Modifications of Inflammatory Pathways in Rat Intestine Following Chronic Ingestion of Depleted Uranium

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The environmental contamination by dispersion of depleted uranium (DU) might result in its chronic ingestion of DU by local populations. The aim of this study was to determine if chronic ingestion of DU at low doses induces inflammatory reactions in intestine, first biological system exposed to uranium after ingestion. Experiments were performed with rats receiving uranium in drinking water (40 mg/l) during 3, 6, or 9 months. Several parameters referring to prostaglandin, histamine, cytokine, and nitric oxide (NO) pathways were assessed in ileum. Concerning the prostaglandin pathway, a twofold increase in gene expression of cyclooxygenase of type 2 was noted after 6 months, with no changes in prostaglandins levels. At the same time, a decrease in mast cell number was observed without any changes in histamine levels. Experiments on cytokines showed increased gene expression of interleukin (IL)-1β and IL-10 at 6 months, and decreased messenger RNA level of CCL-2. This change was associated with decreased macrophage density. An opposite effect of DU was observed on neutrophils, since increased number was observed at 3 (×1.7) and 9 months (×3). The results obtained on NO pathway seemed to indicate that DU exposure inhibited this pathway (decreased endothelial NO synthase messenger RNA, inductive NO synthase activity and NO₂⁻/NO₃⁻ levels) at 6 months. In conclusion, this study demonstrated that chronic ingestion of DU-induced time-dependent modifications of inflammatory pathways, notably in terms of immune cell content. The ultimate effects of DU contamination might be pathogenic by suppressing defense mechanisms or inducing hypersensitivity. Further experiments should be thus performed to determine real consequences on intestinal response to oral antigens.

Key Words: uranyl nitrate; prostaglandins; cytokines; nitric oxide; immune cells.

The biological consequences on health of contamination by ingesting or inhaling a toxic element status are of increasing concern, with regard to the wide dispersal of pollutants in the environment (heavy metals, pesticides, fuel particles, and radionuclides) created by various human activities. For instance, the civilian and military use of uranium, as well as fuel in nuclear power reactors, counterweights in aircraft and penetrators in shrapnel, may lead to the release of this radionuclide into the environment. This was the case in Amsterdam after the aircraft crash in 1992 (Uijt de Haag et al., 2000), around uranium processing areas (Pinney et al., 2003) or following the drop of some 300 tons of depleted uranium (DU) during the Gulf War (Bem and Bou-Rabee, 2004). This uranium dispersion may cause contamination of water wells and/or into the food chain (Di Lella et al., 2005), which may lead to a chronic contamination by ingestion of local populations.

Chronic ingestion of uranium addressed the question of its biological effects. Several studies demonstrated that particles, whatever the element, triggered pro-inflammatory response, characterized by upregulation of cytokine levels and/or immune cell density, in lungs after inhalation of particulate matter. This inflammation was induced by particles of various sizes such as nanoparticles (Inoue et al., 2005), ultra fine particles (Stoeger et al., 2006), or by soluble transition metals (McNeilly et al., 2005). Induction of diverse inflammatory reactions was also reported following uranium contamination in different tissues. For instance, activation of cytokine expression and/or production was noted either in pulmonary tissues following uranium exposure by inhalation (Monleau et al., 2006) or in macrophages after in vitro contamination (Gazin et al., 2004; Wan et al., 2006). Stimulation of prostaglandins was also reported in the kidney after acute contamination (Chaudhari and Kirschenbaum, 1983, 1984). Moreover, more recent studies have demonstrated an effect of DU on the nitric oxide (NO) pathway in the short term in cortex (Abou-Donia et al., 2002) or in the long-term in testicles (Li et al., 2005) following uranium contamination. However, the diversity of these reports in terms of contamination times, uranium doses, contamination pathways, analyzed tissues, and inflammatory pathways studied have made it impossible to obtain an obvious view on the possible effects of uranium on...
induction of inflammation. In addition, it is worthy of note that no effects on inflammation following chronic contamination with radionuclides have been reported on the small intestine that constitutes the entry route in case of ingestion. This point is of particular interest as relationships between ultra fine particles contained in diet and transmural inflammation have recently been discussed (Lomer et al., 2002). Only one study indicated that an acute contamination with DU at high doses modulated expression and/or production of cytokines in the intestine (Dublineau et al., 2006b). Nevertheless, no information was available in the literature concerning the possible effects of uranium following a chronic contamination at low doses by ingestion on inflammatory reactions in the intestine.

