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Environmental exposure to low-doses of ionizing radiation. Effects on early nephrotoxicity in mice

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ABSTRACT

Nuclear accidents of tremendous magnitude, such as those of Chernobyl (1986) and Fukushima (2011), mean that individuals living in the contaminated areas are potentially exposed to ionizing radiation (IR). However, the dose-response relationship for effects of low doses of radiation is not still established. The present study was aimed at investigating in mice the early effects of low-dose internal radiation exposure on the kidney. Adult male (C57BL/6 J) mice were divided into three groups. Two groups received a single subcutaneous (s.c.) doses of cesium (^{137}Cs) with activities of 4000 and 8000 Bq/kg bw. A third group (control group) received a single s.c. injection of 0.9% saline. To evaluate acute and subacute effects, mice (one-half of each group) were euthanized at 72 h and 10 days post-exposure to ^{137}Cs, respectively. Urine samples were collected for biochemical analysis, including the measurement of F2-isoprostane (F2-IsoP) and kidney injury molecule-1 (KIM-1) levels. Moreover, the concentrations of 8-hydroxy-2′-deoxyguanosine (8-OHdG), a sensitive marker of oxidative DNA damage, were measured in renal tissue. Urinary excretion of total protein significantly increased at 72 h in mice exposed to Cs4000. Uric acid and lactate dehydrogenase (LDH) decreased significantly at both times post-exposure in animals exposed to Cs8000. After 72 h and 10 d of exposure to Cs4000, a significant increase in the γ-glutamil transferase (GGT) and N-acetyl-β-D-glucosaminidase (NAG) activities was observed. In turn, F2-IsoP levels increased -mainly in the Cs4000 group- at 72 h post-exposure. Following irradiation (^{137}Cs), the highest level of KIM-1 was corresponded to the Cs4000 group at 72 h. Likewise, the main DNA damage was detected in mice exposed to Cs4000, mainly at 10 d after irradiation. The alterations observed in several biomarkers suggest an immediate renal damage following exposure to low doses of IR (given as ^{137}Cs). Further investigations are required to clarify the mechanisms involved in the internal IR-induced nephrotoxicity.

1. Introduction

Nowadays, more than 400 nuclear power plants are in operation around the world. Despite the strict security measures required by these facilities, four major nuclear accidents occurred in the past century: Kyshtym (Russia, 1957), Windscale Piles (UK, 1957), Three Mile Island (USA, 1979) and Chernobyl (Ukraine, 1986), while one accident occurred in the current century (Fukushima, Japan, 2011). The effects of these serious accidents on individuals and societies have been diverse and enduring (Gudzenko et al., 2015; Han et al., 2011). However, due to the specific characteristics and proximity in time, the recent accident in Fukushima has been particularly shocking. On March 11, 2011, a 9.0-magnitude earthquake struck the northeast coast of the main island of Japan, triggering a tsunami with 14–15 m-high waves hitting the area. The huge earthquake affected the nuclear power plant in the Fukushima prefecture, resulting in large amounts of radioactive materials released into the environment (Hasegawa et al., 2015). The major nuclides released were ^{131}I, ^{134}Cs, and ^{137}Cs, which were early detected in the topsoil, plants and water (Fushiki, 2013; Orita et al., 2016). As a direct consequence of this, almost 170,000 people were evacuated or they should stay indoors.

After accidents of this tremendous magnitude, the populations living in the contaminated areas are potentially exposed to external and internal radiation (Marzano et al., 2001; Ostroumova et al., 2016). The external radiation is mainly caused by the surface contamination of the environment, while the internal contamination is basically due to the presence of radionuclides in the food chain (Aono et al., 2016; Fushiki, 2013; Suslova et al., 2015). Among these radionuclides, ^{137}Cs,

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with a half-life of about 30 years, is the main contributor to exposure of the individuals living in the areas around the accidents (Orita et al., 2016). Although the effects of external exposure to high and low doses of ionizing radiation (IR) have been extensively studied, nowadays information on the health effects of internal irradiation is scarce. A rapid absorption and a widespread systemic distribution of ^{137}Cs have been reported following chronic oral ingestion (Manens et al., 2016). In contaminated areas, the incidence of thyroid cancer and bone, as well as digestive and nervous disorders increases in the children population (Ericson and Kallen, 1994; Sholl et al., 2017). On the other hand, a few animal studies have assessed the biological effects of internal exposure to ^{137}Cs. Biological consequences were observed on small intestinal or secretor functions (Dublineau et al., 2007) and vitamin D_3 and cholesterol metabolism in rats (Souidi et al., 2006; Tissandie et al., 2006). Moreover, in a recent study conducted in our laboratory, mice exposed internally to ^{137}Cs showed impaired learning, and spatial memory, as well as increased anxiety (Heredia et al., 2016).

The harmful effects of radiation can be present in various organs. However, from the standpoint of serious damage, the kidney is probably the most radiosensitive of the abdominal organs (Fuma et al., 2016; Robbins and Zhao, 2004). The tubular epithelial cells appear to be more sensitive to the radiation in comparison to epithelial cells from other tissues. Kidney irradiation induces radiation nephropathy characterized by a reduction in renal function, which is associated with structural alterations in glomerular and tubular cells (Ilhan et al., 2016; Romanenko et al., 2012). It is well known that cancer radiotherapy and accidental exposure to high-doses of external IR induce renal injury (El-Gazzar et al., 2016). Notwithstanding, information on the nephrotoxic effects of internal exposure to IR is certainly scarce. During the period subsequent to the Chernobyl accident, the incidence of malignant renal tumors in Ukraine increased from 4.7 to 10.7 per 100,000 inhabitants (Jargin, 2015), while an association of low-dose IR on renal cell carcinomas (RCCs) was observed in subjects living in ^{137}Cs-contaminated areas of Ukraine (Morell-Quadreny et al., 2011). ^{137}Cs constitutes 80–90% of the internal exposure in people living in radio-contaminated areas, being mainly (approx. 90%) excreted through the kidneys (Ebner et al., 2016; Romanenko et al., 2001).

