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Immunological changes of chronic oral exposure to depleted uranium in mice

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ABSTRACT

Direct ingestion of contaminated soil by depleted uranium (DU) might lead to internal exposure to DU by local populations through hand contamination. The purpose of this study was to assess the immunological changes of long-term exposure to various doses of DU in mice. Three-week-old Kunming mice were divided into the following 4 groups based on the various feeding doses (containing DU): 0 (control group), 3 (DU_3 group), 30 (DU_{30} group), and 300 mg/kg feed (DU_{300} group). After 4 months of exposure, in the DU_{300} group, the innate immune function decreased, manifesting as decreased secretion of nitric oxide, interleukin (IL)-1β, IL-18, and tumour necrosis factor (TNF)-α in the peritoneal macrophages, as well as reduced cytotoxicity of the splenic natural killer cells. Moreover, the cellular and humoral immune functions were abnormal, as manifested by decreased proliferation of the splenic T cells, proportion of the cluster of differentiation (CD) 3^+ cells, ratio of $CD4^+/CD8^+$ cells and delayed-type hypersensitivity, and increased proliferation of the splenic B cells, total serum immunoglobin (Ig) G and IgE, and proportion of splenic $mIgM^+mIgD^+$ cells. Through stimulation, the secretion levels of interferon (IFN)-γ and TNF-α in the splenic cells were reduced, and the levels of IL-4 and IL-10 were increased. By comparison, in the DU_{30} and DU_3 groups, the effects were either minor or indiscernible. In conclusions, chronic intake of higher doses of DU (300 mg/kg) had a significant impact on the immune function, most likely due to an imbalance in T helper (Th) 1 and Th2 cytokines.

1. Introduction

Depleted uranium (DU) is the residue that remains after the refining and enriching of ^{235}U from natural uranium; the content of ^{235}U is usually 0.2–0.3%. Due to its high penetrability and low price as a raw material, DU has been widely used in counterweights, radiation-protective clothing, and military activities (serving as an armour material and an ammunition component) (Bleise et al., 2003). However, during its production and use, uranium may be released into the environment due to failure to follow standard procedures, thus causing environmental pollution. The physical half-life of DU is up to 4.49×10^9 years, and the element remains in the environment for a long time, contaminating soil, groundwater, flora, and fauna, which eventually enter the human body through the food chain, leading to chronic contamination of local residents (Di Lella et al., 2005).

The radioactivity of DU is approximately 60% that of natural uranium, but DU has the same heavy-metal toxicity as natural uranium (Priest, 2001; Squibb et al., 2012). During acute high-dose exposures, the kidney is the main target organ of the chemical toxicity of DU, which may cause severe tubular necrosis (Hao et al., 2012a) and mitochondrial damage (Shaki et al., 2012). Low-dose chronic exposure may cause a series of harmful effects, such as neurobehavioural abnormalities, genetic toxicity, reproductive toxicity, and cancer (Houpert et al., 2005; Lestaevel et al., 2005; Hao et al., 2009, 2012b; Mould, 2001). Gagnaire et al. (2013) reported that low-dose DU exposure had an impact on oxidative stress, detoxification, and the defence system of zebrafish; moreover, the researchers stressed that further research on immunotoxicity (or immune markers) would elucidate these effects of uranium. Our previous research

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCR, B cell surface receptor; BUN, blood urea nitrogen; CCL-2, C–C motif ligand 2; CD, cluster of differentiation; ConA, concanavalin A; CR, creatinine; CTL, cytotoxic T cells; DTH, delayed type hypersensitivity; DU, depleted uranium; ELISA, enzyme linked immunosorbent assay; ICP-MS, inductively coupled plasma mass spectrometry; IL, interleukin; IFN, interferon; Ig, immune globulin; iNOS, induced nitric oxide synthase; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; MTT, 3-(4,5-diamethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium; NK cells, natural killer cells; NO, nitric oxide; OD, optical density; PBS, phosphate buffer solution; PMA, phorbol-12-myristate-13-acetate; SD, standard deviation; SRBCs, sheep red blood cells; TGF, transforming growth factor; Th cells, T helper cells; TNF, tumor necrosis factor.

