

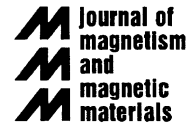


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Antitumor effect of magnetite nanoparticles in cat mammary adenocarcinoma

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Abstract

The antitumor effect of a magnetic fluid was studied after direct inoculation into cat mammary tumors. An external magnetic field was used to retain the nanoparticles in the tumor tissue. After 2 month, the mammary tumor regressed very much in size and the microscopic exam revealed that the tumor cells massively endocytosed magnetic nanoparticles and entered in lysis process.

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1. Introduction

In some previous experiments on mammary adenocarcinoma in bitch, it was observed that the injection of some biocompatible magnetic fluid directly into tumor determined the tumor cells lysis [1]. It was observed that the tumor cells are able to take up from extracellular matrix a high quantity of magnetic nanoparticles. The most important observation that stays at the base of all these experiments is that the magnetic nanoparticles are excessively endocytosed by tumor cells and in the

normal glandular cells the nanoparticles are not present [1–3]. After the magnetic fluid injection, the tumor cells take up the nanoparticles by unspecific endocytosis beginning with 20 min till 12 h. Then the tumor cells are overloaded with magnetic nanoparticles and gradually enter in lysis process [1,2,4]. It was observed that tumor cells take up large amounts of nanoparticles without any dependency on magnetic fluid concentration [2]. In the tumor tissue the tumors cells' environment is changed. The massive tumor cells proliferation and vascular network alteration diminish the food and oxygen supply. In survival competition, the tumor cells lose their specific endocytosis and take up many foreign particles from the extracellular matrix.

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If in the extracellular matrix the magnetic fluid scatters and moves away from tumor tissue, the nanoparticles amount decreases. In order to have a suitable nanoparticles concentration in the tumor tissue and avoid the magnetic fluid dispersion, an external magnetic field is adequate to use. This magnetic field must have the power to retain the magnetic nanoparticles in area until these are endocytosed by the tumor cells.

2. Materials and methods

The experiments were done with aqueous magnetic fluid containing Fe_3O_4 nanoparticles stabilized with a double layer of lauric acid. All compounds were biocompatible. The saturation magnetization of the magnetic fluid was about 80 Gs and the magnetic nanoparticles size was approximately 10–15 nm.

The choice of the biocompatible magnetic fluid was based on the fact that this colloidal magnetic iron oxide can be well-metabolized and excreted from the body [5,6] and is more stable due to the effects by lauric acid. Saturated fatty acids are generally more stable and more difficult to degrade than unsaturated fatty acids. The magnetic nanoparticles' stability is very important as well for endocytosis by the tumor cells as for their intracellular effects. So some observations pointed out that magnetic nanoparticles are taken up by coated pit mediated endocytosis and phagocytosis [3]. After a time estimated at 48 h [4] or less [3] the magnetite shells suffer a degradation process. The nanoparticle stability depends on the magnetic fluid materials. Despite its low cytotoxicity, we did not choose dextran magnetite because it is unstable and the iron is rapidly separated from the dextran coat [3].

The animal experiments were performed with cats selected from a number of cases that were brought for treatment in a private veterinary practice. The adenocarcinoma diagnosis was made after cytohistological examination of fluid samples taken by needle puncture from the tumors of anesthetized animals. The sample smears were stained by the May Grunwald–Giemsa method. A large number of big tumor-specific glandular

cells was identified by microscopic examination (Fig. 1).

The magnetic fluid was inoculated directly into cat mammary tumors after getting permission from the animal owners. The initial mammary tumor size for the three cats of European breed and approximately 4 kg body weight was about 125 cm^3 . Multiple sites of the tumors were injected with 5 ml of magnetic fluid, corresponding to $3.72 \text{ mg magnetite/cm}^3$ tumor and $116 \text{ mg magnetite/kg body weight}$. This dose is not close to toxic or lethal doses. For example, the lethal dose for half of the animals after intravenous ferrite dextran injection in rabbits ($= \text{LD}_{50}$) is about $600\text{--}1500 \text{ mg/kg}$, whereas the LD_{50} for dogs was determined to be between 510 and 900 mg/kg [7]. It is important to mention that the toxicity depends on the magnetic fluid compounds. Some magnetic fluids are based on alcohols and show a higher toxicity [8].

