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The antitumor effect of locoregional magnetic cobalt ferrite in dog mammary adenocarcinoma

Mariana Şincai[a,*], Diana Gângă[a], Doina Bica[b], Ladislau Vékás[b]

[a]*Cell Biology – Histology Department, Faculty of Veterinary Medicine, Calea Aradului no. 119, Timişoara 1900, Romania*
[b]*Laboratory of Magnetic fluids, Center for Fundamental and Advanced Technical Researches, Romanian Academy – Timişoara Branch, Timişoara, Romania*

Abstract

The endocytosis of nanosized magnetic particles by tumor cells led to numerous tests to establish the use of this phenomenon in antitumor therapy. The direct antitumor effect of a biocompatible cobalt-ferrite-based magnetic fluid directly inoculated in bitch mammary tumors was studied. A direct correlation between tumor cell lysis and cobalt ferrite was established in tumors. Massive endocytosis of magnetic particles was observed 1 h after the contact of magnetic fluid with tumor cells.

Keywords: Antitumor effect; Cobalt ferrite; Mammary tumor; Adenocarcinoma; Dog; Breast tumor; Magnetic cobalt particles; Endocytosis

1. Introduction

Lately, a lot of research has been directed at using magnetic fluids in cancer therapy. From all experiments an increased ability of tumor cells to take up magnetic nanoparticles resulted [1–3]. Based on these observations varied methods using magnetic fluids for tumor cells lysis were employed. Some recent studies [4–8] pointed out the lysis of the tumor cells that had endocitosed magnetic particles by, controlled hyperthermia, induced by an external AC magnetic field. But the methods are fastidious and could affect the normal cells [7] from the organs in that the magnetic particles are stored after crossing the blood barrier. Recently, remarkable progress has been obtained in anticancer therapy by using magnetic fluids as antitumor drug carriers [9–11].

The direct intratumor inoculation of antitumor drug magnetic carriers has the advantages of high local drug concentration and of the use of antitumor drugs in smaller doses.

If tumors are difficult to reach, then the only possibility of administering antitumor drugs bound to magnetic carriers is by using the blood circulation. But intravenous inoculation requires higher doses of magnetic drug carriers to compensate for the loss of material, which ends up mainly in liver, spleen and the macrophages system. On the other hand, high doses of cytostatic substances and magnetic fluids intravenously inoculated could induce general toxic effects. The lethal doses are determined by magnetic particle concentration,

* Corresponding author. Tel.: + 40-56-123782; fax: + 40-56-200296.
E-mail address: msincai@teacher.com (M. Şincai).

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complementary substances and animal species [12–14]. Among the animals used for experiments with magnetic fluids, dogs seemed to be more sensitive. For example, the lethal dose of dextran magnetic fluid injected intravenously was five times smaller in mg/kg body weight than in mice.

Coupling anticancer drugs to magnetic particles allows to inoculate these compounds in the vascular network around the tumor and to maintain the magnetic fluid in the area with the help of some magnets, thus allowing the progressive release of antitumor substance [15–18].

Another advantage of employing magnetically targeted antitumor drugs is the accumulation of the anticancer drug effects with cell lysis induced by magnetic nanoparticles.

More and more observations report the destruction of tumor cells due to the blocking of the substances transport through the vascular endothelium because of mechanical clogging of magnetic nanoparticles on the vessels wall. Also, by overloading with magnetic particles due to a massive nonspecific endocytosis the tumor cells are destroyed [13].

The endocytosis phenomenon is a means of transportation of substances through cell membranes with the help of vesicles. The endocytosis may be nonspecific when the inclusion of substances is not made by specific receptors or it may be specific when it is mediated by specific membrane receptors.

The most familiar type of specific endocytosis occurs during low-density lipids (LDL) formation. This mechanism controls the intracellular cholesterol concentration. The LDL size (20–25 nm) is close to that of magnetic particles in the fluid structure.

Endocytosis is more intense in cells with higher proliferative ability such as fibroblasts, epithelial cells and committed blood cells where more nutritive substances are needed and the transmembrane transport is faster.

In the case of hyperplasia and hypertrophy of these cells (as modification characteristic to tumor process) the endocytosis is intensified and loses its specificity. Cells with high turn-over must get large supplies to be able to replicate, and if nanoparticles, which are similar in size to organic compounds, are present in the extracellular matrix, then they can be endocitosed fast. This also happens to magnetic particles, which are taken up fast and overload the cell, blocking the cell multiplication and leading to lysis [1,19].

