

INVESTIGATION OF OXIDATIVE STRESS IN SHEEP BRED IN AREAS EXPOSED TO DEPLETED URANIUM (DU) AMMUNITION

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The paper presents the preliminary results of the study on environmental and health effects due to the use of depleted uranium (DU) ammunition during NATO bombing of Serbia and Montenegro in 1999. The blood samples of sheep were collected randomly in the region of Bujanovac, in 2003. To assess the possible effects of DU on animal health, some of the relevant biochemical and hematological blood parameters were analyzed: concentration of RBC MDA (red blood cells malondialdehyde), erythrocytes superoxid dismutase (SOD) activity, hemoglobin concentration and number of erythrocytes (Er). Functional activity of leukocytes (Le) was performed using NBT reduction and adhesion test. The results are considered in correlation with the data on

the content of DU that entered the environment during the bombing. The obtained results on peripheral blood hematological and biochemical parameters indicated that animals were under violent oxidative stress. However, there were not enough data, especially on the content of DU in the environmental samples in the region (soils, vegetation, feed) to enable a conclusive correlation of the blood data with the effects of DU.

Key words: Depleted uranium (DU), oxidative stress, environment, health effects, peripheral blood parameters, sheep.

INTRODUCTION

Depleted uranium (DU) is radioactive material, a by-product of uranium processing in nuclear reactors, that came into military use as ammunition into the second half of the 20th century. DU particles from the missiles could be buried deep in the soil, adding to the content of natural uranium in the environment (LEE *et al.*, 2001). The impact of DU on the environment includes initial exposure of local population and animals due to DU particles resuspended from contaminated soils and food/feed, and long-term exposure from contaminated water or dust containing DU particles (WRENN *et al.*, 1985; RIBERA, 1996). Ingested or inhaled, DU appears in the bloodstream (WRENN *et al.*, 1985; LA TOCHE *et al.* 1987., RIBERA 1996), primarily in erythrocytes (FISENNE and PERRY, 1985), and subsequently, it accumulates in kidneys, skeleton, lungs, liver and heart (PRIEST *et al.*, 1982; WRENN *et al.*, 1985; LA TOCHE *et al.*, 1987; PELLMAR, 1999; Duraković, 1999), leading to increased production of reactive oxygen species - ROS (BOGDAN *et al.*, 2000). When ROS production is not followed by effective antioxidative defense, proteins, lipids and nucleic acids are damaged (SUTHERLAND, 2002; LORIMORE and WRIGHT, 2003). This provokes a wide spectrum of injuries and different clinical symptoms (PETKAU, 1971; PETKAU, 1972; GRAEUB, 1994; NUSSBAUM and KOHNLEIN, 1994).

During NATO bombing in the spring of 1999, there were 11 locations on the south of Serbia and in Montenegro hit by DU missiles, yielding a total activity over 10¹⁰ Bq (FM Report, 2000). Therefore, the aim of this study was to determine the concentration of basic marker of oxidative stress, red blood cells malondialdehyde (RBC MDA), as well as activity of RBC SOD. The obtained results are correlated with complete blood count and the functional activity of leukocytes.

MATERIAL AND METHODS

Samples of animal blood were collected randomly on 6 locations in the region of Bujanovac, in the spring of 2003. Blood was drawn from 20 clinically healthy sheep (Merinos/Svrljig, age 3-6), by puncture of v. jugularis (anticoagulant: acid-citrate dextrose). Randomly selected animals were held in pastures with free access to water, near the area of the strike. Control group of animals consisted of 10 sheep from locations not exposed to DU. There were no significant differences among the groups that could affect the examined parameters in blood (age, health status, nutrition, etc.).

Complete blood counts were determined according to standard laboratory procedures. RBC MDA concentrations and SOD activity were determined spectrophotometrically, on Specord M40 (Karl Zeiss, Jena, A 480-540 nm).

Leukocytes functional activity was evaluated by cytochemical assay for the respiratory burst (MONBOISSE *et al.*, 1991), as a measure of an intracellular reduction of nitro blue tetrazolium (NBT). To measure the spontaneous or induced reduction of NBT, leukocytes were incubated in the medium only (spontaneous reduction) or in the presence of 50 ng/ml PMA (induced reduction), respectively. Adhesion of leukocytes to plastic was assessed by a modified assay initially described by OEZ *et al.* (1990).

Statistical analysis was performed in Excel, using descriptive statistic tools and unpaired student T test.

RESULTS

The results on the number of erythrocytes, hemoglobine concentration, packed cell volume, SOD activity and RBC MDA concentration are presented in Table 1. The results are expressed as "means \pm standard error" (MV \pm SE).

All exposed sheep had a significantly lower number of Er ($p<0.001$), hemoglobin concentration ($p<0.001$) and PCV values ($p<0.001$) then the animals in the control group. Calculated main cell volume MCV (44.76 ± 1.89) and main cell hemoglobin concentration MCHC (275.40 ± 2.13) values indicate macrocytic hypochromic anemia. The animals had a significantly increased RBC MDA ($p<0.001$) and SOD activity, compared to the control, too.

