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# Lysosomes of Brain Cells: A Site of Handling of Lanthanum

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## Abstract

The brain serves as the center of the nervous system. Physiologically, it centralized control over the other organs of the body and acts on the rest of the body both by generating patterns of muscle activity and by driving the secretion of hormones. In the present ultrastructural investigations, we undertake to figure out the subcellular impact of Lanthanum (La) on brain of Wistar rats after its intraperitoneal injection under soluble solution. Our data, using Transmission Electron Microscopy (TEM), attempted to locate that the administered element was selectively concentrated under insoluble form within the lysosome of nerve cells with their axons and oligodendrocytes from brain ultrathin sections of lanthanum treated rats. While, neither electron dense surcharge, nor ultrastructural modifications were identified in brain ultrathin sections from rats given intraperitoneal saline solution. In addition to the presence of lanthanum deposits in the nerve and oligodendrocytes cells, visible changes in tissue and cells such as histology one involving cytoplasmic vacuolization and rarefication, marked expansion of the endoplasmic reticulum and mitochondrial damages were also identified as consequences of the presence of lanthanum. Although during our experiments we found that there were cases of death weight loss and mother's abortions. These results speculated the toxicity of lanthanum at the used dose. The observed signs of toxicity allowed concluding that the important role of lysosomes in the sequestration of this element under an insoluble form in all categories of cells in the studied tissues does not seem to be efficient.

**Keywords: Alterations; Lanthanum; Lysosome; Nerve cell; Oligodendrocytes; TEM**

## Introduction

Lysosome is a heterogeneous cellular organelle, whose most important functions have been well known, in particular those concerning the breakdown of organic molecules such as glycolipids and glycoproteins, since the studies of De Duve. These functions are linked to the presence of enzymes, among which acid phosphatases could be mentioned [1]. More recently, along the introduction of new highly sensitive physical methods allowing the localization of mineral elements within cells, another function of lysosomes has been discovered, namely, the active concentration of elements. The first reported study describing this phenomenon of mineral element concentration was performed on the kidney with gold and uranium. This study showed that proximal cells had the ability to selectively concentrate gold as insoluble salts, using the arylsulfatase enzyme, after the subcutaneous injection of this metal to rats as a soluble solution [2].

## Materials and Methods

### Animal experiment

Twelve virgin females from Wistar strain with an average body weight of 170 g were purchased from the Pasteur Institute of Tunis, and acclimatized for 1 week before the beginning of the experiment. Rats were kept in polystyrene cages and they were maintained in a controlled room under the temperature of (22°C ± 2°C), with 12:12 h light/dark cycle humidity of 75% and ventilation of (10 to 20) times/h. Feed and water were provided *ad libitum*.

One group of 6 Wistar rats received an intraperitoneal dose of 48 mg of lanthanum/Kg of body weight in 4 chronic injections of 1 ml each. The injections were administered over a four days period. Control rats were injected with saline solution (NaCl at 9%; 154 mmol/L $Na^+$; 285 mosm/L Sigma-Aldrich), in the same experimental conditions.

24 h after the last injection, all rats were anesthetized, sacrificed and brains were removed from control and experimental groups.

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All procedures involving animal care and experimental procedures were performed according to the approved animal care protocols of the Ethics Committee on Animal Welfare in accordance with the international principles for the use of animals in Toxicology.

### Samples treatment

Histological and ultrastructural studies were performed using regular techniques of conventional Transmission Electron Microscopy (TEM): brain samples measuring 1 mm were fixed by immersion at 4°C for 48 h in glutaraldehyde (3%) with cacodylate buffer (0.2 M) and kept at 4°C. Samples are then , post fixed for 2 days in 1% osmium tetroxide, dehydrated in increasingly concentrations of ethyl alcohol (70°, 80°, 90°, 95°, 100°), and embedded in epon. Semithin sections of 100 nm to 150 nm thicknesses were obtained. Ultra-thin sections of about 70 nm of diameter were obtained with a Leica Ultra cut E Ultramicrotome and put on copper grids. One group was stained with uranyl acetate and lead citrate, the second one was unstained. The ultrathin sections were contrasted in two steps in order to increase the contrast of specific structures of the cells and tissues.