The aims of the present study were thus to determine the effects on the small intestine of a chronic contamination with DU on different inflammatory pathways including prostaglandins (PGE2), histamine, cytokines (interferon [IFN]-γ, tumor necrosis factor [TNF]-α, transforming growth factor [TGF]-β, interleukin [IL]-1β, IL-10, IL-13, CCL-2), and NO synthases. These experiments were performed in rats receiving DU in drinking water during 3, 6, or 9 months. The uranium concentration used here in the drinking water was close to maximal environmental doses found in Finland (20 mg/l; Junutunen, 1991).

The results of this study will be used to determine whether chronic uranium ingestion led to a generalized inflammatory reaction in the intestine or a more specific effect to a single pathway. More particularly, the question was to understand whether uranium ingestion induced successive or simultaneous effects on the various inflammatory pathways (prostaglandins, histamine, cytokines, and NO) and whether the induction time as well as the duration time of the DU effects were similar for the four pathways.

MATERIAL AND METHODS

Animals

Experiments were performed on male Sprague–Dawley rats weighing 300 g at the beginning of experiments obtained from Charles River (L’Arbresle, France). They were kept under constant temperature (21 ± 2°C) with a 12:12-h (light:dark) cycle and had free access to normal rat food (105, CERJ, France) and water throughout the experimental period. The experiments were conducted according to the French regulations for animal experimentation (Ministry of Agriculture Act No. 2001-464, May 2001) and with the agreement of the local animal welfare committee of Institut de Radioprotection et de Surete Nucleaire.

In Vivo Contamination Procedures

Contamination of animals began after 2 weeks of acclimation, which corresponded at 12 weeks old. Animals were contaminated during 3, 6, or 9 months (10 individuals per group) with DU contained in drinking water at the quantity of 40 mg/l (AREVA-COGEMA, France). This corresponded to a daily ingestion of about 1 mg in rats. Previous studies carried out with the same contamination protocol indicated that this dose of DU was not toxic with regard to food intake, weight gain, or the general health status of animals.

Determination of immune cells. Histological analyses were performed on terminal ileum. For the estimation of neutrophil infiltration, some ileal segments obtained at euthanasia were rinsed with NaCl 0.9% and fixed in 4% formaldehyde solution (Carlo Erba, Rueil Malmaison, France) at room temperature. They were then dehydrated, embedded in paraffin, and cut into 5-μm-thick sections. The tissue neutrophil infiltration was estimated on paraffin sections incubated with rabbit antibody directed against rat myeloperoxidase (MPO, d = 1/200, Novocastra, Newcastle upon Tyne, UK) and LSAB2 HRP kit (Dako, Trappes, France). The macrophages were visualized with mouse antibody against CD68 (Serotec, Cergy Saint Christophe, France) followed by histoimage simple stain mouse MAX PO (Microm Microtech, Francheville, France). For mast cell coloration, the ileal segments were fixed in Carney solution. Histological slices of 5 μm were then stained with Alcian Blue method.

Quantitative analysis was performed by determining the number of mast cells and neutrophils per crypt-villus axis. The results were expressed as mean ± SEM of six animals.

Gene expression. Total RNA was prepared from 50 mg tissue with the RNeasy mini kit (Qiagen Courtabeuf, France) following the manufacturer’s instructions.

Reverse transcription. One microgram of total RNA was reverse transcribed at 42°C for 50 min with random hexamers. 20 μg: deoxyribonucleoside triphosphate, 1mM; dithiothreitol, 10mM; ribonuclease inhibitor (RNase out), 20 U and Superscript reverse transcriptase, 200 U.