Our experiments are preliminary investigations regarding the effect of cobalt ferrite magnetic fluids 250 G on the tumor cells of bitch mammary adenocarcinoma during a 24 h period.

2. Materials and methods

Water-based magnetic fluid with saturation magnetization 250 G having $CoFe_2O_4$ nanoparticles was used. These were obtained by chemical coprecipitation of Co^{2+} and Fe^{3+} ions in NaOH solution 6 N, the particles being sterically stabilized by oleic acid double layer. The size of nanoparticles was about 110 Å. This type of magnetic fluid (MF) was selected from a large variety of magnetite nanoparticle samples currently prepared [20] for technical [21] and biological applications [22].

The toxic effect of cobalt ferrite MF was experimented on cow follicular cells culture. The 0.4 and 0.2 mg cobalt ferrite/1 ml cell culture doses were well supported until 24 h when the cell lysis was observed (paper submitted).

The magnetic fluid was inoculated directly into the mammary tumors of bitches brought for treatment at the Surgery Clinic of the Faculty of Veterinary Medicine Timisoara and at two Private Veterinary Practices. The experiments were made after getting the agreement of animal owners. Mammary tumors of about 100–120 cm^3 were chosen to provide enough tumor tissue. The animals were between six and eight years old and their body weight between 15 and 17 kg.

The magnetic fluid was inoculated in four symmetrical points of the tumor, releasing the magnetic fluid slowly while extracting the needle from the tumor.

The applied doses of magnetic fluid took into account the dog's higher sensitivity for magnetic fluid. We took into consideration the observations regarding magnetic dextran microspheres. The lethal dose in dogs after intravenous injection is

0.5–0.9 g/kg body [15] and we thus used 10 and 5 times smaller concentrations.

In a first group of four tumors 2.5–3 ml of cobalt ferrite magnetic fluid was inoculated, corresponding to 7.25 mg cobalt ferrite/cm^3 tumor and 48.33 mg cobalt ferrite/kg body. The amount of magnetic particles in the tumor tissue (cells and extracellular matrix) never came close to or exceeded the whole body lethal doses.

Five-ml cobalt ferrite magnetic fluid and 14.5 mg cobalt ferrite/cm^3 tumor and 96.6 mg/kg body weight, respectively, were inoculated to a second group of fourth mammary tumors.

From each group of bitches a tumor fragment was resected at 20 min, 1 h, 6 h and 24 h after magnetic fluid inoculation. The tumor fragments were taken both from near the MF injection points and from peripherical zones. The tumor fragments were fixed in formalin and prepared for cytohistological studies by H&E and Perls methods. Perls iron stain is the classic method for demonstrating iron in tissues. The section is treated with dilute hydrochloric acid to release ferric ions from binding proteins. These ions then react with potassium ferrocyanide to produce an insoluble blue compound (the Prussian blue reaction).

The percentage of tumor cells, which had endocitosed cobalt ferrite particles, was calculated microscopically for every magnetic fluid dose and the state of tumor cells was also studied.

From every tumor as many cells as possible (approximately 1000) were counted on serial slides and the percentage of tumor cells with endocitosed magnetic particles calculated. The resected tumor glands had a weight between 130 and 150 g.

3. Results and discussion

The cytohistological investigations of mammary tumors inoculated with cobalt ferrite magnetic fluid revealed that after 20 min almost half of the tumor cells endocitosed magnetic nanoparticles. The normal glandular cells and other cell types did not take up magnetic nanoparticles.

Twenty minutes after the intratumor administration of the two magnetic fluid doses there was only a slight difference between them regarding the percentage of tumor cells with endocitosed nanosize particles. After 1 h the difference between the two groups increased. The percentage of tumor cells with included nanosize particles was higher than 90% in the case of 14.5 mg cobalt ferrite/cm^3 tumor dose. In the case of tumors inoculated with 7.25 mg cobalt ferrite/cm^3 tumor, the tumor cells that included cobalt ferrite were close to 64% (Table 1).

