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Research Article

The Effects of Gadolinium in the Uterus and in the Ovary of Pregnant Rat: Ultrastructural Study

Mhamdi Marwa[1,2]*, Badri Nedra[1,2], Florea Adrian[2], Matei Horea[2], Maghraoui Samira[1] and Tekaya Leila[1]

[1]Laboratory of Physiology, Faculty of Medicine of Tunis, University of Tunis El Manar, Baab Saadoun, Rabta, Tunis, Tunisia

[2]Department of Cell and Molecular Biology, Faculty of Medicine, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

***Corresponding Author:** Mhamdi Marwa, Laboratory of Physiology, Faculty of Medicine of Tunis, University of Tunis El Manar, Baab Saadoun, Rabta, Tunis, Tunisia.

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Abstract

The subcellular behavior of gadolinium (Gd), used in medical and industrial fields, was studied in the uterus and in the ovary tissues after its intraperitoneal administration to pregnant rats. The ultrastructural investigations using the Transmission Electron Microscopy (TEM) revealed the presence of numerous heterogeneous clusters with very electron dense inclusions in the lysosomes of uterus (myometrium, endometrium cells), and of ovary (internal theca cells, granulosa cells), in addition to that there were cytoplasmic vacuolization, expansion of the endoplasmic reticulum, mitochondrial alterations and apoptosis foci were also highlighted. Moreover, huge phagolysosomes and high number of eosinophils as signs of inflammation produced by the damaged cells were also identified. On the other hand the sections of control pregnant rats showed a normal ultrastructure. These results demonstrated the toxicity of Gd and the important role played by lysosomes in the sequestration of Gd under insoluble form in all cells of studied tissues (uterus and ovary).

Keywords: *Gadolinium; Uterus; Ovary; Lysosomes; Apoptosis*

Introduction

Gadolinium (Gd) is a lanthanide that exists only in trace in nature. It has several chemical properties and an important paramagnetic effect that allows it to be used in many fields like medicine especially in the Magnetic Resonance Imaging (MRI). Gd may be a toxic element when it is administered intraperitoneally because it enters in competition with calcium and it has polarizing effect, that's why in this study we use the same route of administration to pregnant rates. The purpose of this work is to study the impact of gadolinium in the ovary and uterus tissues of a pregnant rat.

There are many studies interested to localize this element in the lysosomes of different organs, such as like, liver, spleen, bone marrow, kidneys, lung [1], hepatocytes and Kuppfer cells, mammary glands [2,3]. However, no study has been undertaken on the localization of this element in the uterus and in the ovary of pregnant rat.

The aim of this study was to determinate the behavior and the repercussions of the prolonged accumulation of gadolinium in the reproductive system of pregnant rates after its intraperitoneal administration.

To realize our aim we used the simple Transmission Electron Microscopy (TEM).

Materials and Methods

Experimental protocol

For all conducted experiments, we used 24 pregnant females rats weighing each approximately 200g ± 20. These rats were randomly divided into two groups (G1 and G2), each group consists of 12 rats, they were kept for 15 days on a 12h dark/12h light cycle in the department of Experimental Medicine of Faculty of Medicine of Tunis in order to be acclimated. The rats were fed ad libitum and have no restriction to water. Each rat from the first group (G1) was given a total dose of 28 mg of a soluble solution of gadolinium as seven chronic intraperitoneal injections of 1 ml each spread over two weeks. The second group (G2) used as control received physiological serum (NaCl 9%) in the same experimental conditions.

Twenty four hours after the last injection, all rats were anesthetized with ether. The organs were removed and finally the rats were sacrificed.

The animals used in this study were maintained and treated according to internal institutional rules, based on the principles of ethics in animal experimentation.

Method of sampling

The uterus and the ovary were cut into fragments of 1 mm3. These fragments were immediately immersed in a 3% glutaraldehyde solution in sodium cacodylate buffer for 24 hours at 4°C. After rinsing in the same buffer, the fragments were post-fixed in 1% osmium tetroxide for 1.5 hour in an ambient temperature 25°C. All fragments were dehydrated in ethanol baths of increasing concentration (70, 80, 95 and 100°) and in two baths of propylene oxide then embedded in Resin Epoxy (Epon) and incubated for 48h at 45°C and for 24h at 60°C. Finally, these fragments were included in epon. Semithin sections of 100 to 150 nm thicknesses were obtained with a Bromma 8800 Ultratome III (LKB, Sweden). Tissues areas selected on semithin sections were then cut to obtain ultrathin sections that were collected on 300 mesh copper grids. These cuts were contrasted with uranyl acetate and lead citrate, and examined with a Jeol JEM1010 electron microscope.