Polymerase chain reaction. PCR amplification was performed using Syber PCR master mix (Qiagen, Courtabeuf, France) in a final volume of 25 μl. The primer sets were previously reported (Dublineau et al., 2006a; Gremy et al., 2006). Real-time semi-quantitative analysis was performed with the Abi prism 7000 sequence detection system (Applied Biosystems, Courtabeuf, France). For the relative quantification of the target gene, data were normalized to an endogenous reference (HPRT, hypoxanthine-guanine phosphoribosyltransferase, a housekeeping gene), and the uncontrolled analysis was performed for three months. Data were then expressed as mean ± SEM.

Determination of Inflammatory Mediator Tissue Levels

Mucosal samples obtained by scraping the rat terminal ileum at euthanasia were kept at −80°C until analysis. Mucosal protein extracts were obtained from tissue homogenates performed using ribolyser (COGER, Paris, France) in phosphate buffer (pH = 7.4, PBS, Gibco, Invitrogen, Cergy Pontoise, France) containing a protease inhibitor cocktail (0.5 ml/100 ml, Sigma, L’Isle D’Abeau Chesnes, France). This step was followed by a centrifugation step (10,000 × g, 15 min). Tissue levels of PGE2 were measured in supematants with an ELISA (enzyme-linked immunosorbent assay) Quantikine kit (R&D, Lille, France), and tissue levels of CCL-2 were quantified with a kit from Biosource (CCL-2 or MCP-1, Montrouge, France). Nitrites/nitrates in intestinal tissues were measured using a R&D kit after filtration (< 10 kDa). For histamine quantification, tissue ribolysis was performed in HCl 0.1M. Neutralization with NaOH 1M was made after the 10,000 × g centrifugation. Tissue histamine was measured with a kit from Neogen Corporation (Ayrshire, Scotland, UK). The rat mast cell protease type of 2 (RCMP II) was also measured in ileal tissue samples at (d = 1/5000) using the ELISA kit originating from Moreduc (Moreduen Scientific, Midlothian, Scotland, UK). The results were expressed per milligram of protein determined with Bradford assay (Sigma).

Measurement of NOS Activity

The total (endothelial NO synthase [eNOS] + inductive NO synthase [iNOS]) activity was determined by the L-[14C] arginine to L-[14C] citrulline conversion assay, using a commercial kit (Cayman Chemical Company, Ann Arbor, MI). Briefly, 10 μl of samples was incubated at room temperature during 30 min with reaction buffer and calmodulin (0.1μM). The samples were then eluted with resin in spin cups by centrifugation. The L-[14C] citrulline was then eluted with resin in spin cups by centrifugation. The L-[14C] citrulline was then measured by scintillation (Ultima Gold AB, Perkin Elmer, Courtabeuf, France) in a beta counter (Tri-Carb 2300TR, Packard Instruments, Perkin Elmer). The results were expressed in percentage of the respective control group.
Statistical Analysis

Data are expressed as the mean ± SEM of 6–10 animals per group depending on the considered analyses. Statistical comparisons between different groups were performed using an unpaired Student’s t-test or a one-way analysis of variance (Kruskal–Wallis or Tukey test) when necessary. A p-value of less than 0.05 was considered as significant.

RESULTS

The inflammatory pathway leading to the production of prostaglandins was studied first. The Figure 1 reports the results on the relative gene expression of inductive enzyme cyclooxygenase of type 2 (COX2) and the tissue level of PGE2 in ileal mucosal extracts after 3, 6, and 9 months of DU chronic contamination. The relative expression of COX2 was decreased at 6 months in control animals as compared with at 3 months in the control group (Fig. 1A). This decrease was not observed in DU–contaminated animals, which resulted in a significant difference between control and DU-contaminated animals at 6 months. However, this effect on gene messenger RNA (mRNA) levels of COX2 was not followed by a change in tissue level of PGE2, regardless of contamination time (Fig. 1B).

