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Oral Fe_3O_4/salicylic acid nanoparticles: a rational option to the parenteral delivery

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ABSTRACT

In the last decades, the magnetic nanoparticles are interesting tools for the scientists, and they are tested either for parenteral or oral delivery. The most common delivery route is the parenteral one, but the oral administration of nanoparticles presents some important advantages: easy and cheap administration, and also patient compliance. For oral delivery, the nanoparticles must be designed to withstand the harsh condition of the digestive tract. Our work presents the possibility to oral administration of the Fe_3O_4/salicylic acid nanoparticles on the murine model. The modified Massart method has been used to synthesize Fe_3O_4/salicylic acid nanoparticles with core diameter around 15 nm, and mean hydrodynamic diameter of 43.5 nm. Two milliliters of aqueous dispersion of Fe_3O_4/salicylic acid nanoparticles (iron concentration 356 mg/L) were administered by gavage, to each Wistar rat. Perls' Prussian blue staining and the light microscope examination were used for the nanoparticles deposits identification. The results of this study showed the feasibility of oral administration of Fe_3O_4/salicylic acid nanoparticles, and also that the nanoparticles passed into the bloodstream and can be conducted under magnetic field action, in the interest areas.

Keywords: *Magnetic nanoparticles, Salicylic acid, oral delivery, murine model.*

1. INTRODUCTION

Within the last four decades, the use of magnetic nanoparticles (MNPs) as drug delivery nanosystems was reported in scientific literature, but still now the most important bioapplication of MNPs is as MRI agents. The biodistribution of MNPs and the targeting of the interest areas are closely related on their physicochemical and biological factors (i.e. the diameter and the stability of the nanoparticles, the surface functionalization and their physical characteristics, the electrophoretic mobility, the administration route, the dosage and their toxicity) [1, 2]. The most used route of MNPs administration is the parenteral route (intravenous, intramuscular, intraperitoneal, and intratumoral injection) [3-7]. The MNPs oral administration becomes an attractive route due to its advantages: patient convenience and compliance, easy administration, and cheap method (without sterile conditions at administration) [8, 9].

However, in order to be used to oral delivery the MNPs must be designed to resist to the harsh environment of the gastrointestinal tract: the low pH in the stomach, the degradative enzymes and the mucus layer that lines the surface of the gastrointestinal tract [10, 11].

In this study we tested and evaluated, on the murine model, the oral delivery as a route for Fe_3O_4/salicylic acid nanoparticles aqueous dispersion administration. We followed the potential to administer the dispersion of nanoparticles by gavages, and the possibility of giving it a targeted direction under the action of a static magnetic field.

2. EXPERIMENTAL SECTION

2.1. MNPs synthesis and characterization.

Fe_3O_4/salicylic acid MNPs aqueous dispersion was synthesized by modified Massart method [12] using reagents with analytical purity. The MNPs were characterized by High Resolution Transmission Electron Microscopy (HRTEM) – JEOL ARM 200F electron microscope, Induced Coupled Plasma/Optical Emission Spectrometry (ICP) – Thermo I CAP 6000 dual view, and Dynamic Light Scattering (DLS) – Zetasizer Nano ZS.

2.2. Murine model.

We tested the aqueous dispersion of nanoparticles on four Wistar male rats, age 6 months and a weight of between 250-300 g. The animals were feeding ad libitum and maintained in proper condition (220C and 46% humidity). This experiment was advised by the Ethical Committee of University of Medicine and Pharmacy of Craiova.

The rats were anesthetized by Ketamine and Xylazine administration. Following induction of anesthesia, each rat was administered by gavages a dose of 2 mL of Fe3O4/salicylic acid aqueous dispersion with a concentration of 0.19%. After MNPs administration, two rats were exposed to a static magnetic field by introducing a 0,18T NdFeB magnet in a small surgically created pocket under the skin in the left flank region. The magnets were maintained for 24 hours, after which the rats were sacrificed and autopsied to study the distribution of nanoparticles in the digestive tract and the area exposed to the magnetic field. Another two rats were observed and sacrificed after a period of 48 hours for viscera harvesting (liver, spleen, gut, heart, lung, and kidney).

2.3. Histological analysis.

Sacrificed rats were fixed in formalin and autopsied for morphological examination and viscera harvesting. Formalin fixed visceral tissue samples were processed for paraffin embedding, cut into 5 μm thick sections and stained with Hematoxylin & Eosin

and Perls' Prussian blue in order to identify intravisceral MNPs deposits.

3. RESULTS SECTION

The ICP-OES quantitative analysis of the Fe_3O_4/salicylic acid nanoparticles aqueous dispersion showed a concentration of 356 mg/L Fe. HRTEM analysis of MNPs samples showed nanostructurated morphology with a core Fe_3O_4 nanoparticles diameter around 15 nm. DLS analysis showed an average Zeta potential of 35.83 mV (±4.81 mV) and a hydrodynamic diameter of 43.5 nm (± 0.026 nm) (Figure 1).

