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Gold Deposits within Lysosomes of Placental Cells: Ultrastructural and Quantitative Study

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Abstract

The placenta is a temporary organ, which has a dual transport function in that it facilitates the passage of some bio-substances to the fetus while acting as a barrier to other materials including metals which may interfere with placental function at many levels. Therefore, any deviation from normal placental development may constitute a potential threat to fetus growth.

In this work we report investigations of the histological and ultrastructural effects induced by gold, a metallic earth, at the level of the placenta cells from gestating Wistar rats. Using TEM, gold inclusions were identified respectively in maternal connective tissus as well as in syncytiotrophoblast and cytotrophoblast from fetus side of placenta. The lysosomes of these varieties of tissues displayed varied shapes and sizes, and most important, they were loaded down with an electron-dense material. Moreover, some of them have lost their membrane. Control cuts showed a normal aspect of the organelles, no overloaded lysosomes were observed in different territories of placenta.

The transplacental passage of gold from mother to fetus was already confirmed by the spectrometric analyzes using the ICP-AES. In fact, different quantities of gold within the maternal side (12,1 ppm-42,72 ppm) and the fetal side (8,51ppm-17,25pm) were successfully measured. Indeed, both are found to be the seat of handling of gold.

The placental cells responded with degenerative changes to the experimental treatment with the used doses (32 mg/kg). In fact, several disruptions of cells architecture like vacuolations, significant expansion of the rough endoplasmic reticulum, extensive mitochondrial alterations and presence of many destroyed cells especially on fetal part of placenta, were noted. Finally the present work strongly suggests the transplacental passage of gold salt and its accumulation on fetal placental part may cause pathological effects in fetus.

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Introduction

Exposure to heavy metals leads to deposits at the level of a wide variety of cell from many Biological systems such as the female reproductive [1] system, the male reproductive system [2], the digestive system [3,4], the renal system [5] and some endocrine glands such testicles [6,7]. Indeed, the subcellular behavior of such elements was successfully carried out since the introduction of new particularly sensitive techniques of observation and analysis whatever they were introduced into the body by natural or accidental ways.

Despite their effect in the medical and industrial fields, toxic and even carcinogenic and teratogenic effects of foreign mineral elements are still unclear especially on placenta: temporary organ, which delivers all-necessary nutrients to the fetus, as well as a barrier to prevent passage of toxic substances, including metals, which may interfere with placental function at many levels. Therefore, any deviation from normal placental development may constitute a potential threat to fetus growth. Not many investigations are seen for Gold in Placenta.

Gold is a heavy and valuable metal, usually found in nature as a metallic element or as salts. Its Medical properties have been explored throughout the history as an anti-inflammatory medicine for rheumatoid arthritis as well as component in dental restorations and in implant materials.

Besides the therapeutics effects attributed to gold, several toxic side effects were also observed. In fact, heavy metals are known to influence the endocrine system at several levels including hormone secretion, hormone activation, and binding to target tissue.

There is a need for more experimental and clinical research of the neuropharmacology and neurochemistry of gold, and for the exploration of gold possible role as a trace element [8].

Following intraperitoneal injection of gold salt, the present work was undertaken to investigate the consequences of the administration of gold in the cellular structure of the studied tissues (focused on the detection of signs of toxicity in the studied tissues) and if it is able to across the placental barrier also if the fetus concentrated gold salt in specific organs: liver, kidney, etc (unpublished data).

Experimental

Animal experiment

Twelve virgin females with an average body weight of 170 ± 30 g and eight fertile rats of Wistar strain were obtained from the Animal Experimentation Unit of the Medical School of Tunis (Tunisia) and used for experimentation.