The possible effects of DU on mast cells (Figs. 2 and 3A) and subsequent histamine liberation (Fig. 4) were also evaluated in this study. Concerning mast cell population, a decrease in the number of mast cells per crypt-villus axis was noted at 6 months in contaminated animals as compared with the control group (p < 0.01) (Fig. 3A). This was mainly due to an increased number in control animals (1.8-fold) at 6 and 9 months as compared with the control group at 3 months without similar age-dependent increase in contaminated animals. The measurement of the RMCP II in ileum of control and DU noncontaminated animals corroborated the results obtained on the mast cell number, since a diminution of tissue level of RMCP II was induced at 6-months postcontamination as compared with the age-matched control group (Fig. 3B). The basal level of histamine was also measured in the mucosal extracts of intestine of DU-contaminated animals as compared with control rats following 3, 6, or 9 months of chronic uranium ingestion (Fig. 4). There were no significant differences in DU-contaminated animals, regardless of contamination time.

In addition to mast cells, different immune cell populations (neutrophils and macrophages) present in the intestinal wall were analyzed after DU contamination (Fig. 2). A comparison of contaminated animals with controls did not show major modification in localization of these different cell populations, which were present in the lamina propria. The cell counting of neutrophils was performed in ileum after 3, 6, or 9 months of chronic contamination (Fig. 5). The results showed a regular decrease in neutrophil number dependent on the age of animals for control rats. Such an age-dependent decrease was not observed in contaminated rats, for which the neutrophil number measured per villus remained elevated, especially at 9 months. The real quantification of macrophages was not realizable because of the high density of these immune cells in rat ileum (see Fig. 2). However, the examination of histological slides seemed to provide evidence of a decrease in the density of macrophages at 6 and 9 months of DU contamination without change in localization.

The mRNA and protein levels were measured for the monocyte attractant CCL-2 (or MCP-1) (Fig. 6). No change was observed after a 3-month DU contamination. The mRNA level of CCL-2 was increased in control animals at 6 months. Such an increase was not observed in age-matched control rats. Consequently, a 50% decrease was induced at 6 months of DU contamination. The decrease was maintained at 9 months. However, the protein level was not modified by uranium as shown in the Figure 6, regardless of the contamination time considered.

![Graph](https://example.com/graph.png)

**FIG. 1.** Effects of chronic contamination with DU on prostaglandin pathway. Measurements were carried out in mucosal extracts of rat ileum in control (○) and DU-contaminated (●) animals during 3, 6, or 9 months. (A) Enzyme gene expressions of inductive COX2 were expressed as a ratio to the reference gene HPRT. Data were mean ± SEM (n = 7–10). §§§p < 0.001: significantly different from control values at 3 months. *p < 0.05: significantly different from uncontaminated animals at 6 months. (B) Intestinal levels of prostaglandins after chronic ingestion of DU. Tissue levels were expressed in ng/mg protein. Values were mean ± SEM (n = 8).
Relative mRNA levels of some pro-inflammatory (IFN-γ, TNF-α, TGF-β, IL-1β) and anti-inflammatory (IL-10, IL-13) cytokines were measured in ileal tissues after 3, 6, and 9 months of DU ingestion. The results indicated in the Figure 7 show evidence of marked effects of age on mRNA levels of some studied cytokines. Firstly, a drastic decrease in IFN-γ gene expression was noted in older animals (9-month contamination). This decrease was similar in uncontaminated and DU-contaminated animals. Concerning the uranium effects, diminution or elevation of mRNA levels was obtained dependent on the cytokine type. A decrease was observed at 3 months for TNF-α, and a rise in gene expression (< twofolds) was induced for IL-1β and IL-10 at 6-months DU contamination.

The second inflammatory pathway studied was that involved in the NO production. We measured the gene expression of the two enzymes responsible for the synthesis of NO (Fig. 8), iNOS and constitutive eNOS, their enzyme activity (Fig. 9), and the ileal level of nitrites/nitrates representative of NO production (Fig. 10) at 3, 6, and 9 months of chronic uranium ingestion. No effect was reported for iNOS mRNA levels mainly due to the huge variability between animals in the 3-month control group (Fig. 8A). The results on eNOS mRNA levels indicated an age-dependent increase in control animals (respectively, five and ninefolds at 6 and 9 months) (Fig. 8B). Such a drastic increase was less pronounced in DU-contaminated animals, which resulted in a diminution in mRNA levels of eNOS following a chronic ingestion of DU of 6 and 9 months as compared with their age-matched control. A stimulatory effect of DU was observed on the total enzyme activity of NO synthases, notably at 6 and 9 months of contamination (×2.5) (Fig. 9). This rise was mainly due to an effect of DU on constitutive NO synthases. Conversely, a decrease at the limit of significance (p = 0.057) was observed in the activity of iNOS at 6 months of uranium contamination. The changes in enzyme activity of NO synthases (increased eNOS and decreased iNOS activities) resulted in a diminution of nitrites/nitrates levels and NO metabolites in ileal mucosal extracts of contaminated animals at 6-months DU contamination (Fig. 10).