Results

Effects of the intraperitoneal administration of a soluble solution of gadolinium in the female reproductive system

Uterus. Two varieties of cells were studied: endometrium and myometrium cells

Endometrium cells

Ultrastructural study of endometrium cells from rats which received intraperitoneally a soluble solution of gadolinium during 15 days revealed a numerous lysosomes present within the cytoplasm; they displayed varied shapes and sizes, and they were charged with an electron-dense material. The mitochondria in the studied cells were swollen, with no visible cristae, and containing a very electron lucent matrix. In some cells, the endoplasmic reticulum profiles were also altered (Figure 1). The general architecture of these cells remained otherwise unchanged.

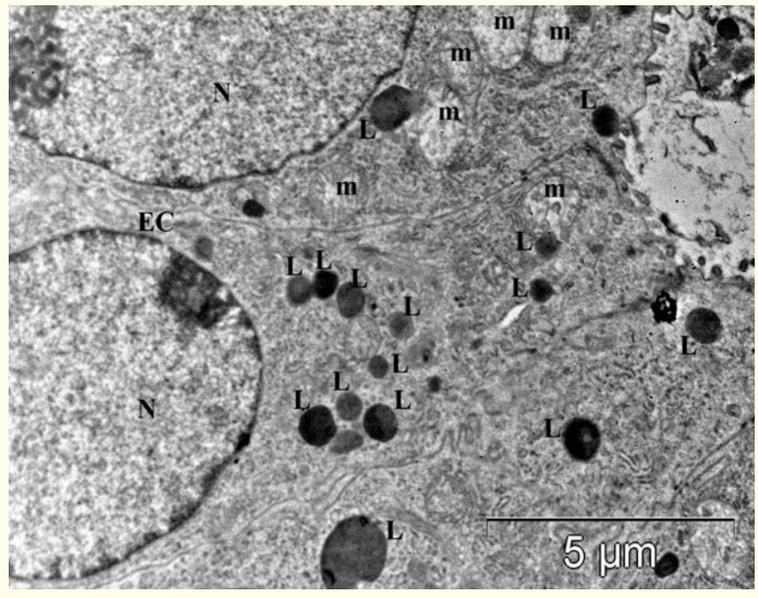

Figure 1: *(Magnification X 6000). Ultrastructural micrograph of endometrium cells of gadolinium-treated rats. The image shows electron-dense deposits in lysosomes (L) of epithelial cells (EC) with their round, euchromatic nucleus (N), dilated rough endoplasmic reticulum (RER) and altered mitochondria (m).*

In other zone of endometrium we observed a large number of death cells with probably apoptosis phenomenon (Figure 2).

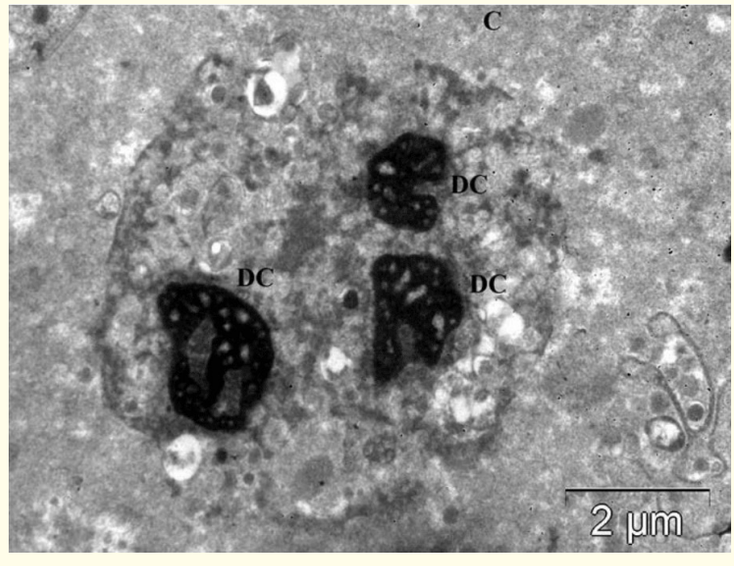

Figure 2: *(Magnification X 10000). In other zone in endometrium tissue many cells (C) died with apoptosis phenomenon (DC).*

On the other hand, the endometrium cells from control rats displayed a normal ultrastructure of nuclei and cell organelles. No over-loaded lysosomes were observed in different territories of endometrial cells and no altered mitochondria (Figure 3).

