Effects of depleted uranium on immune parameters of zebrafish, *Danio rerio*, measured by flow cytometry

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Introduction

Uranium is ubiquitous in natural waters at concentrations ranging from a few ng/L to low µg/L depending on the composition of surrounding rocks and up to 1 mg/L at the vicinity of uraniferous sites.[1] Depleted uranium (DU) is a byproduct of enriched uranium and is used in military, aviation, medical and research applications. Freshwater ecosystems may constitute the final receptor areas of DU to which they may be chronically exposed both to its chemical toxicity.[2]

Among all physiological processes possibly disturbed by pollutants, the immune system is likely to be one of the more sensitive physiological systems.[3] Fish innate immune responses, which are the first line of immune system defence of these organisms may be suppressed by xenobiotics and seem to represent relevant immunotoxic endpoints.[4] Keller *et al.*[5] reported effects of cadmium, copper and zinc on immune parameters including total white blood cells, macrophage activity, and oxidative burst in several fish species. Mercury and selenite were also proved to be toxic to lysozyme activity and lymphocyte proliferation in blue gourami.[6] Phagocytosis and oxidative burst were also reduced in the presence of cadmium or mercury in rainbow trout.[7] However, data on effects of DU on fish immune system are scarce.

The objective of this work was to test the potential impacts of DU on zebrafish immune system using several endpoints measured by flow cytometry: cell population, cell mortality, reactive oxygen species (ROS) production, lysosome integrity and phagocytosis activity. Two separate experiments were conducted on zebrafish in order to assess DU effects *in vivo* on adults and *ex vivo* on leucocytes.

Materials and Methods

Freshly isolated leucocytes from naïve fish were exposed *ex vivo* during 17 h to 0, 20, 250, 500 µg DU/L. In *in vivo* experiment, adults were exposed during 3 days to 20 and 250 µg/L; a control with no DU was also added. Adult animals were sacrificed for sampling after 3 days in order to assess the activity of different immune parameters: cell sub-population, cell mortality (propidium iodide exclusion test), oxidative burst [H_2DCFDA before and after stimulation by phorbol 12-myristate 13-acetate (PMA)], lysosomal membrane integrity (LMI) (acridine orange) and phagocytosis (fluorescent beads). At the end of the *ex vivo* contamination. The same biomarkers were measured on leucocytes exposed to DU, except for LMI. Results were expressed as percentage of positive cells for cell sub-population, mortality and phagocytosis, and as the mean fluorescence intensity for ROS production and LMI.

Results and Discussion

In the *ex vivo* experiment, ROS basal level was higher in cells exposed to DU for all tested concentrations (Table 1). ROS stimulated level showed no difference between control and DU concentrations (Table 1). ROS stimulation index was therefore lower in cells exposed to DU for all tested concentrations (Table 1). No other significant effect of DU was detected on other parameters tested.

In the *in vivo* experiment, ROS basal level was higher in fish exposed to DU for all tested concentrations (Table 2). ROS stimulated level was higher in fish exposed to 250 µg DU/L (Table 2). ROS stimulation index was therefore lower in cells exposed to DU for all tested concentrations (Table 2). LMI was lower in fish exposed at 250 µg DU/L (Table 2). No other significant effect of DU was detected on

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Table 1. Effects of depleted uranium on leucocyte parameters during the *ex vivo* experiment. Values are means of 20 replicates, except for reactive oxygen species values for technical reasons (n comprised between 8 and 14); standard error is presented.

[DU] (µg/L)	ROS basal level (MFI)	ROS activated level (MFI)	ROS index
0	12.2±1.1[a]	9.3±0.7	0.90±0.1[b]
20	16.5±1.1[ab]	7.6±1.1	0.46±0.1[a]
250	19.2±2.3[b]	7.5±1.1	0.43±0.1[a]
500	20.4±3.2[b]	7.8±1.0	0.48±0.1[a]

DU, depleted uranium; ROS, reactive oxygen species; MFI, mean fluorescence intensity. [a,b] significantly different from control at P<0.05; a<b.

Table 2. Effects of depleted uranium on leucocyte parameters during the in vivo experiment. Values are means of 20 replicates, except for controls values (n=18); standard error is presented.

[DU] (µg/L)	ROS basal level (MFI)	ROS activated level (MFI)	ROS index	LMI (MFI)
0	5.8±0.5[a]	12.9±3.5[a]	2.3±0.7[b]	167.1±8.7[b]
20	12.2±1.1[b]	11.5±1.3[a]	1.0±0.1[a]	149.8±7.3[b]
250	14.9±1.4[b]	17.3±1.2[b]	1.2±0.1[a]	130.3±5.5[a]

DU, depleted uranium; ROS, reactive oxygen species; MFI, mean fluorescence intensity; LMI, lysosomal membrane integrity. [a,b] significantly different from control at P<0.05; a<b.

other parameters tested.

LMI decreased in fish exposed to 250 µg DU/L after 3 days. In earthworms, lysosomal stability decreased with increasing concentrations of U or DU in soils.[8] In rat hepatocytes, DU induced lysosomal membrane rupture.[9,10] Therefore, our study confirmed that DU can affect the lysosomal membrane integrity in adult zebrafish.

DU increased ROS basal level with no modification on ROS PMA-stimulated level, leading to a reduction of ROS stimulation index in both *in vivo* and *ex vivo* experiments for all concentration tested. In rats, uranyl acetate and DU increased ROS basal level in kidney mitochondria.[9,10,11] In a previous study, we showed an increase of ROS fold induction index in living whole kidneys removed from zebrafish exposed to 20 µg DU/L during 28 days.[12] It appears that DU induces the ROS basal production in zebrafish kidney leucocytes, showing a similarity to the mechanism of action of uranium known in mammals. This phenomenon could lead to an oxidative stress (inflammation) on the whole organism.

In conclusion, our study examined the effects of DU on immune parameters in zebrafish, *Danio rerio* for the first time. We showed that at environmentally-relevant concentrations of DU, an increase in ROS basal production, decrease of ROS stimulation by PMA and lysosomal membrane integrity in kidney leucocytes were produced. We conclude that DU could pose of risk to fish health in contaminated environments.

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