**DISCUSSION**

This study demonstrated that DU at low doses induced changes in the inflammatory pathways in rat intestine following
chronic ingestion. All inflammatory pathways, i.e. prostaglandins, histamine, cytokines, and NO, seemed to be affected by the DU contamination, although the effect levels, induction, and duration times were somewhat different depending on the pathway considered.

The minor effects of uranium on prostaglandin pathway were not surprising. Some studies reported that uranium could interfere with this pathway following acute administration of a high dose of uranyl nitrate (Chaudhari and Kirschenbaum, 204).

FIG. 3. Evaluation of mast cell activation following chronic contamination with DU. (A) Ileal density of mast cells at different times of chronic DU contamination. The positive cells were estimated per villus-crypt axis, along the 30 villus-crypt axis per animal. Data were mean ± SEM of six animals. **p < 0.001: significantly different from age-matched control values. §§§p < 0.001: significantly different from control values obtained at 3 months (unpaired Student’s t-test). (B) Measurement of RMCP II in mucosal extracts of rat ileum in control (■) and DU-contaminated (●) animals at 3, 6, or 9 months. Data were mean ± SEM of six animals. §§§p < 0.01: significantly different from the 3-month control group. **p < 0.05; ***p < 0.001: significantly different from age-matched control values.

FIG. 4. Intestinal tissue levels of histamine after chronic ingestion of DU. Measurements were performed in mucosal extracts of rat ileum in control (■) and DU-contaminated (●) animals during 3, 6, or 9 months. Tissue levels of histamine were expressed in ng/mg protein. Values were mean ± SEM (n = 10).

FIG. 5. Evaluation of the ileal density of neutrophils at different times of chronic DU contamination. The positive cells were estimated per villus-crypt axis, along the 30 villus-crypt axis per animal, in control (■) and DU-contaminated (●) groups during 3, 6, or 9 months. Data were mean ± SEM of six animals. §p < 0.05: significantly different from the 3-month control group. *p < 0.05: significantly different from age-matched control values.
In these studies, the authors demonstrated that uranium increased PGE$_2$ production in the short term. This rise in prostaglandin liberation resulted from a decreased PG metabolism (via the PGE$_2$-9-ketoreductase pathway) rather than from an increase in its synthesis (Chaudhari and Kirschenbaum, 1983). This increased PGE$_2$ production seemed to be a protective mechanism against the effects of vasoconstrictor factors released following uranium-induced renal failure. However, such tissue failure was not induced in the present study because of the low dose used. In fact, the uranium quantity in kidneys, reference tissue for uranium accumulation, reached 0.06 $\mu$g/g tissue after a 9-months DU ingestion (Paquet et al., 2006) while the minimal toxic dose was estimated at 0.7 $\mu$g/g tissue (Diamond et al., 1989). The conclusion therefore is that chronic ingestion of DU at non toxic doses did not have a marked effect on prostaglandin pathway in the intestine.

The first point to note concerning the results on intestinal mast cell number is the increase in age-related mast cell number in control animals with an augmented number of mast cell at 6 and 9 months as compared to 3-month animal (Fig. 9). This result was similar to the one obtained in a previous study that show evidence of an increase in mast cell numbers of adult animals compared with young (Matsson, 1993). This age-dependent increase was abolished in DU-contaminated animals, since mast cell quantification was not significantly different between the three contamination periods studied (3, 6, and 9 months). Consecutively, a diminution of mucosal mast cell number corroborating an intestinal content of RMCP II was noted at 6 months in DU-contaminated animals. Two studies reported mast cell alteration after administration of thorium or plutonium (Rowley, 1963; Sanders and Adee, 1969). These data, combined with the results of this study, indicated therefore that mast cells were sensitive to internal contamination with actinides. The absence of changes in histamine content either after uranium contamination or dependent on age may appear surprising. Nevertheless, this lack of histamine content variations was compatible with the slight biological significance of mast cell secretion in the absence of immunological stimuli.