In the case of mammary tumors inoculated with 7.25 mg cobalt ferrite/cm^3 tumor the percentage increased progressively at 1 h and 6 h. After 6 h a lot of tumor cells with endocitosed magnetic nanoparticles were observed in various necrosis processes. After 24 h most of the tumor cells were destroyed, but intact tumor cells were still detected, especially, far from the point of magnetic fluid inoculation (Fig. 1).

Table 1
Endocytosis of magnetic cobalt ferrite particles over time

Time of tumor resection	Cobalt ferrite doses					
	7.25	mg/cm^3	Tumor	14.5	mg/cm^3	Tumor
	Total number of inspected tumor cells	Number of endocitosing cells	Fraction of endocitosed cells (%)	Total number of inspected tumor cells	Number of endocitosing cells	Fraction of endocitosed cells (%)
20 min	1018	543	53	1003	572	57
1 h	1076	692	64.3	1024	931	90.91
6 h	1022	789	77.2	1015	996	98.12
24 h	Most tumor cells are in lysis					

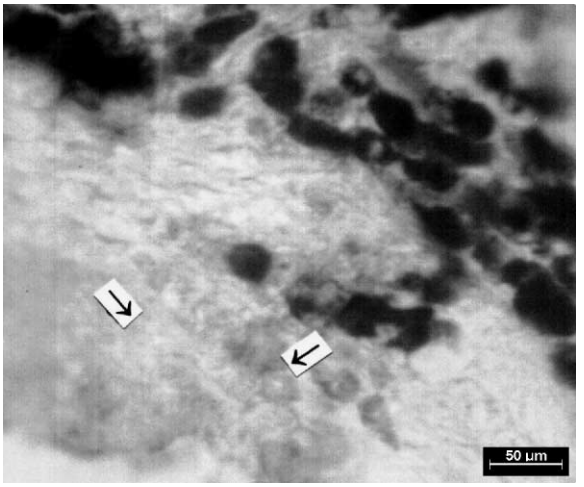

Fig. 1. Bitch mammary tumor inoculated with 7.5 mg cobalt ferrite/1 cm^3 tumor, after 24 h. Arrows indicate tumor cells that have not taken up cobalt ferrite nanoparticles.

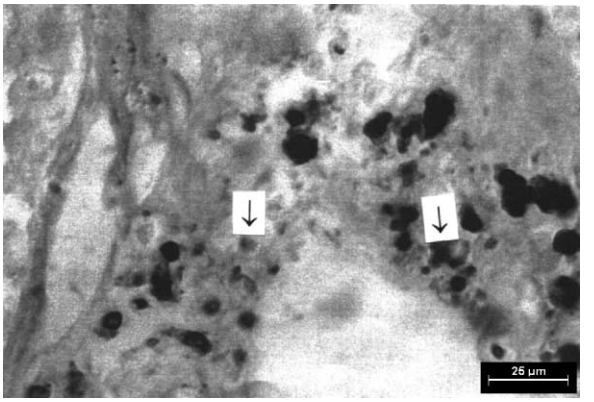

Fig. 2. Necrosis of the tumor cells at 24 h after inoculation of 14.5 mg cobalt ferrite/1 cm^3 tumor.

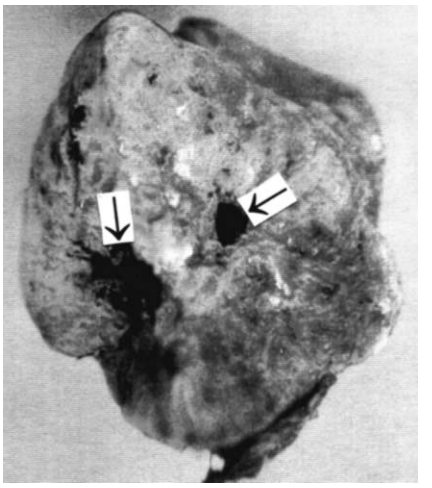

Fig. 3. Sagital section of the mammary tumor inoculated with 14.5 mg cobalt ferrite/1 cm^3 tumor after 24 h. Arrows indicate necrosis foci.

The presence of intact tumor cells could be noticed both at of a low concentration and at a slow diffusion of magnetic nanoparticles in cellular matrix. We must also take into consideration the magnetic fluid, which passed in blood stream. These phenomena diminish the uptake of cobalt ferrite particles and allow the tumor cell's proliferation.