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(Hao et al., 2012a) has confirmed that in addition to primary accumulation in the kidney, DU also accumulates in the liver and spleen, suggesting that DU might have certain effects on the immune system.

Several studies have confirmed that DU has a toxic effect on immune cells *in vitro*. Kalinich et al. (2002) found that macrophages can uptake uranium and subsequently undergo apoptosis. Gazin et al. (2004) determined that DU causes abnormal expression and release of tumour necrosis factor (TNF)-α and interleukin (IL)-6 from macrophages. Wan et al. (2006) demonstrated that exposure to low-dose DU affects the immune function through regulation of the expression of cytokines (*e.g.*, involved in signal transduction, interleukin expression, chemokines, chemokine receptors, and neurotrophic factors).

However, few published studies exist on the impact of DU on immune function and inflammation in live animals. Monleau et al. (2006) found that inhalation of insoluble DU causes a time-dependent increase in a variety of inflammatory cytokines in rat lung tissue. A rat model of chronic exposure was established by long-term intake of uranium-containing water (40 mg/l); at 3, 6, and 9 months, the effect of uranium exposure on various inflammatory pathways [prostaglandins, histamine, cytokines and nitric oxide (NO)] was evaluated. The results revealed that chronic ingestion of DU causes time-dependent changes in a variety of inflammatory pathways (Dublineau et al., 2007). DU enters the body through the oral route. Direct ingestion of contaminated food and soil should also be considered in addition to drinking contaminated water. However, there is still no report on the effects of ingesting DU-contaminated food on the immune system.

In addition, it is a speculated that the "Gulf War Syndrome" might be caused by the systematic shift of T helper (Th) 2 cytokines by Th1 cytokines because the clinical symptoms are markedly similar to those of autoimmune diseases (Rook and Zumla, 1997). *In vitro*, after cluster of differentiation (CD) 4^+ T cells and macrophages are exposed to DU, there is increased expression of IL-5 and IL-10, which strongly suggests a shift to Th2 cells during the initial stages of T cell differentiation (Wan et al., 2006). For other heavy metals, such as lead, studies on mouse bone marrow-derived dendritic cells also revealed a shift to Th2 cells during the immune response (Gao et al., 2007). In this study, we hypothesised that DU may modulate immune cell cytokine expression, especially Th1 and Th2 cytokines, to influence the immune system function. However, Dublineau et al. (2006) reported that, there was no biological consequences in the cytokine expression [IL-10, transforming growth factor (TGF)-β, interferon (IFN)-γ, TNF-a] in Peyer's patches and in mesenteric lymph nodes of rats after chronic ingestion of DU by drinking water (40 mg/l).

Therefore, the objective of this study was to establish a mouse model in which mice were exposed to long-term ingestion of DU-containing feed, to evaluate the overall impact of DU exposure on the entire immune system of the mice after 4 months, and to verify whether the DU exposure caused an imbalance between Th1 and Th2 cytokines. We set up 4 different dose groups based on the DU concentration. The control group consumed normal feed with a uranium concentration of approximately 0 mg/kg. The uranium concentration that was used in the DU_3 group (3 mg/kg) was mainly based on the average concentration of uranium in the natural soil (3 mg/kg; Bleise et al., 2003). The uranium concentration that was used in the DU_{30} groups (30 mg/kg) was mainly based on the concentration range of uranium in the topsoil of the western Kosovo region (0.69–31.47 mg/kg; Di Lella et al., 2005) and on the uranium concentration (40 mg/l) that is the uranium concentration commonly used in drinking water in studies (Wade-Gueye et al., 2012; Barillet et al., 2011) of chronic exposure [which was twice the highest environmental concentration in Finland (Juntunen, 1991)]. Finally, in accordance with the 10-fold uranium concentration gradient for each dose group, the DU_{300} groups were exposed to 300 mg/kg; this 300 mg/kg concentration was still far lower than that of the highest uranium concentration in the topsoil of the Kosovo region (assessed in November 2000), which was approximately 18,000 mg/kg (Sansone et al., 2001). Four months after the animals were fed the DU-containing feed, immunotoxicological experiments were conducted to comprehensively evaluate the animals' innate immunity and cellular and humoral immune function to analyse the subtypes of the immune cells and the expression of the cytokines and thus further explore the molecular mechanisms of the immunotoxicity of DU. The results of this study suggest that DU plays a role in increasing the incidence of autoimmune diseases, infectious diseases, and tumours, which lays the foundation for future studies of the biological effects of chronic DU exposure.