**Contrast with uranyl acetate:** In a Petri dish, four drops of uranyl acetate (3.5%) in 50° ethanol were poured on a paraffin parafilm. The grids were placed with the sections against the solution, for 15 min. The cuts were then rinsed by placing the grid in three successive baths of alcohol at 50°. Finally, the grids were dried on filter paper at room temperature.

**Contrast with lead citrate:** Several drops of 2.8% lead citrate were placed into a different Petri dish. On each drop, a grid was placed for 20 min. The sections were then rinsed in three baths of distilled water. Finally, the grids were dried on filter paper, at room temperature. The contrasting sections were the last processing phase prior to ultrastructural study. Ultrastructural observations were carried out using a Jeol JEM1010 (Jeol, Tokyo, Japan). The following operating conditions were used: accelerating voltage 80 Kv and various magnification powers.

### Statistic study

The results from the various measurements performed by the ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrometry) technique have been technically and biologically validated.

Statistical calculations were performed using the Anova test and the results were considered significant from the significant $p<0.03$.

## Results

### Nerve cells

The ultrastructural study of ultrafine sections of the brain from lanthanum treated rats revealed the presence of numerous lysosomes rich in electron-dense granulations; severe neuronal sufferance was clearly demonstrated by the transformation of the nucleus, which became polymorphic, comparable to straight-line extensions. A cytoplasm vacuolization and a significant dilatation of the endoplasmic reticulum within the cell body have been also reported (Figure 1a). However, the ultrastructural study of ultrafine brain sections of control rats revealed a normal architecture of this tissue. No loads down lysosomes were detected (Figure 1b).

### Axon

Ultrastructural observations of ultra-thin sections of the brain from pregnant rats treated with lanthanum showed axonal presence

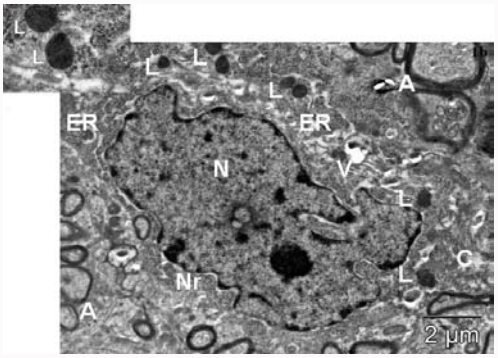


**Figure 1a: (GX10000):** The micrograph shows a Neuron (Nr) with its highly Polymorphic (N) nucleus with an irregularly shaped membrane and a highly dilated perinuclear space, numerous Lysosomes (L) with more or less rounded electron-dense inclusions and variable size occupying the Cytoplasm (C) which contains Vacuolization (V) as well as dilated Endoplasmic Reticulum (ER), a Cell Body (CC). Axons (A) likewise have been identified.

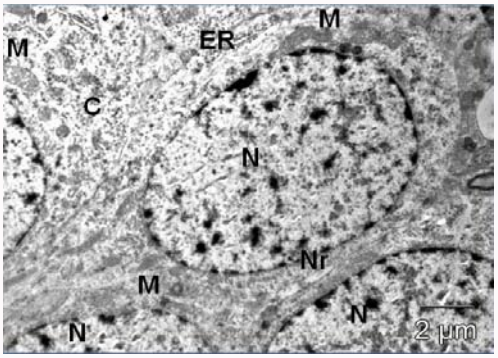


**Figure 1b: (GX8000):** The Nerve cells (Nr) observed appears to be normal in appearance and devoid of electron-dense granulations. Each nerve cell is provided with a Cell Body (CC) containing a Nucleus (N), Mitochondria (M) and Endoplasmic Reticulum (ER).