Rats were housed in polystyrene cages which were adequate for accommodating three rats each (ratio of 1 male: 2 females to ensure gestation), under controlled conditions of temperature (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. Vaginal smears were carried out daily to detect pregnancy then the selected pregnant females were divided equally into two groups (n = 6). The first group received 1 ml of soluble solution of gold (sodium 3-aurothio-2-hydroxypropane-1-sulfonate at 30% of gold ($AuSCH_2$-CHOH-CH_2SO_3Na-Sarbak, Turkey)), for 4 days. Each ml contains 2 mg of gold salt so a total cumulative dose of 32 mg of gold salts / kg of body weight per animal. Used doses were very low to the lethal doses and were obtained after a very long preliminary study where we fixed all doses for different mineral elements.

Control ones received saline solution (NaCl at 9%; 154 mmol/L Na+; 285 mOsm/L Sigma-Aldrich), in the same experimental conditions.

From the 16th day of gestation, the experimentation phase began

- D16: 1st day of injection
- D17: 2nd day of injection
- D18: 3rd day of injection
- D19: 4th day of injection

24 hours after the last injection (day 20), all rats were anesthetized, sacrificed and fragments of placenta were removed from control and treated females.

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.

Methods of sampling

Histological and ultrastructural studies were performed using regular techniques of (TEM):

The various biological specimen obtained were immediately cut into small fragments of 1 mm3 volume and then immersed in a fixer solution (3% glutaraldehyde) for two day at 4C°.

Two successive washing steps of the fragments were performed in 0.5 M cacodylate buffer then post fixed with osmium tetroxide at 1% was released, for 2 days, and covered only half of the fragments. At 37C°, all the samples were dehydrated in baths of ethyl alcohol with increasing concentrations (70, 80, 95 and 100°) then included in pure epon and placed in an oven until polymerization to obtain blocks that were ready to achieve cuts. 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, collected on 300 mesh copper grids and contrasted with uranyl acetate and lead citrate, and examined with a Jeol JEM1010 electron microscope. The stained sections were finally ready for the ultrastructural study.

Statistic study

The results from the various measurements performed by the ICP-AES technique (Inductively Coupled Plasma Atomic Emission Spectrometry) 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

TEM:

Fetus side of placenta

Using Transmission Electron Microscopy, gold deposits were identified respectively in syncytiotrophoblast **(Figure 1)** and cytotrophoblast **(Figure 2)** from fetus side of placenta. The

lysosomes of these varieties of tissus displayed varied shapes and sizes, and most important, they were loaded down with an electron-dense material. Moreover, some of them have lost their membrane. Our results provided additional informations about the toxicological effects of gold on the cellular architecture. We noted many disruptions of cells accompanied with several changes like vacuolations, significant expansion of the rough endoplasmic reticulum, extensive mitochondrial alterations and presence of many destroyed cells especially on fetal part of placenta, demonstrating clearly the toxicity of gold with the used doses.

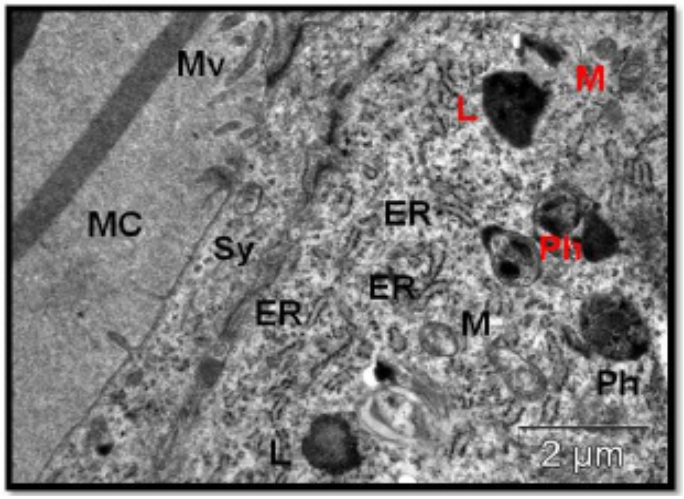

Figure 1: Transmission Electron Microscopy (GX20000).

TEM of a portion of the syncytiotrophoblast (Sy) shows the presence of a wide range of loaded phagolysosomes (Ph) and lysosomes (L), a cellular suffering marked by the presence of vacuolization (V) and altered mitochondria (M) without tubular crests. Marked expansion of endoplasmic reticulum (ER), a portion of maternal capillary (Mc) and microvilli (Mv) were also highlighted.