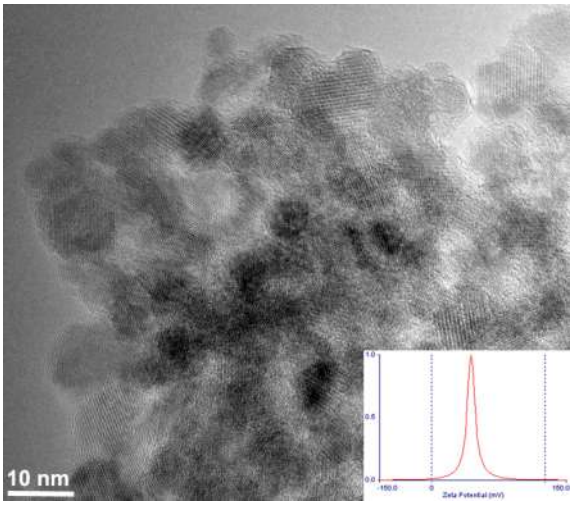

Figure 1. Image of Fe_3O_4/salicylic acid nanoparticles characterized by HRTEM, and DLS (box).

The rats' autopsy revealed no residual MNPs in the digestive tract that suggests the full absorption of the MNPs administered dose. In addition, no gross pathological changes were noted in the viscera harvested from the rats.

The absorption and the presence of MNPs in the blood vessels of rats were demonstrated by their accumulation at 24 hours in the skin vessels adjacent to the magnet's implant (Figure 2, Figure 3 F). Histological examination of the viscera taken from the rats showed the presence of the MNPs deposits (Perls staining +) in the liver, spleen, heart, lung, kidney. We do not identified MNPs deposits on the gut sections of all examined rats, and we considered that another proof for completely absorption of the gastric administered MNPs.

Most deposits of MNPs were identified into Kupffer cells of the liver, lung macrophages and reticuloendothelial cells of the spleen and kidney (Figure 3A-D). Also, we observed MNPs aggregates between subendocardic muscular fibers of the heart (Figure 3E) and as free MNPs accumulation in the blood vessels.

After microscopic examination of the kidneys nanoparticles were only identified in the renal arteries and veins, without being able to reveal the presence of nanoparticles at the level of the urinary tract. All these observations indicate that oral administered nanoparticles were absorbed from the gastrointestinal tract, crossed the liver filter, then passed the pulmonary circulation and the systemic circulation and finally go through the kidney without being urinary filtered.

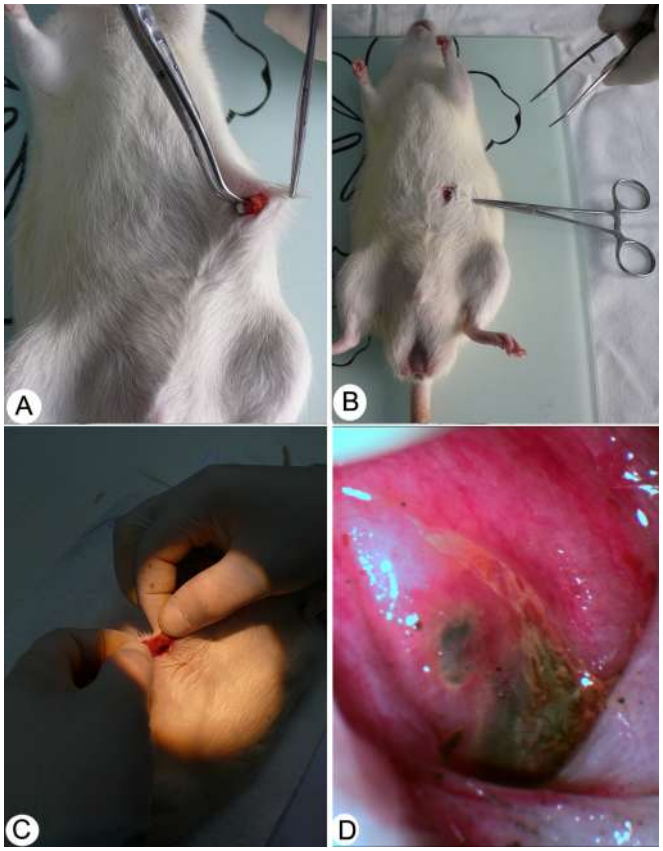

Figure 2. The technique of the magnet subcutaneous implantation in rats after administration by gavage of the aqueous dispersion of Fe_3O_4/salicylic acid nanoparticles (A, B). The accumulation of nanoparticles at subcutaneous implantation site of the magnet (C, D).