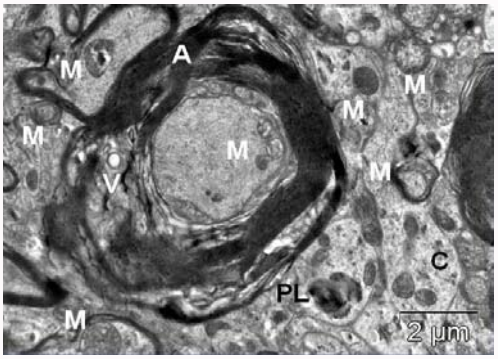


**Figure 2a: (GX 15000):** The image shows Axons (A) with their suffering Mitochondria (M), the Cytoplasm (C) is occupied by very large Vacuolization (V) as well as by Phagolysosomes (PL).

of altered mitochondria, phagolysosomes containing electron-dense deposits, and intense cytoplasmic vacuolations. In addition, a significant change in the shape of the axons was observed (Figure 2a). Control samples didn't reveal any surcharge or histological modifications (Figure 2b).

### Oligodendrocytes

The ultrastructural study of brain slices treated with lanthanum was focused on another cell variety: oligodendrocytes. In the same

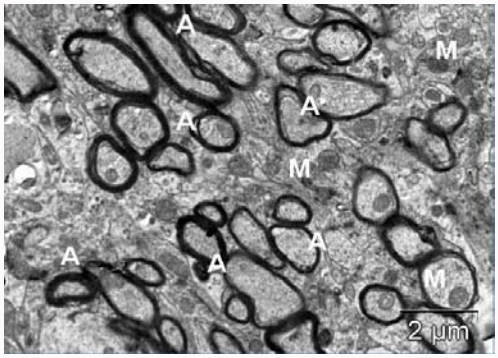


**Figure 2b:** (G: X 12000): The Axon (A) of the neuron appears to have a normal structure with Mitochondria (M) having normal aspect. No overload was detected.

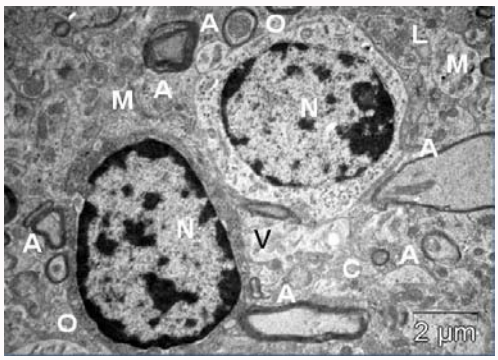


**Figure 3a:** (GX 10000): The image shows two Oligodendrocytes (O) with their Nuclei (N), their Cell Bodies (CC), and a Vacuolization (V) of the Cytoplasm (C), destroyed Mitochondria (M) as well as two altered Axons (A).

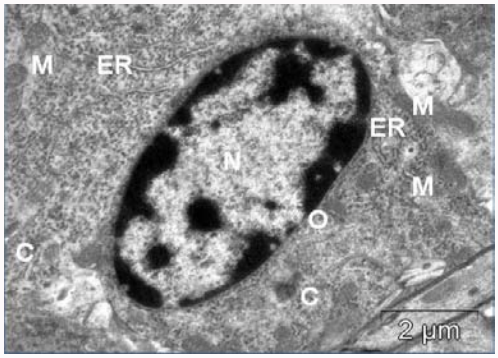


**Figure 3b:** (GX15000): This image shows an Oligodendrocyte (O) containing a Cell Body (CC) with a Nucleus (N), a Cytoplasm (C) with Mitochondria (M) and a normal Endoplasmic Reticulum (ER).

territory, an oligodendrocyte may appear normal in appearance with all its cellular constituents, while others appear with an irregular outline, a cytoplasm totally devoid of its cellular constituents. Mitochondrial damage and vacuolization of the cytoplasm have been noted. In the same area a severe alteration of the axons was also observed (Figure 3a). The ultrastructural study of brain from rats given intraperitoneal saline solution showed their normal appearance (Figure 3b).

### Statistic study

ICP-AES quantified significant lanthanum concentrations in the brain from rats treated with lanthanum compared to those found in

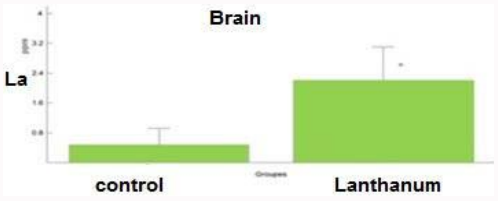


**Figure 4a**: Lanthanum was observed in the brains of treated rats compared to control ones.
*: significant difference ($p<0.03$)

control rats. The difference between the two groups was significant ($p<0.03$) ($n=6$) (Table 1 and Figure 4a).