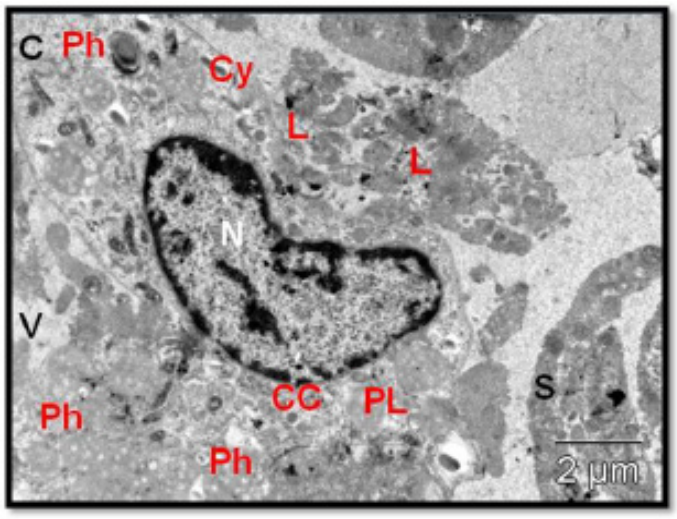

Figure 2: Transmission Electron Microscopy (GX10000).

The micrograph of a section of cytotrophoblast (Cy) shows a cytotrophoblastic cell (CC) , nucleus (N) with irregular contour, a disorganization of the cytoplasm (C) translated by the presence of vacuoles (V), overload lysosomes (L) as well as phagolysosomes (PL) a as well as a part of the syncytiotrophoblast (S).

Maternal side of placenta

The ultrastructural study of maternal side of placenta of gestating gold-treated rats allowed the presence of electron dense deposits within the lysosomes of maternal connective tissus. Highly swollen mitochondria as singe of gold toxicity were in addition highlighted **(Figure 3)**.

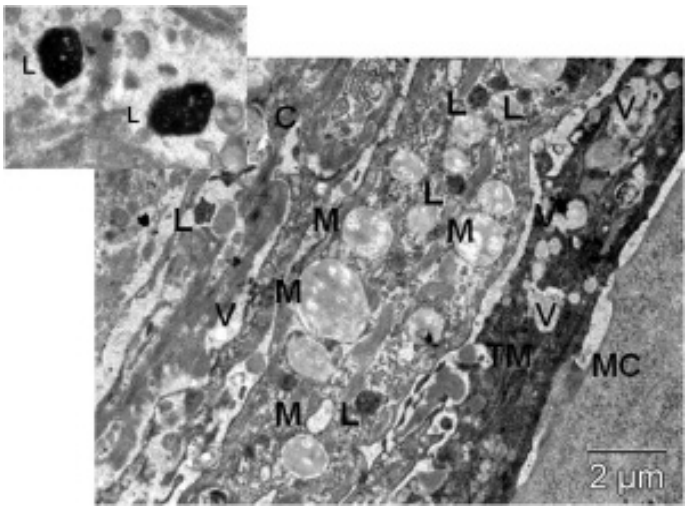

Figure 3: Transmission Electron Microscopy (GX10000).

This micrograph of the maternal tissue (TM) shows the cytoplasm (C) occupied by large vacuoles (V), mitochondria (M) completely altered lysosomes (L) and a portion of the maternal capillary (CM).

Control Placenta

The ultrastructural study of placental cells from rats given intraperitoneal saline solution showed their normal appearance **(Figure 4)**.