After the magnetic fluid injection, a magnetic field of 0.1 T was maintained close to the mammary tumor gland for 20–30 min.

From one animal, a tumor sample was removed 1 h after magnetic fluid injection. The smear was stained by Perls' method and showed a lot of tumor cells loaded with magnetic nanoparticles (Fig. 2).

The animals were returned to their owners and closely watched. All animals maintained a good appetite and normal behavior. After 2 months, two of the animals had their tumors removed for

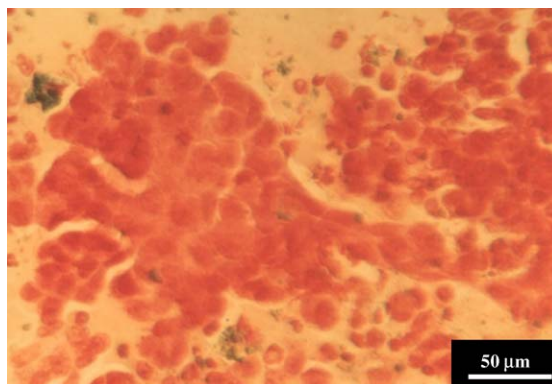


Fig. 1. Smear from cat mammary tumor with tumor glandular cells, before magnetic fluid injection, stained by Perls method.

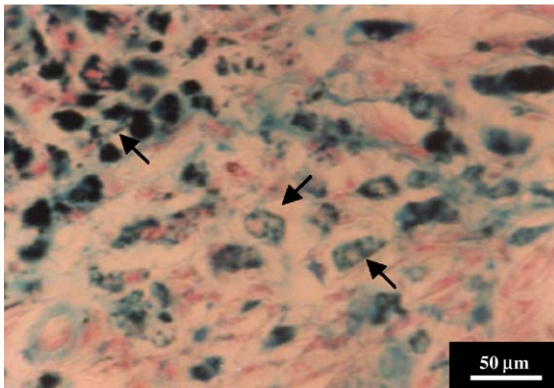


Fig. 2. Biopsy from cat mammary tumor at 1 h after magnetic fluid injection; arrows—tumor cells overloaded with magnetic nanoparticles agglomerated into solid clumps.

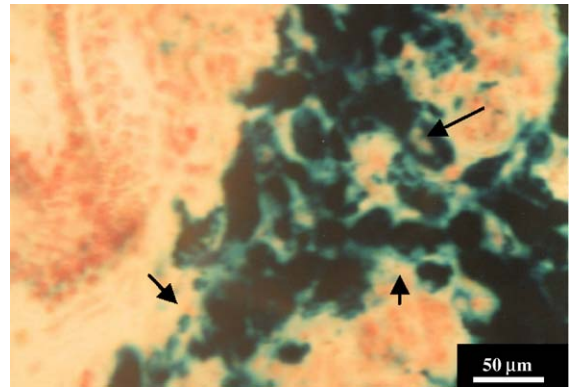


Fig. 3. Section through cat mammary tumor after two months from magnetic fluid inoculation; arrows—tumor cells, overloaded with magnetic nanoparticles.

further analysis. The third cat owner refused any further procedures because the tumor had diminished so much in size. Small tumor fragments were prepared into serial slides and stained for cytohistological studies by Perls' method, a specific stain for iron.

3. Results and discussions

The clinical examination of the cat mammary tumors revealed that, in the first 3 weeks, the tumors progressively diminished in size. The tumors decreased from approximately 125–84, 79 and 62 cm³, respectively. After three more weeks, the tumor size reduction was even more pronounced, with one of them decreasing to 2 cm³. This was one of the tumors that was rejected and examined by microscope.

After rejection in the tumor tissue of both mammary glands, some necrosis foci and black clusters of organic and ferrite residua were observed. A very interesting observation was that at high magnification, these ferrite clusters appeared to be agglomerated into solid clumps (Fig. 3).