## Discussion

This work is the first paper detailed a subcellular accumulation of lanthanum within the lysosomes of brain cells using the TEM and the ICP-AES. Thus, after four intraperitoneal administrations of a soluble solution of lanthanum to Wistar rats, our data showed that lysosomes of nerve cells with their axons and oligodendrocytes were the seat of electron density and huge lipid droplets. Our results are similar with those found previously with the same element and using the same techniques of observation. In fact, electron-dense clusters were found in lysosomes of medullar and alveolar macrophages [3-5], proximal renal cells [6], ovarian and uterine cells [7] and maternal and fetus side of placenta [8]. The presence of lanthanum within the nerve cells suggests that this rare earth is able to cross the blood-brain barrier. These results confirm those previously published on a number of lanthanides such as gadolinium [9], neodymium [10], samarium [11], and Ytterbium [12] showing that these mineral elements are able to cross the hematoencephalic barrier, remain in the brain and accumulate in the striatum, hippocampus and cortex with particularly high concentrations in the hypothalamus.

The ICP-AES came to confirm our results obtained with the MET since the precise determination of the different quantities of the lanthanum within the brain was carried out. Indeed, the brain is found to be the seat of concentration of lanthanides, since values between 1.23 ppm and 3.83 ppm for lanthanum were found within it. Our results suggest that lanthanides are able to cross the blood brain barrier but in small amounts. Our results are also reminiscent of those using other lanthanides such as; terbium, intralysosomal inclusions loaded with electron-dense deposits of this rare earth were found in renal proximal cells, spleen, spinal macrophages [3-5] and lactating mammary gland [13]. As for gadolinium, a second rare earth, it has been detected in deposits located in several cell types such as alveolar macrophages [3-5] Kupffer cells and hepatocytes [3,4,13-15], spinal [3-5,14], lymph node cells [14] and epithelial cells of the lactating mammary gland [16]. In addition, intralysosomal localization of cerium has been observed in lysosomes of hepatocytes, proximal renal cells and testicular cells [17]. At the used doses (48 mg/Kg), lanthanum was found to cause multiple alterations at the cellular level. Indeed, neuronal damages have been clearly demonstrated by the transformation of the contour of the nuclei with finger-shaped extensions, vacuolization and cytoplasmic rarefaction, extensive dilation of the endoplasmic reticulum and mitochondrial alterations. For oligodendrocytes, histological changes resulting in an irregular cell contour, a cytoplasm totally devoid of cellular constituents, swollen mitochondria, and a significant change in the shape of axons were noticed.

**Table 1:** The difference between the two groups.

| | Rate 1 | Rate 2 | Rate 3 | Rate 4 | Rate 5 | Rate 6 |
|---|---|---|---|---|---|---|
| **Brain (lanthanum)** | 3.83 ppm | 1.36 ppm | 1.3 ppm | 2.23 ppm | 1.23 ppm | 3.25 ppm |
| **Brain (control)** | 0.014 ppm | 0.015 ppm | 0.18 ppm | 0.35 ppm | 1.04 ppm | 1.23 ppm |

Our results confirm our previously published data showed that lanthanum cause similar effects in ovarian, uterin and placental cells [7,8]. Indeed, our results showed similarity with those of [18] demonstrated that lanthanum was capable of causing brain development disorders pregnant rats. In addition, lanthanum could affect certain functions of the brain, in particular hippocampus activity in rats receiving a dose of 40 mg of lanthanum/kg for 6 months [19].

## Conclusion

In conclusion, our study showed that lanthanum, a rare earth is sequestrated under insoluble form within brain cells such as nerve cells with their axon and oligodendrocytes. This mechanism was performed very probably as a defensive process to avoid cell injury when these exogenous elements are present in a soluble and free form.

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