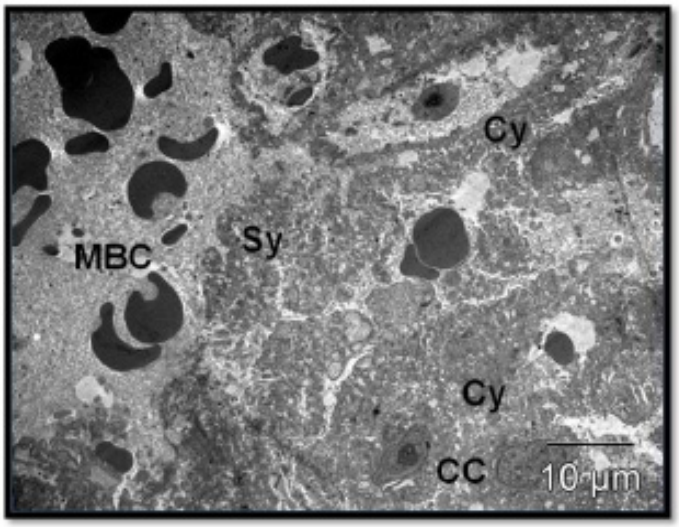

Figure 4: Transmission Electron Microscopy (GX5000).

Normal ultrastructural aspects of placenta of control rats. TEM showed maternal blood capilary (MBC), syncitiotrophoblast (Sy), Cytotrophoblast (Cy) with their caracteristic cells (CC).

ICP-AES

Placenta

ICP-AES analyzes performed on the placenta of gold-treated rats have also shown very high ppm concentrations in this type of tissue. The difference between the control group and that treated is significant ($p < 0.03$) ($n = 6$) **(Figure 5)**.

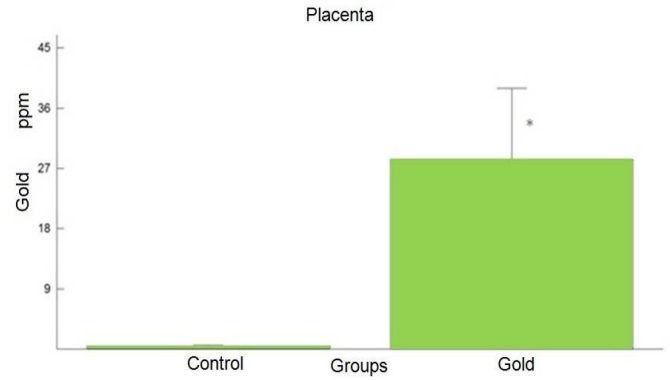

Figure 5: High concentrations of gold were observed within the placental tissue, compared to the controls (~30 times).

*: significant difference (p <0.03).

Fetal tissue

The results obtained by the ICP-AES show that the fetal tissues from treated rats concentrate very large quantities of gold. The difference between the two groups is significant (p <0.03) (n = 6) **(Figure 6)**.

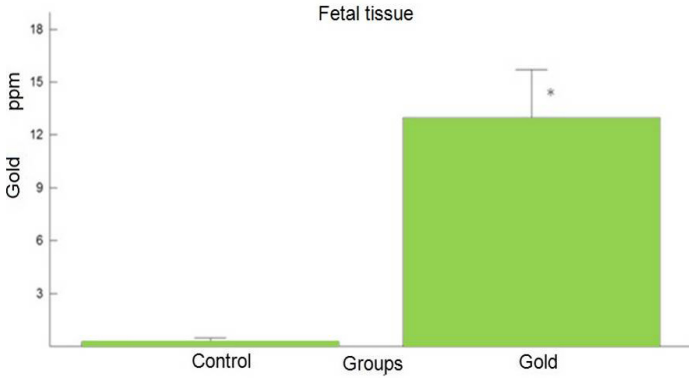

Figure 6: Significant amounts of gold were detected in the fetal tissue of treated rats (15 times).

*: significant difference (p <0.03).

Discussion and conclusion

In the present study, our experimental investigations were based essentially on ultrastructural and quantitative studies, exploring the effects of an intraperitoneal injection of gold on rat placenta. Using the approach of Transmission Electron Microscopy, gold deposits were identified respectively in maternal connective tissus from maternal side of placenta as well as in syncytiotrophoblast and cytotrophoblast from fetus side of placenta. The lysosomes and the phagolysosomesnoticed in these varieties of cells displayed varied shapes and sizes, and most important, they were loaded down with an electron-dense material. Moreover, some of them have lost their membrane. However, neither electron dense surcharge nor ultrastructural modifications were observed in control ones.