The microscopical investigations revealed near MF injection points some clogging of magnetic particles on the vessels wall.

In the case of mammary tumors inoculated with 14.5 mg cobalt ferrite/cm^3 tumor the spreading of magnetic fluid was faster and extensive. After 20 min more than 57% of tumor cells endocitosed magnetic nanoparticles. The magnetic fluid diffusion was faster and the vessel wall was full of magnetic particles. After 1 h 90% of tumor cells were full of magnetic nanoparticles and after 6 h practically all the tumor cells were overloaded with nanoparticles. This nonspecific endocytosis of cobalt ferrite particles is proportional to their concentration in the extracellular matrix. Most often the endocytic vesicles can fuse with other vesicles and the magnetic material was stored in the cell. After 6 h the necrosis of tumor cells was intense (Fig. 2). Frequently, cell membrane and nuclear disintegration were noticed. The sagital section of the tumor revealed a pronounced dispersion of magnetic fluid in the whole tumor and a lot of necrosis foci (Fig. 3).

The microscopical examination showed massive necrosis of tumor cells in the mammary tumor resected 24 h after magnetic fluid inoculation. Tumor cells without endocitosed cobalt ferrite particles were not observed any longer. The microscopic examination of this last tumor pointed out a fibrocarcinoma type. Besides glandular tumor cells, many hypertrophied and hyperplasic

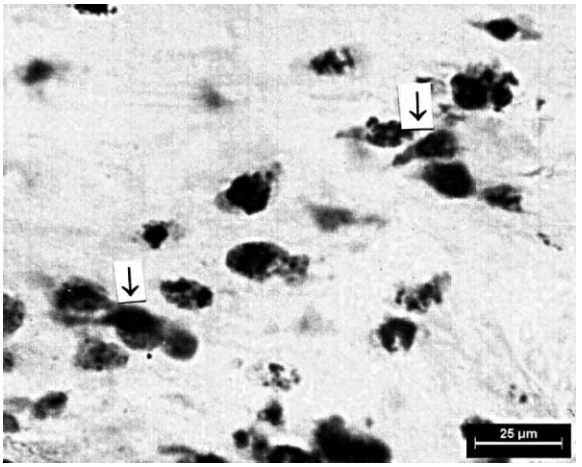

Fig. 4. Glandular tumor cells overloaded with magnetic particle vesicles. Fibrocarcinoma mammary tumor inoculated with 14.5 mg cobalt ferrite/1 cm^3 tumor.

fibroblasts that endocitosed cobalt ferrite were observed, too (Fig. 4).

These observations led to the conclusion that magnetic fluids with a certain magnetic particles concentration and nanosize (especially 20–25 nm) realize cell membrane transport troubles of vascular network that blocks the O_2 and nutrient supply of cells. The capillaries transcytosis is affected mostly.

Under these circumstances, the tumor cells which continuously multiply and need large amounts of nutrient supply lose their functional specificity, including that of endocytosis. So, such tumor cells endocitosed a lot of substances from extracellular matrix whose nanometrical sizes are not too big and, more than that exhibit a hydrophobic cover resembling LDL.

The massive uptake of magnetic nanoparticles overloads the tumor cells that cannot get rid of them. So, the magnetic particles are like 'Trojan horse', leading to tumor cell lysis. Supposedly, the tumor cells lysis could also be a magnetolysis. Indeed, subdomain-sized cobalt ferrite nanoparticles have a permanent magnetic moment, $m = M_s V$(M_s — saturation magnetization of $CoFe_2O_4$, V — particle volume). Once these little magnets arrive in cells, they can modify orientation and function of inner organelles and of structures with obvious polarity such as microtubules. So, the magnetic nanoparticles clog polymerization and depolymerization of microtubules, which leads to cytoskeleton alteration and hinders the formation of division spindle, blocking the multiplication. More than that, the magnetic fluid remanence in the tissue and in the blood stream prevents the development of tumor tissue.

The cells destruction is obviously due to magnetic particle endocytosis. However, it is necessary to study the behavior of tumor tissue for a longer period of time after magnetic fluid inoculation. One must also establish whether tumor cells destruction is complete, which is the magnetic fluid remanence in the tissue as well as the recovery ability of affected tissue, objectives which we will take into consideration in the next stages.

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