The microscopic exam of the tumors revealed that, after 2 months, the majority of tumor cells were destroyed or turned into stone. The tumor cells destruction could be due to the overloading with nanoparticles from which they could not rid

of. The tumor cells have a 9- or 10-fold higher uptake of magnetic nanoparticles than normal cells [3]. It is very possible that tumor cells lysis is determined by the magnetic properties of the nanoparticles, too. These little magnets entered in the cells can alter the plasmalemma's electric and chemic potential and to affect the intracellular metabolism. An important effect of these little magnets could be the stopping of microtubule assembly and spindle division blocking [1,2,4].

The growth of the tumor stopped after magnetic fluid injection and progressively the tumor cells entered in lysis process, being removed by macrophages. In magnetite nanoparticles phagocytosis the macrophages generate reactive oxygen radicals that alter the normal tissue [9].

In the mammary gland, the tumor tissue was gradually replaced by dense connective tissue (Fig. 4). In the extracellular matrix were detected macrophages and fibroblasts overloaded with magnetite, cloggy ferrite residua, remains of the destroyed cells and tumor cells turned into stone (Fig. 4).

In the mammary gland a glandular tissue restoration was not noticed but in some areas normal glandular tissue was observed (Fig. 4).

A very interesting observation was the presence in the mammary tissue of some areas with small atypical cells (Fig. 4). However, these cells were not observed in the mature stage. These cells when wander in the connective tissue and move away

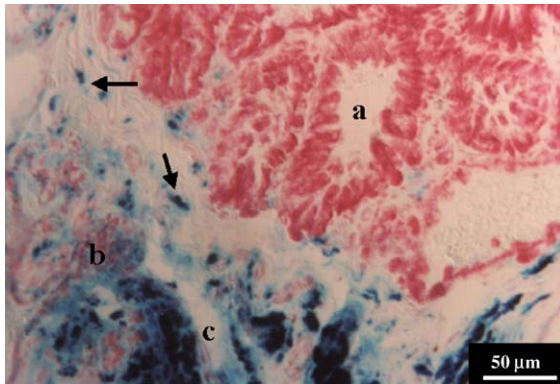


Fig. 4. Section through cat mammary tumor after two months from magnetic fluid inoculation; (a) normal glandular tissue; (b) area with small atypical cells, (c) cloggy ferrite residua; arrows—macrophages and fibroblast overloaded with magnetite.

from native site are rapidly destroyed. The changed environment and the iron compounds that still exist in the mammary gland tissue are not proper for tumor cells development. Some of these cells were full of magnetic nanoparticles.

The fibrous connective tissue extension, the ferrite clusters, and possibly some intact magnetite nanoparticles or other iron compounds hinder the tumor development. This aspect is unusual because some references claim that the nanoparticles of most biocompatible magnetic fluids lose their coating in the first 48 h [3,4]. Magnetic nanoparticles stabilized with the saturated fatty acid laurel acid are more stable to oxidative processes, especially in the extracellular matrix. This might be the reason why magnetic fluids containing as a stabilizer, laurel acid injected into a tumor, might inhibit tumor growth for a longer period. The histological examination showed that iron is present in the extracellular matrix not only as ferrite clusters, but also as absorbent compounds.

A serious problem is the long remanence of large ferrite storages in the animal tissues, though

the magnetic field was used only for a short time. In these cases, surgical rejection might be advisable, though no toxicity signs were observed in cats.

Another possibility could be, in order to avoid the necrosis phenomena, the reduction of the magnetic nanoparticles concentration that is injected per cubic centimeter tumor, and the time extending for the external magnetic field.

4. Conclusions

After intratumor injection of a biocompatible magnetic fluid, the size of cat mammary tumors significantly diminished in 2 months. A cytohistological examination showed that a large quantity of the magnetic nanoparticles had been endocytosed by the tumor cells and then caused cell lysis. The reduction in size of the cat mammary tumors was due to tumor tissue lysis and a necrosis process. The magnetic fluids were only slowly removed from the mammary tumor tissue after injection of a dose of 3.72 mg of magnetic nanoparticles per cubic centimeter of tumor. Even after 2 months there was still a large quantity of ferrites detectable.

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