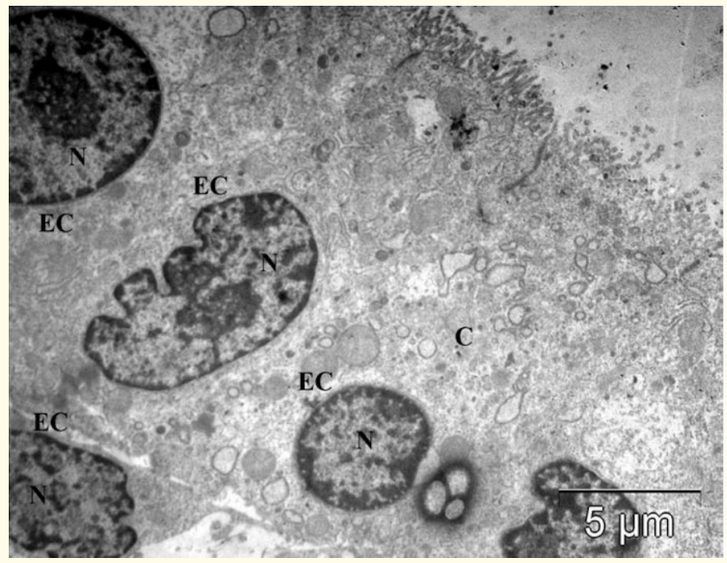

Figure 3: *(Magnification X 6000). Ultrastructural micrograph of endometrium of control rats showing the presence of normal structure of endothelial cells (EC) with their nuclei (N).*

Myometrium cells

Investigations of the myometrium cells from rats treated with gadolinium showed the presence of round-elongated cells, with euchromatic nuclei more or less indented, and relative dense, and compact cytoplasm. The main feature of the myometrium cells was the presence of a large lysosomes, characterized by various sizes and shapes also charged with an electron-dense material (Figure 4).

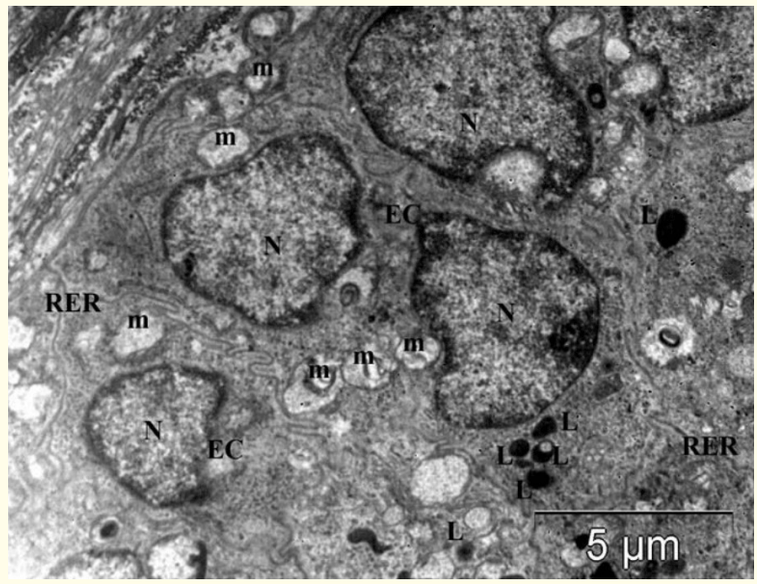

Figure 4: *(Magnification X 6000). Ultrastructural micrograph of myometrium cells of gadolinium-treated rats. The image shows electron dense deposits in lysosomes (L). The cells have nuclei (N) with irregular outline, altered mitochondria (m) and dilated rough endoplasmic reticulum (RER).*

In some cells, a high number of eosinophils were found, as signs of inflammation produced by the damaged cells (Figure 5).