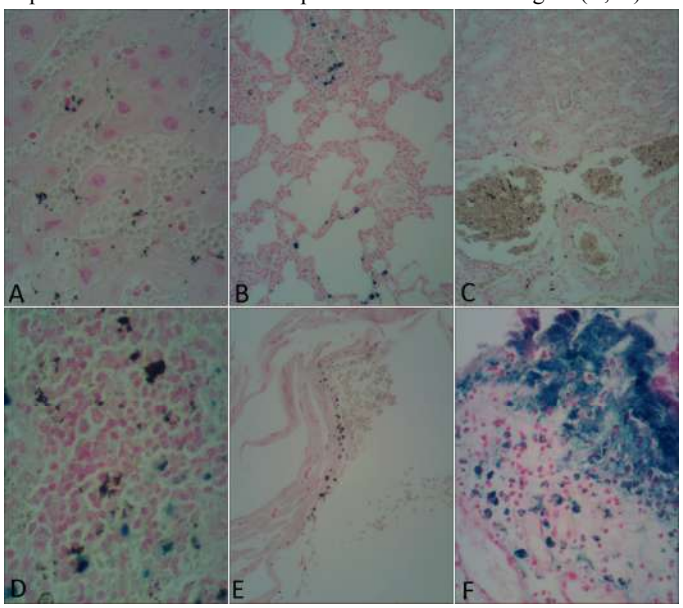

Figure 3. The distribution of Fe_3O_4/salicylic acid nanoparticles administered by gavage at the level of the liver (A, obx40), lung (B, obx20), kidney (C, obx20), spleen (D, obx20), heart (E, obx20) and the exposed skin at the action of the magnetic field (F, obx20). Perls' Prussian blue staining.

The most important barrier to the MNPs oral administration is the harsh acidic environment of the gastrointestinal tract, which pH values varies from 1 – 2.5 at the stomach through 6.4 - 7.5 at the small intestine [13].

In order to verify the possible administration by gavage of the Fe_3O_4/salicylic acid nanoparticles aqueous dispersion, we tested the stability of the dispersion making changes in its pH range 1-6, variations obtained by the addition of concentrated HCl.

The results of this experiment showed that the dispersion of nanoparticles is very stable in that pH range, and the lack of a deposition on the walls of the tubes, even after 24 hours of the experiment, can be considered as proof of the strength and stability of the nanocomposite in the acidic environment (if degradation of the salicylic acid coating would be occurred, then iron oxide nanoparticles would be precipitated).

A major problem to be avoided in the intravenous administration of the MNPs dispersion is the presence of particles of size greater than 200 nm, which have an increased embolic potential. In this regard, the oral administration of MNPs dispersion is a more convenient method of administration because uses the natural filter of MNPs digestive absorption. Some studies have shown that only nanoparticles with a diameter below 200 nm can diffuse through the mucus that coats the lining of the digestive tract [14].

Our results were similar with other studies that showed that into the blood stream the magnetic and the nonmagnetic nanoparticles suffer a process of opsonization and phagocytosis in the reticulo-endothelial system, which plays a role in the recognition and elimination of foreign substances in the body, and usually accumulate in the liver and spleen [15, 16]. The processes of phagocytosis, biodistribution and pharmacokinetics are closely related with their shell structure. Uncoated nanoparticles are very quickly removed after parenteral administration by the reticuloendothelial system or by renal excretion [2, 16], while nanoparticles coated with biocompatible substances have the advantage of a longer period to stay in the blood stream, which is due to the fact that macrophages do not recognize these foreign particles in a short time [17].

The presence of MNPs visceral deposits and lack of MNPs in urinary tract can be explain by their 50 nm hydrodynamic diameter, knowing that the clearance of the injected magnetic nanoparticles consists in quickly renal elimination for diameters smaller than 20 nm and the macrophages uptake and tissue deposits for the magnetic nanoparticles larger than 40 nm (high MNPs levels in liver 80 - 90% and spleen 5-8%) [18-20].

No structural changes were observed on histological analysis of the visceral parenchyma with MNPs deposits after gavage administration of Fe_3O_4/salicylic acid nanoparticles aqueous dispersion with 356 mg/L iron concentration. However, in the exposed skin area to the action of the magnet the necrotic lesions can be observed, probably due to the increased concentration of MNPs. In 2008, Karlsson et al., states that the concentration of the magnetic nanoparticles greater than 100 mg/mL can produce cytotoxicity [21]. Toxicity studies showed that the nanoparticles functionalized with various coatings have low toxicity due to the presence of a biocompatible coating [22, 23].

4. CONCLUSIONS

Oral administrated MNPs were completely uptake by the rat gastrointestinal mucosa, gone further than liver filter and entered the bloodstream. The bloodstream MNPs can be magnetic targeted into interest area. The results of the study confirmed the viability of the method of administration by gavage of the aqueous dispersion of Fe_3O_4/salicylic acid nanoparticles.

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