2. Materials and methods

2.1. Animals

Male Kunming mice weaned at 3 weeks of age were obtained from the Institute of Zoology [The Third Military Medical University, SCXK (Chongqing) 2007-0003, China]. The mice were acclimated to the laboratory for 7 days prior to the start of the experiment and found to be in good health were selected for use. The mice's weights were in the range 18–21 g at the beginning of the experiments. The mice were housed in plastic cages (ten mice per cage) under controlled conditions with a 12:12-h (light:dark) cycle, an ambient temperature of 20–25 °C, and a relative humidity of 55%. The mice had free access to water and food throughout the experimental period. Food intake, water intake, body weight, and health status were recorded daily. Over the four months after ingestion of DU, the mice were euthanised by rapid decapitation or anaesthetised with ether for blood collection. The animal experiments were conducted in conformity with the National Institutes of Health guidelines (NIH Pub. No. 85-23, revised 1996) and with the agreement by the Animal Care and Use Committee of the Third Military Medical University.

2.2. Contamination

DU (^{238}U: 99.75%, ^{235}U: 0.20%, and trace ^{234}U, specific activity of 1.24×10^4 Bq/g) was purchased from the China National Munitions Corporation, Beijing. The preparation of DU-spiked food followed as previous study (Hao et al., 2009). In brief, DU was dissolved in nitric acid as uranyl nitrate and then spiked in food evenly. The resulting chemical speciation of uranyl nitrate mixed with food was uranyl nitrate hexahydrated [$UO_2(NO_3)_2{\cdot}6H_2O$]. For animal exposure, four different solutions were prepared to obtain four concentrations of uranium in food: 0 mg/kg (control group), 3 mg/kg (DU_3 group), 30 mg/kg (DU_{30} group) and 300 mg/kg (DU_{300} group). After food consumption and weight were considered, the mice were exposed to DU in their food at approximate doses of 0, 0.4, 4, and 40 mg/kg body weight/day for four months, respectively.

2.3. Relative weight of spleen and thymus

Over the four months after ingestion of DU, the mice of each group ($n = 10$) were anaesthetised with ether and blood samples were collected from femoral vein. Serum was prepared for biochemical assays below. Then spleen, thymus and sternum from mice were lightly dissected and spleens and thymus were weighed and normalised to the body weight. Spleen, thymus and sternum were used for uranium analyses below.

2.4. Biochemical assays

Serum concentrations of urea nitrogen (BUN), creatinine (CR), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured by an automated spectrometric system (Konelab 20, Thermo Electron Corporation, Cergy-Pontoise, France) with the manufacturer's biological chemistry reagents.

2.5. Uranium analyses

The uranium content was measured in the kidney, sternum, thymus and spleen. Samples (25–400 mg) were digested by the addition of 3 ml of concentrated nitric acid in a CEM MARS Xpress Microwave Accelerated Reaction System (CEM Corporation, Matthews, NC, USA) using following procedure: (1) microwave power at 1600 W, ramp 5 min to reach 120 °C and remained at 120 °C for 2 min; (2) microwave power at 1600 W, ramp 2 min to reach 150 °C and remained at 150 °C for 2 min. Uranium content in samples was determined using an inductively coupled plasma mass spectrometer (ICP-MS, Thermo Finnigan MAT, Bremen, Germany). The limit for the instrument was 0.002 ppb. Values are expressed as ng g^{-1} of fresh sample material.