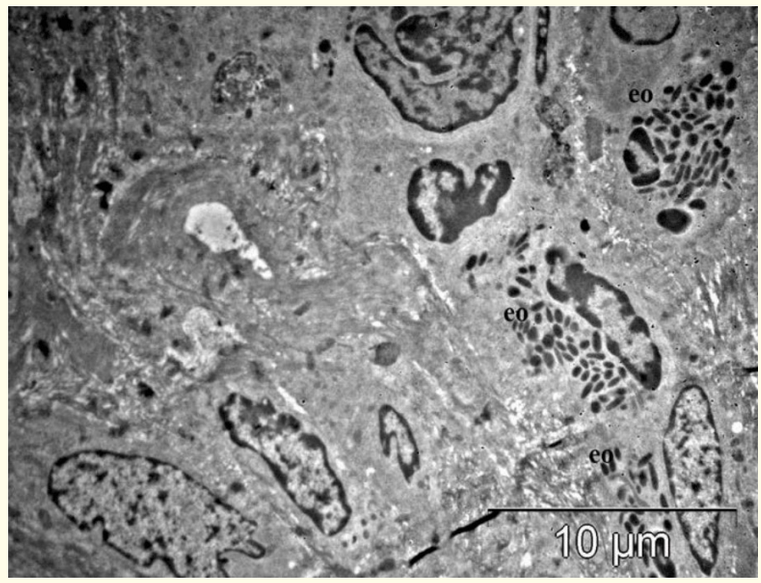

Figure 5: *(Magnification X 3000). In another zone of myometrium tissue of treated rats, we observed many eosinophils (eo) indicating a marked inflammatory reaction.*

The ultrastructural study of myometrium cells from rats given an intraperitoneal saline solution showed a normal appearance, with large, oval euchromatic nuclei and few organelles in the cytoplasm and absence of charged lysosomes (Figure 6).

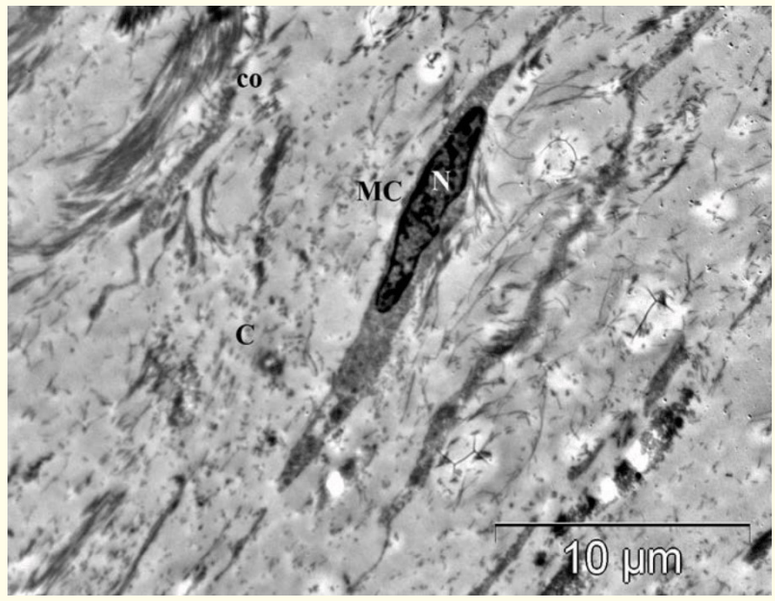

Figure 6: *(Magnification X 3000): the investigation of myometrium tissue of control rats showed the presence of normal structure of myometrium cells (MC) with their nucleus (N), cytoplasm (C) and collagen fibers in the extracellular matrix (co).*

Ovary. Two varieties of cells were studied: internal theca cells and granulosa cells

Internal theca cells

The ultrastructural study of the internal theca cells from rats receiving intraperitoneally a soluble solution of gadolinium, showed the presence of lysosomes characterized by various shapes and sizes and moderately charged with an electron-dense material. The nuclei were elongated and polymorphous, with random distribution of heterochromatin. Mitochondria were round, sometimes swollen, with normal appearance of their matrix, and endoplasmic reticulum profiles were either in low number, or altered (Figure 7).