Table 1. Number of Er, hemoglobin concentration (Hb), packed cell volume (PCV), SOD activity and RBC MDA in sheeps

	Experimental group		Control group	
	MV	SE	MV	SE
Er ($\times 10^{12}/L$)	5.41***	0.18	10.95	1.03
Hb (g/L)	78.7***	2.18	115.00	3.87
PCV (%)	26.7***	0.7	35.1	1.57
SOD (U/gHb)	4022***	202	2497	202
RBC MDA (nM/gHb)	2.199***	0.348	0.046	0.004

Significance: * $p<0.05$; *** $p<0.001$ vs. control

Total number of leukocytes, relative contribution of lymphocytes, monocytes and all granulocytes of examined sheep were within physiological range. The rate of NBT reduction *in vitro* (Table 2) indicated no changes in spontaneous and PMA-stimulated NBT reduction in leukocytes of the exposed animals. On the contrary, stimulated adhesion of leukocytes was significantly higher ($p<0.01$) in the exposed group vs. the control one.

Table 2. Spontaneous and PMA-stimulated NBT reduction test and adhesion of peripheral blood leukocytes

	NBT Reduction Test PMA (0 ng/ml)		PMA (50 ng/ml)		Adhesion PMA (0 ng/ml)		PMA (50 ng/ml)	
	Control group	Experimental group	Control group	Experimental group	Control group	Experimental group	Control group	Experimental group
MV	0.48	0.469	0.745	0.735	0.13	0.129	0.199	0.36**
SE	0.02	0.02	0.1	0.03	0.02	0.01	0.04	0.02

Significance at ** $p < 0.01$ vs. control

DISCUSSION

In our study, the examined animals had a smaller number of erythrocytes and significant decrease in hemoglobin concentration and PCV than the controls. Hematological MCV and MCHC values indicated macrocytic hypochromic anemia. Macrocytosis and hypochromia due to increased reticulocyte number, suggested responsive anemia. The results showed significantly higher RBC MDA concentration in the exposed vs. control group. Since the concentration of RBC MDA indicates severe oxidative damage of erythrocyte plasma membrane, one could conclude that pathogenesis of established anemia is partially provoked by this mechanism (MATTIA *et al.*, 1993a, 1993b; MYHRE *et al.*, 2001).

Extremely high activity of SOD (COTGREAVE *et al.*, 1988; BOGDAN *et al.*, 2000) indicates its increased antioxidant action. In our case, although SOD activity was significantly increased, obviously it was not enough to neutralize such intensive production of free oxygen radicals.

All examined sheep had the number of leukocytes and the differential Le-count within the physiological range. No changes in the spontaneous and PMA-stimulated leukocytes NBT reduction test suggested that in examined animals, granulocyte respiratory burst mechanism was not activated. Increased Le PMA stimulated adhesiveness pointed to the increased leukocytes functional activity.

However, the radioactivity measurements of the environmental samples from the region (BOŽIĆ *et al.*, 2004), have shown that the concentrations of radionuclides in soils and feed sampled at locations the examined animals were bred on, were within the range of the average radionuclides concentrations for the country, while the activities of uranium were under 10^3 Bq/kg (TODOROVIĆ *et al.*, 2001).

To conclude, the results on biochemical and hematological parameters indicated an existence of an oxidative stress effects and macrocytic hypochromic anemia in sheep bred in the region exposed to DU ammunition. While total leukocyte number and differential Le count were within physiological values, the increased PMA stimulated Le-adhesiveness in the exposed sheep pointed to their increased functional activity. NBT reduction test indicated absence of leukocytes oxidative burst. As all examined sheep were clinically healthy, the obtained results indicate that animals were exposed to severe oxidative stress, due to some

disturbances in the environment. However, as the study was performed on a small group of animals, and there are no data indicating the higher content of uranium in soils and feed in the region, we cannot conclusively correlate the obtained data with the effects of DU that entered the environment.

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ISPITIVANJE OKSIDATIVNOG STRESA KOD OVACA U OBLASTIMA IZLOŽENIM DEJSTVU OSIROMAŠENOG URANIJUMA

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I z v o d

U radu su prikazani preliminarni rezultati ispitivanja mogućih efekata municije sa osiromašenim uranijumom upotrebljene tokom NATO bombardovanja Srbije i Crne Gore 1999. godine, na zdravlje životinja i životnu sredinu.

Uzorci krvi su sakupljeni od ovaca, metodom slučajnog izbora, u proleće 2003 godine. U cilju ispitivanja mogućeg dejstva osiromašenog uranijuma na zdravlje životinja, analizirani su neki od relevantnih biohemijskih i hematoloških parametara: koncentracija malondialdehida u eritrocitima, aktivnost eritrocitne superoksid dismutaze, koncentracija hemoglobina, broj eritrocita i dr.

Funkcionalna aktivnost leukocita je ispitana testom NBT redukcije i adhezije. Rezultati su razmotreni u korelaciji sa podacima o sadržaju osiromašenog uranijuma koji je dospao u životnu sredinu tokom bombardovanja.

Hematološki i biohemijski parametri periferne krvi ukazuju da su životinje bile izložene jakom oksidativnom stresu. Međutim, nepostojanje pouzdanih podataka o sadržaju DU u području sa koga su ispitivane životinje, ne omogućava ustanovljavanje pouzdane korelacije između dobijenih rezultata i efekata osiromašenog uranijuma koji je dospao u životnu sredinu.

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