Ageing is associated with a decline of neutrophil number and/or functions (Plackett et al., 2004; Schroder and Rink, 2003), which corroborated the results obtained in uncontaminated animals (slow decrease throughout the entire life). One very interesting result reported in this study was the abolishment of the decreasing age-dependent number of neutrophils in the intestinal wall after DU contamination (Fig. 5). Data currently available in the literature indicated an increase in blood neutrophil leukocytes in workers occupationally exposed to lead (Di Lorenzo et al., 2006; Queiroz et al., 1993). This suggested that uranium effects were similar to those obtained with lead, despite the difficulty in correlating the neutrophil number in tissue and in blood.

A modification in macrophage population seemed also to be induced following chronic DU contamination, leading to a decrease in the macrophage density at 6 and 9 months. The decrease in macrophage population agreed with the decrease in gene expression of CCL-2 (monocyte attractant) observed at the same time. Decrease in protein levels was also reported in ileal mucosa in the short term following acute contamination with DU (Dublineau et al., 2006b). Uranium uptake by alveolar macrophages has been shown to occur after inhalation of soluble and insoluble compounds (Berry et al., 1997), and it has been demonstrated that the subsequent uranium accumulation may lead to increased production of cytokines/chemokines and apoptosis (Gazin et al., 2004; Lizon and Fritsch 1999; Wan et al., 2006). The effects obtained in the present study on macrophages thus indicated that soluble uranium could also alter this immune population in intestine after chronic ingestion.
Chronic DU contamination also induced some modifications in cytokine/chemokine expression: chemokine CCL-2 (see above), pro-inflammatory cytokines IL-1β and TNF-α, and anti-inflammatory cytokine IL-10. The decrease in TNF-α expression was quite surprising, as previous studies reported increases in mRNA or protein levels of this cytokine following uranium contamination (Gazin et al., 2004; Monleau et al., 2006). Another study demonstrated no significant molecular effects of DU on TNF-α (Dublineau et al., 2006a). Concerning IL-10, an increase in gene expression was already described after uranium exposure (Wan et al., 2006), which suggested that uranium induced an anti-inflammatory reaction.

The NO pathway seemed to be a preferential target for uranium effects: chronic ingestion of DU-induced decrease in gene expression, stimulation of enzyme activity of eNOS and slight decrease in iNOS activity, as well as diminution of NO metabolite content. Two publications reported effects of uranium on the NOergic system. Increase in nitrite levels was
observed in some brain regions in rats after administration of 0.1 mg/kg uranyl acetate for 7 days (Abou-Donia et al., 2002). Other study indicated an increase in the gene expression of iNOS in testis of rats following intratracheal instillation (Li et al., 2005). These contamination protocols were still too different to compare their results with those obtained in the present study following long-term chronic contamination. However, an extensive series of publications was available in the literature on the effects of lead on the NO pathway (Barbosa et al., 2006; Kong et al., 2000; Zhu et al., 2005). The results of all these studies showed evidence that the NO pathway was a target for lead exposure even in the case of chronic exposure at low doses, which resulted in an inhibition of NO synthesis leading to functional impairment of the tissues involved (Carmignani et al., 2000; Zhu et al., 2005). The lead-induced inhibition of NO production was reported in the central nervous system (Zhu et al., 2005), blood (Barbosa et al., 2006), in kidneys (Dursun et al., 2005), in cardiovascular system.

FIG. 8. Effects of chronic contamination with DU on mRNA levels of NO synthases in rat intestine. Measurements were carried out in mucosal extracts of rat ileum in control (□) and DU-contaminated (●) animals at 3, 6, or 9 months. The mRNA levels of iNOS (Part A) and eNOS (Part B) were expressed as a ratio to the reference gene HPRT. Data were mean ± SEM (n = 6). §§§p < 0.001: significantly different from 3-month control group; *p < 0.05: significantly different from the age-matched control group.