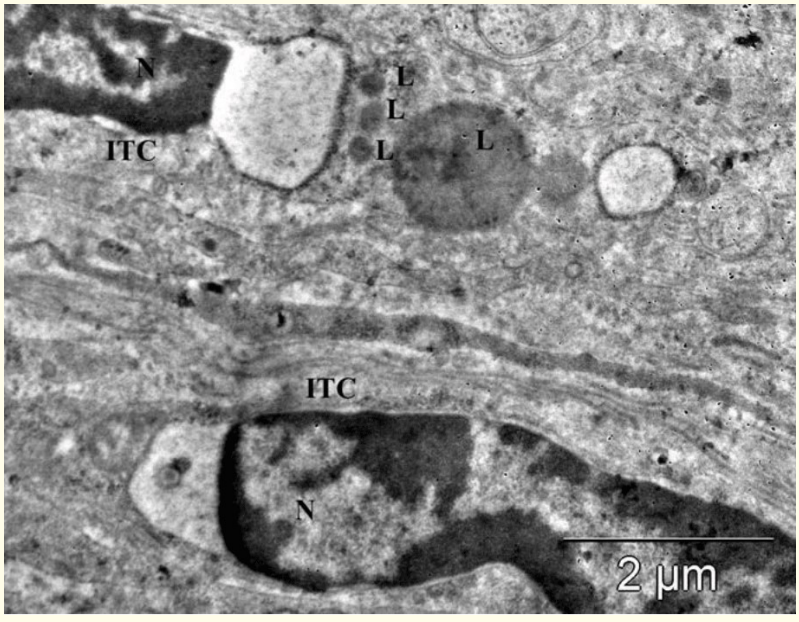

Figure 7: *(Magnification X 10000): This figure presents the internal theca cells (ITC) of treated rats with their nuclei (N), showing the presence of lysosomes (L) with electron dense deposits, altered mitochondria (m) and dilated rough endoplasmic reticulum (RER).*

Granulosa cells

In another part of the interstitial tissue we found granulosa cells as round-polyhedral shaped cells with euchromatic, indented nuclei. In their cytoplasm, all organelles suffered degenerative ultrastructural changes. Almost all mitochondria were polymorphous or swollen, with abnormal, altered cristae and electron lucent matrix. A large number of lysosomes loaded with an electron-dense material were found in the cytoplasm of granulosa cells (Figure 8).

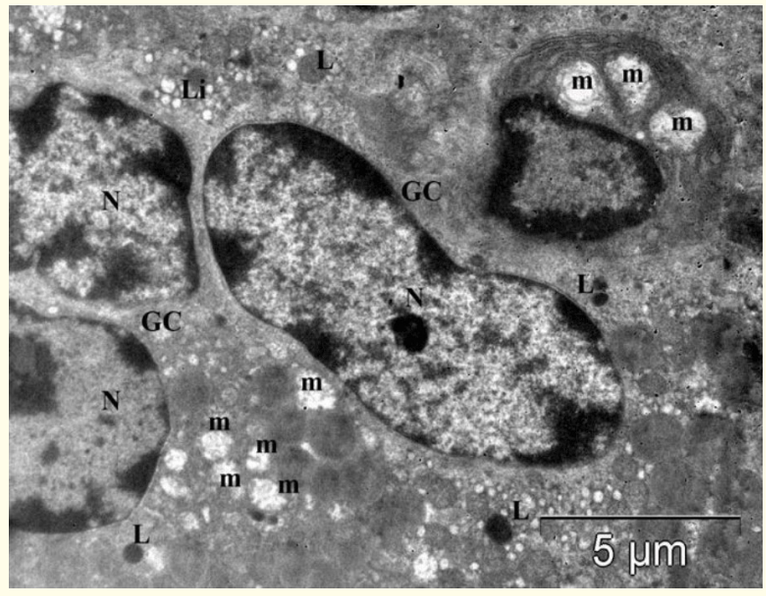

Figure 8: *(Magnification X 6000): The ultrastructural micrograph of granulosa cells (GC) of treated rats with their nucleus (N) showing charged lysosomes with electron dense deposits (L), altered mitochondria (m) and lipids (Li).*

The investigation of other zone from granulosa cells showed an alteration of mitochondria and endoplasmic reticulum that conferred a rarefied aspect to these cells (cytoplasmic rarefaction) (Figure 9). But a normal ultrastructural aspect was observed in the internal theca cells, in the granulosa cells of ovary from control rats, and no electron-dense materials were found in lysosomes (Figure 10).