Our ultrastructural findings provided additionalinformations about the consequences of the administration of gold on the cellular structure of the studied tissues. Indeed, we noted many disruptions of cells accompanied with several changes like vacuolations, significant expansion of the rough endoplasmic reticulum, extensive mitochondrial alterations and presence of many destroyed cells especially on fetal part of placenta, demonstrating clearly the toxicity of gold with the used doses.

Our Resultats regarding the impact of intraperitoneally administered gold salt on the behavior of placenta cells completely agree with our previous published works demonstrating that the same element precipitated within the lysosomes of oocytes, cells of theca interna, theca externa, and interstitial cells of ovary as well as endometrium and myometrium cells of uterus [9]. Identical accumulations of gold have been described in other cells such as hepatic cells of patients suffering from rheumatoid arthritis and treated with allochrysine [10] as well as in suprarenal gland cells of rats after allochrysine injections [12,13]. Furthermore, in comparison with previous researches using the TEM and EPMA, we found that the intralysosomal appearance of dense inclusions already observed in placenta of treated rats was quite reminiscent, in form and density, to those reported for the same element, most often associated with sulfur, in other cells like steroidogenic cells: adrenal cells [5,6] and interstitial testicular cells [2,6] when it was administered following intraperitoneal route.

In addition, our data are inaccordance with those reported by Hocine and al as well as by Manoubi and al for other mineral elements such as lanthanum. These works demonstrated that, after its intraperitoneal administration, this rare earth was foundaccumulated within the lysosomes of alveolar macrophages, bone marrow and spleen [11], as well as hepatocytes and Kupffer cells [12,13].

A mother- fetus transplacental passage of gold to be already confirmed by the spectrometric analyzes using the ICP-AES. In fact, different quantities of gold within the maternal placenta (12,1ppm- 42,72 ppm) and the fetus (8,51ppm-17,25pm) were successfully measured. Indeed, both are found to be the seat of handling of gold. Our data strongly suggests the transplacental passage of gold and its accumulation on fetal placental parts may cause pathological effects in fetus. Indeed, during our experimental period we found that there were cases of death respectively of the mother and fetus, congenital malformations were observed in the fetus. Weight loss and mother's abortions were also highlighted. All these results confirmed that gold across trough the placental barrier from the mother to the fetus demonstrating that the placental barrier is not completely impermeable to the passage of harmful substances. Our results confirm previous work relating to the quantification of mineral elements following their oral administration. Indeed, the spectrometric studies of Pragya and al. showed a significantly high concentration of aluminum in the placenta (20 µg/g), maternal blood (20 µg/ml) and the fetal brain 10 µg / g in pregnant rats receiving a total dose of 345 mg/kg of aluminum chloride from the first to the sixth day of gestation [14].

Also, our results are reminiscent to those tried to quantify certain heavy metals on human populations. Studies of Iyengar and al. concerned rather toxic heavy metals such as lead (Pb), mercury (Hg) and cadmium (Cd). These studies made it possible to find high concentrations of these elements, 5-60 ng / g, 2-13 ng / g and 1-6 ng / g respectively in the placental samples from pregnant women living near contaminated areas [15].

Finally, our investigations obtained with gold showed similar side effects obtained with lanthanum [16]. Furthermore, available studies showed that cadmium accumulated in the placental tissues and in high levels of exposure could alter endocrine function, causing various reproductive problems and changes in the cells may be the cause of the death of the fetus by placental dysfunction or failure [17].

Our results have shown that the used techniques (TEM and ICP-AES) seem to be complementary. Indeed, if the Transmission Electron Microscopy (MET) informed us about the presence of gold deposits within the lysosomes of the different cell categories, the ICP-AES determines precisely the different amounts of gold within each cell type studied.

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