDH (U/ml)</td>
<td>14.5±0.21</td>
<td>89.00±3.30</td>
<td>12.7±0.24</td>
<td>82.7±1.28</td>
<td>25.60±2.09</td>
<td>108.20±3.69</td>
<td>21.11±0.32</td>
<td>93.00±2.90</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>21.4±1.46</td>
<td>60.0±2.9</td>
<td>23.8±1.22</td>
<td>55.7±2.08</td>
<td>31.95±1.6a</td>
<td>88.9±3.1a</td>
<td>26.15±1.58</td>
<td>62.0±2.22</td>
</tr>
</tbody>
</table>

proportional to the number of necrotic cells present in cardiac tissue (Farvin et al., 2004). The recorded protection to the heart enzymes is attributed to that adenosine inhibits superoxide radical production by activated human neutrophils (Marton et al., 2001). The exogenous administration of larger doses of adenosine is reported to prevent ischemia-reperfusion injury in the heart and brain (Hasko et al., 2004).

The present study revealed significant aleration in XOR system of irradiated rats. The changes in XOR was manifested by significant increase in the activity of XO associated with significant decrease in XDH activity as compared with control rats. XOR is a member of the molybdoenzyme family that catalyze purine degradation, hypoxanthine and xanthine metabolism to uric acid with concomitant generation of ROS (Berry and Hare, 2004). Uric acid, the end products of DNA catalosm was elevated significantly in serum of rats exposed to gamma rays in the present study, indicating the increased rate of purine bases degradation and DNA damages. It was suggested that serum uric acid correlates with circulating markers of inflammatory process (Struthers et al., 2002). This implies a relation between increase in XO activity and endothelial injury secondary to increase of oxidative damages (Farquharson et al., 2002). Ionizing radiation has been shown to convert XDH into XO and contribute to increase ROS release which leads to cell damage (Srivastava et al., 2002). XO and XDH are two inter convertible forms of XOR. Administration of adenosine after exposure to gamma radiation induced significant decrease in XO and significant increase in XDH activities associated with significant decrease in serum uric acid ,postulating the inhibitory effect of adenosine on ROS generation systems (Kudina et al., 2003). Adenosine is an endogenous purine nucleoside that modulates many physiological processes. Modulation of XOR system by adenosine is due to that cellular signaling by adenosine occurs through four known adenosine receptor subtypes (A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub>, and A<sub>3</sub>). In regard to stress or injury, the function of adenosine is primarily that of cytoprotection preventing tissue damage. Activation of A<sub>2A</sub> receptors produces a constellation of responses that in general can be classified as anti-inflammatory (Mustafa et al., 2009).

Regarding the histological changes in the heart recorded in the present study, it was found that exposure of rats to whole body gamma irradiation caused patchy necrosis of muscle fibers with infiltration of acute and chronic inflammation cells. Marked interstitial edema was also noted. These results coincide to some extent with the results of (Said et al., 2002; Mansour and Abou El-Nour, 2009; Gorg, 2014) who concluded that this damage may be due to generation of oxidized, reactive lipoproteins and through direct attacks on the DNA of the arterial wall cells. In this study, irradiation of rats induced the formation of structural changes in their aortas, degeneration of the endothelial cell layer of the tunica intima, changes in the endothelium of the intima that was the cause for the development of edema, fibrosis and increase of vascular permeability, as well as degeneration and decrease of the number of smooth muscle cells of the tunic media of the aorta: the results agreed with (Soliman, 1997).

The significant protection observed for the heart tissue support the role of adenosine in minimizing radiation-induced damage. The treatment with adenosine before radiation exposure might lead to reduction of the damaging effect of oxygen free radicals acting as oxygen scavengers, which by its turn preserved the normal like
appearance of the heart tissue. This was observed upon the use of different free radical scavenging agents (Soliman, 2007; Gaur et al., 2011). Adenosine as a natural antioxidant prevents oxidative damage to DNA, decreases the generation of radical oxygen species and protects the animal against gamma-radiation induced death, and could especially enhance the survival of animals when administrated shortly after irradiation (Gudkov et al., 2005b). It could be concluded that adenosine could protect against radiation-induced oxidative stress in experimental rats via affecting ROS generation system and salvage of antioxidant status thus it can be conveniently incorporated in the diet as a nutritional supplement.

Statement of conflict of interest
Authors have declared no conflict of interest.

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Hartz, 1972. Tissues were fixed in bouins solution for 24h according to hartz technique Am. J. clin. Pathol., 17: 750.