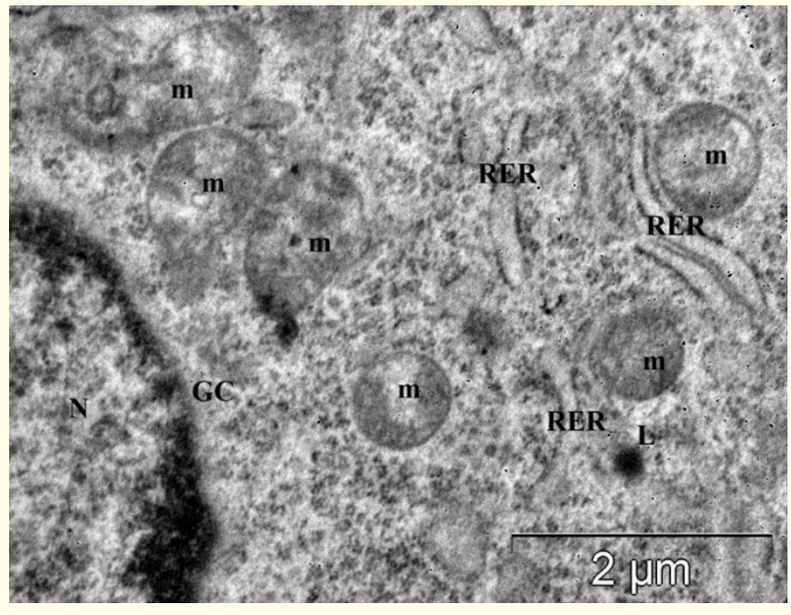

Figure 9: *(Magnification X 15000) in other area of granulosa cells (GC) with their nucleus (N). A high number of altered mitochondria (m), lysosome (L) charged with electron dense deposits and dilated rough endoplasmic reticulums (RER) were observed.*

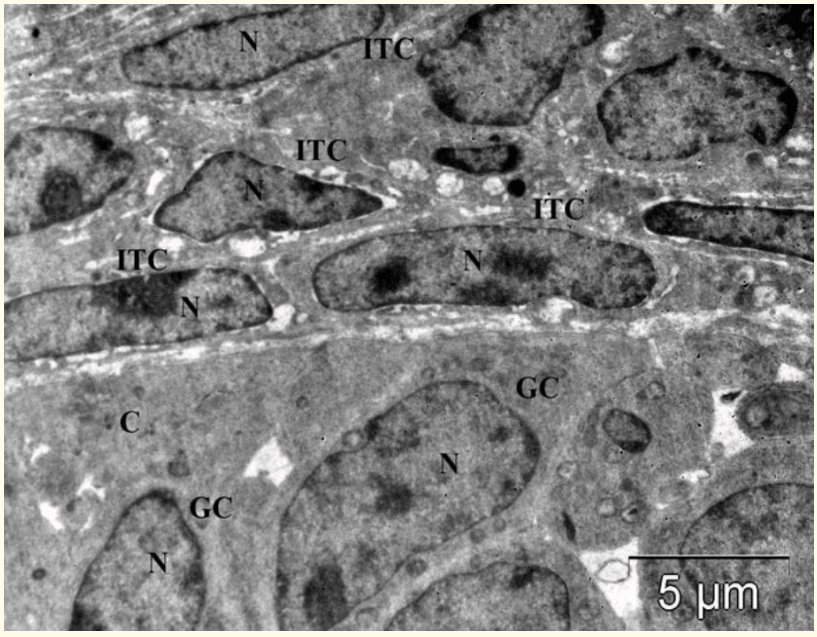

Figure 10: *(Magnification X 6000): Internal Theca Cells (ITC) of control rats with their nuclei (N), cytoplasm (C) and Granulosa Cells (GC) with their nuclei (N).*