Most of the radiation-induced damage to biomolecules in aqueous media is caused by the formation of free radicals resulting from the radiolysis of water (Ekici et al., 2016). The generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) predominantly mediates the harmful effects of IR (El-Gazzar et al., 2016). In turn, IR-induced oxidative stress (OS) may produce ROS, which are reported to be the main cause of tissue injury. Therefore, if ROS/RNS are not scavenged, they can lead to widespread lipid, protein and DNA damage (Havas, 2017; Ozyurt et al., 2014) Overproduction of free radicals reacts with cell membrane fatty acids and proteins impair their function. As a result of this, various investigations have suggested that the measurement of F2-isoprostane (F2-isoP) levels is a reliable and useful approach to assess lipid peroxidation and OS in vivo (Alonso et al., 2010; Clayton et al., 2014). Upon kidney function disorders, alterations in urinary levels of parameters such as albumin, creatinine or urea can be used as biomarkers of kidney failure (Belles et al., 2007). Recently, a novel biomarker for renal injury is the Kidney Injury Molecule-1 (KIM-1). KIM-1 is a type I transmembrane glycoprotein discovered in renal tubular epithelial cells. KIM-1 is undetectable in healthy kidneys, but it is highly expressed and can be found at very high levels after acute kidney injury (Nan-Ya et al., 2015; Pianta et al., 2017; Yin and Wang, 2016). Partial or total body exposures to radiation doses as low as 5 Gy in a single fraction, can lead to radiation nephropathy, while exposure to even lower doses of radiation have been associated with renal disease.

As a consequence of the nuclear accident of Chernobyl and more recently in that of Fukushima, the effects of low-dose IR, especially internal exposure, are at the forefront of everyone's attention. Renal diseases increases human morbidity and mortality. Therefore, renal function must be part of the immediate and long-term follow-up of individuals who have had been subjected to accidental radiation exposures. In the present study, we investigated the nephrotoxic effects of low-dose IR exposure in order to assess the initial events after irradiation. The main objective was to evaluate the diagnostic capacity of biomarkers of kidney injury and OS in predicting renal damage.

2. Materials and methods

2.1. Animals

Male C57BL/6J mice (2 months of age) were purchased from Charles River (Criffa, Barcelona, Spain). Housing conditions included a temperature of 22 ± 2 °C, relative humidity of 50 ± 10% and 12 h light-dark cycle. Food (Panlab rodent chow, Panlab, Barcelona) and tap water were offered ad libitum throughout the experiment. The experimental protocol was approved by the Animal Care and Use Committee of the Universitat Rovira i Virgili (Tarragona, Catalonia, Spain) following the “Principles of Laboratory Animal Care”, being carried out in accordance with the European Union Directive 2010/63/EU for animal experiments.

2.2. Groups and treatment

After a quarantine period of two weeks, mice were randomly classified into three experimental groups (n = 16 per group). Two groups received a single subcutaneous (s.c.) dose of cesium (^{137}Cs, provided by CIEMAT, Madrid, Spain) with activities of 4000 (Cs4000) and 8000 (Cs8000) Bq/kg bw, respectively. The ^{137}Cs doses were based on the estimate of the ^{137}Cs dietary intake of the surrounding populations during the years following the Chernobyl accident (Belles et al., 2016; Grison et al., 2012; Lestaevel et al., 2008). A third group (control group) received a single s.c. injection of 0.9% saline. To evaluate the acute and subacute renal effects, mice (half of the animals in each group) were euthanized at 72 h and 10 d post-exposure to ^{137}Cs, respectively. Twenty-four hours before being sacrificed, animals were individually housed in plastic metabolism cages. Urines (24 h) were collected to determine various biochemical parameters, KIM-1 and F2-isoP levels. After urine collection, animals were sacrificed with an overdose of ketamine–xylazine given intraperitoneally. Kidneys were removed and cleared of the adhering tissues. Kidney tissue was used to quantify DNA damage.

2.3. Urine biochemical analysis

In fresh urine, the 24 h volume, and the concentrations of urea, uric acid, levels of creatinine, total protein, as well as lactate dehydrogenase (LDH), N-acetyl-β-D-glucosaminidase (NAG) and γ-glutamil-transferase (GGT) activities were analyzed using a Cobas Mira automatic analyzer (Roche Pharmaceuticals, Basel, Switzerland) (Belles et al., 2007).

The levels of 8-iso-prostaglandin F2α (8-iso-PGF2α), one isomer of the F2-isoP family, were analyzed in urine for assessment of oxidant stress in vivo. In the present study, urine samples were stored in aliquots of 1 mL containing 10 μL of butylated hydroxytoluene at −80 °C until analysis. The measurement of urinary F2-isoprostane (8-iso-PGF2α) concentrations was performed based on the protocol of the competitive enzyme-linked immunosorbent assay (ELISA) kit from Oxford Biomedical Research (Deltaclon, Spain). The level of 8-iso-PGF2α in each urine sample was normalized to that creatinine level [8-iso-PGF2α (ng)/creatinine in urine (mg)] (Alonso et al., 2010).

For the measurement of KIM-1 in urine (stored at −20 °C), ELISA test kit for the detection of KIM-1 in mouse (Aviscera Bioscience, Deltaclon, Spain) was used according to the manufacturer's instructions. The amount of KIM-1 in each urine sample was normalized to that creatinine level [Kim-1 (μg)/creatinine in urine (g)].