FIG. 9. Effects of chronic contamination with DU on activity of constitutive and inducible NO synthesis enzyme. Measurements were carried out in mucosal extracts of rat ileum in control (□) and DU-contaminated (●) animals during 3, 6, or 9 months. (A) Determination of total enzyme activity of NO synthesis (iNOS + eNOS) measured in presence of Ca2+. (B) Determination of iNOS enzyme activity measured in absence of Ca2+. NO synthase activities were expressed as percentage of age-matched control group. Data were mean ± SEM (n = 6). μp = 0.057; *p < 0.05; **p < 0.001: significantly different from age-matched control values.
(Carmignani et al., 2000), in immune cells (Bishayi and Sengupta, 2006), and in intestine (Kong et al., 2000), suggesting a common mechanism present for these tissues. The results presented in the present study in intestine indicated that chronic ingestion of DU had similar effects than those observed with lead on the NOergic pathway. Several hypotheses may be postulated to explain these inhibitory effects of uranium on the NO pathway. The first one involved direct interference of uranium with calcium. An exchange of uranyl ion with calcium has been assumed at bone surface and inhibition of different calcium transporters by uranium was reported several years ago (Desmedt et al., 1993; Thompson and Nechay, 1981). Interactions between metals and calcium were already suggested for lead with transporters or calcium-binding proteins (Kern and Audesirk, 2000; Simons, 1993). Several authors demonstrated that Pb²⁺ could be substituted for Ca²⁺ in the activation of calmodulin, leading to negative effects on iNOS activity (Gribovskaja et al., 2005; Simons, 1993). It can be thus suggested that uranium inhibits the NO production via interaction with calmodulin, but further experiments have to be performed to validate this hypothesis. Another hypothesis for NO inhibition is the activation of inhibitors of iNOS, such as IL-4 and IL-10 (Simmons and Murphy, 1993). Such uranium effect was actually conceivable since an increased gene expression of IL-10 was observed at 6 months of DU contamination. Thirdly, an inhibitory effect of uranium-induced oxidative stress could be involved in the decrease of intestinal NO production after ingestion. In fact, oxidative stress has been observed following uranium contamination (Linares et al., 2006; Periyakaruppan et al., 2006). Here again, similarity between uranium and lead may be noticed because lead-induced oxidative stress has been shown as causing NO inactivation (Vaziri and Ding, 2001). Finally, it has been demonstrated that iNOS was activated by CCL-2 (Biswas et al., 2001). Thus, the decrease in gene expression of CCL-2 observed at 6-months DU ingestion could be explained by the diminution in iNOS activity induced at the same contamination time. In conclusion, the conditions to inhibit the NO production in tissues following uranium ingestion seemed to be present altogether: decrease in NO production cells (macrophage), diminution in iNOS activators (CCL-2), increase in oxidative stress, and elevated levels in NO inhibitors (IL-10).

In summary, this study demonstrated that DU-induced effects on different inflammatory pathways in the intestine following chronic ingestion at low doses. Firstly, the effects of uranium ingestion on the intestine were the modifications in age-dependent evolution of some molecular and cellular actors of the mucosal immune system. Secondly, inhibition of NO pathway was induced by chronic ingestion of DU at low doses probably due to convergent pro- and anti-inflammatory processes. In addition, most of DU effects were similar to those observed after an exposure to lead, indicating that the DU effects were mainly due to its chemical toxicity and not to its radiological properties. These DU effects were observed in adult organisms after chronic contamination during their mature state. It seems now important to define the biological consequences in animals contaminated during their early stages of life, when they were more sensitive to metal intoxication.

The ultimate effects of DU chronic contamination could be pathogenic, by either suppression of defense mechanisms or induction of hypersensitivity, following the increased neutrophil content and the decreased macrophage population. Several current studies have demonstrated that oral tolerance mechanisms to alimentary antigen (ovalbumin) after administration of lead were disturbed (Goebel et al., 2000). The perspectives of the present study are thus to evaluate the consequences of
DU-induced inflammatory effects on the response of the mucosal immune system to pathogenic or aluminant antigens.

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