Discussion

Previous studies

Gadolinium has several physicochemical properties and especially its paramagnetic effect that allows it to be used in many fields. In the medical field it is used as a contrast agent in the Magnetic Resonance Imaging (MRI) in form of Gadolinium-Diethylene-Triamine-Pentaacitic-Acid (Gd-DTPA), gadolinium-tetraazacyclododecane tetraacetic-acid (Gd-DOTA) and gadolinium-ethlenediamine tetraacetic-acid (Gd-EDTA), to detect liver tumors, brain etc. Gd is also widely used in industries mainly in the most modern ones, such as the manufacture of computer memories, video recorders readers [4], etc. This brings the humans in contact with this metal, which can be toxic to the health. In addition, gadolinium contributes to the pollution of the environment following the rejection of some products comprising this element.

Many studies showed the important role of an intracellular organelle, the lysosome, involved in the phenomenon of concentration of rare earths in the form of insoluble phosphate salt after intraperitoneal injection, such as gadolinium, which precipitated in different varieties of cells like liver, spleen, lungs, being always associated to phosphorus [1,5,6], in the lysosomes of lymphatic cells [7], and mammary gland cells [3]. Another element belonging to the same group: Lanthanum was also localized in the medullar macrophages, spleen and in lysosomes of the liver cells [5,7,8]. As for terbium, deposits have been located in the spleen macrophages, spinal cord, lung [5] as well as in liver lysosomes [2,4,5] and mammary gland cells [2]. Samarium meanwhile has been located in macrophages, spleen, spinal cord as well as in the lysosomes of liver cells [7].

This phenomenon of sequestration was generalized to other elements of group III-A of periodic chart; such as aluminum after its intraperitoneal administration to rats. This element was concentrated in the lysosomes as an insoluble form, in different varieties of cells

always associated to the phosphorus such as liver, kidneys, brain, bone marrow [7], testicles cells [9]. Indium which is an element belonging to the same group of aluminum precipitated in electron dense deposits in the lysosomes of testicles, liver [10] after its intraperitoneal administration. In addition to that, gold is a heavy metal precipitated in the lysosomes of the interstitial cells of Leydig [10].

Contribution of this work and discussion

The female reproductive system is one of the most complex systems in the body taking into account the hormonal fluctuations associated with ovarian and uterine menstrual cycles. In addition, few studies tackled the phenomenon of concentration of this element in this system especially in ovary and uterus.

The ultrastructural study of the uterus and the ovary cells of pregnant rats, receiving a total dose of 112 mg/kg of body weight of a soluble solution of gadolinium administrated by intraperitoneally way, was performed.

The results obtained revealed a numerous lysosomes present within the cytoplasm; they displayed varied shapes and sizes, and most important, they were charged with an electron-dense material in the uterus (endometrium and myometrium cells) and in the ovary (internal theca and granulosa cells). On the other hand, we found the endoplasmic reticulum dilated in many areas, and mitochondrial alteration, cell deaths with probably apoptosis phenomenon, cytoplasmic vacuolization, inflammatory reaction marked by the presence of eosinophils. The investigation recalling those obtained for this element in the lysosomes of different cell varieties like: liver, rate, lung [1,5,6], lymphatic cells [7], and mammary gland cells [3]. Where Gd was always associated with phosphorus.

The results obtained in the uterine cells with Gd were in agreement with other studies using aluminum and in which this element belonging to group IIIA was already solved in the same cell types [11].

Our studies confirmed earlier works performed by microanalytical methods and showing that gadolinium is concentrated in the lysosome of other cell categories such as lung and mammary gland cells always associated with phosphorus [1,3,5,6]. Cerium, an element belongings to the some group of gadolinium was concentrated in the zona pellucida, being responsible for the alteration of mitochondria as well [12]. Another study proved that gold was also concentrated in the granulosa cells [13].

The precipitation phenomenon and sequestration of a mineral element in the form of an insoluble phosphate salt was already demonstrated for aluminum [14], indium [15], and cerium [16] when it was administrated by parental way. This is due to the presence of intralysosomal enzyme activity highlighting the concentration of minerals within living cells [7].

The direct demonstration with TEM that these deposits are formed by phosphorus and gadolinium is impossible as long as this technique has the objective of a just an ultrastructural study, but the presence of gadolinium is in the cells is indicated by observation of deposits in the lysosomes of the treated rats corroborated with and their absence in the lysosomes of the control rats and following the comparison with previous work. We could therefore assume that the deposits observed in a large variety of cells are most likely gadolinium associated with phosphorus.

Conclusion

This work allowed us to determine, the fundamental role played by the lysosome in the phenomenon of concentration of the Gd. This can be considered one of the body's defense mechanism against foreign elements or having not known physiological role. The severe toxicity of gadolinium in the female reproductive system was demonstrated with the structural modification of the different tissues of uterus and ovary of the rat. Long-term accumulation of this mineral element can cause functional damage to the female reproductive system.

This work deserves to be further investigated by verifying that the deposits observed are actually Gd associated with phosphorus. For this, microanalytical techniques of observation and analysis should be used: Electron Probe Microanalyses, Analytical ion microanalysis or mass spectrometry.

Bibliography

1. N Hocine JP., *et al*. "Subcellular localization of gadolinium injected as soluble salt in rats: a microanalytical study". *Cellular and Molecular Biology* 41.2 (1995): 271-278.

2. A Ahlem., *et al*. "Rôle des lysosomes des cellules duodénales et hépatiques dans le phénomène d'accumulation du gadolinium". *Comptes Rendus Biologies* 331.5 (2008): 357-362.

3. A Ahlem., *et al*. "Localisation intracellulaire du gadolinium dans la glande mammaire en lactation: etude ultrastructurale". *Revue Maghrébine D'endocrinologie-Diabéte et de Reproduction* 16.1-2 (2010): 48-53.

4. A Ahlem., *et al*. "Ultrastructural study of intracellular behavior of four elements in the lactating mammary glands cells: study using conventional transmission electron microscopy". *Microscopy Research and Technique* 71.12 (2008): 849-855.

5. N Hocine JP., *et al*. "Mapping and localization of gadolinium. A study using ion microanalysis and electron microprobe". Les éditions de physique (1994a): 841-842.

6. N Hocine., *et al*. "Intracellular localization of gadolinium, lanthanum and terbium. Three rare earths. A microanalytical study using ion microanalysis and electron microprobe". Metal ion in biology and medicine. Eds Ph. Collery LA Poirier, NA Littlefield, JC Etienne. John Libbey. Eurotext. Paris (1994b): 193-198.

7. JP Berry. "The role of lysosomes in the selective concentration of mineral elements. A microanalytical study". *Cellular and Molecular Biology* 42.3 (1996): 395-411.

8. L Manoubi-Tekaya., *et al*. "Rôles des lysosomes dans le comportement intracellulaire d'éléments minéraux : cas du lanthane". *La Tunisie Medicale* 78.3 (2000): 195-200.

9. Maghraoui S., *et al*. "Role of parietal and principal gastric mucosa cells in the phenomenon of concentration of aluminium and indium". *Microscopy Research and Technique* 75.2 (2012): 182-188.

10. Maghraoui S., *et al*. "Comparison of the intracellular behavior of gold (Au) and indium (In) in testicle after their parental administration". *Microscopy* 62.3 (2013): 397-403.

11. Fu Y., *et al*. "Effects of subchronic aluminum chloride exposure on rat ovaries". *Life Sciences* 100.1 (2014): 61-66.

12. C Blandine., *et al*. "Ultrastructural interactions and genotoxicity assay of cerium dioxide nanoparticles on mouse oocytes". *International Journal of Molecular Sciences* 14.11 (2013): 21613-21628.

13. R Stelzer and RJ Hutz. "Gold nanoparticles enter rat ovarian granulosa cells and Subcellular organelles, andalter in-vitro estrogen accumulation". *Journal of Reproduction and Development* 55.6 (2009): 685-690.

14. JP Berry., *et al*. "Aluminium phosphate visualization of acid phosphatase activity". *Journal of Histochemistry and Cytochemistry* 30.1 (1982): 86-90.

15. P Galle. "Mécanisme d"élimination rénale de deux éléments du groupe III A de La classification périodique: aluminium et l indium". C. R. Acad. SC. Paris (1981): 292.

16. L Manoubi., *et al*. "Subcellular localization of cerium in intestinal mucosa, liver, kidney, suprarenoland testicle glands, after cerium administration in the rat". *Journal of Trace and Microprobe Techniques* 16.2 (1998): 209-219.

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