COMPARATIVE STUDY OF ENDOCYTOSIS BETWEEN NORMAL CELLS AND THOSE THAT HAD BECOME TUMOR CELLS

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Summary

Comparative studies were made regarding endocytosis between some normal cells and the same type of cells with tumoral changes. Cells with a high endocytosis capacity (secretory and absorbing epithelial cells and fibroblasts) were chosen. These types of cells, both normal and tumoral, were placed in direct contact with magnetic nanoparticles. It was observed that the tumor cells lost their specific endocytosis capacity and to survive they took a high quantity of foreign particles from the extracellular matrix.
Gradually, the tumor cells were overloaded with magnetic nanoparticles that could no longer be eliminated from the cell. In time a part of the tumor cells were destroyed and some were petrified. The main observation is that the tumoral process is stopped.

Key words: endocytosis, magnetic nanoparticles, tumor cells

Endocytosis is a type of transport through cell plasmalema that is specific in most cases or unspecific. Endocytosis does not have the same intensity for all cell types. For macrophages and absorbing cells endocytosis it is the main activity. Also, the secretory cells and fibroblasts have a high endocytosis capacity.
Endocytosis can suffer changes depending on the cell metabolism. A very interesting phenomenon was observed in tumor cells regardless of their cellular origin. They lose their specific endocytosis capacity and take up many foreign particles from the extra cellular matrix. These cells only eat up and are in a continual multiplication. In the last decade a lot of researchers tried to find possibilities to destroy tumor cells as a result of unspecific endocytosis of some nanoparticles carrying therapeutic substances. (1, 2, 3, 4, 5,).

In our experiments we wanted to point out the endocytosis of magnetic nanoparticles by some cell types compared with the same cells that became tumoral. Also we wanted to observe what happens to the magnetic nanoparticles in the tumor cells.

Materials and methods

The experiments were done with aqueous magnetic fluid containing cobalt ferrite nanoparticles ($CoFe_2O_4$) stabilized with a double layer of laurel acid. The saturation magnetization was about 80 Gs corresponding with 93mg of magnetic nanoparticles/1ml magnetic fluid. The size of magnetic nanoparticles was approximately 10 – 15 nm.

The experiments were done on guinea pigs and dogs.

In the first phase the behaviour of normal cells with a high endocytosis capacity that were put into contact with magnetic nanoparticles was noticed.

Four groups of guinea pigs were organized. Each group was composed of four animals to which the magnetic fluid was administered in the following way:

- batch I, oral administration of 1 ml magnetic fluid;
- batch II, intradermal inoculation of 0,1 ml magnetic fluid;
- batch III, with four females that were intrammary injected with 0,1ml magnetic fluid
- batch IV, intraepidermal inoculation with 0,1 ml magnetic fluid.

From each group two animals at 1 h and 6 h were euthanasied. Small fragments of intestine, skin and mammary gland were taken and prepared for cytohistological exam by hematoxiline-eosine and Perls methods.

In the second stage the same type of magnetic fluid was injected in various tumor.types. The experiments were done in two Private Veterinary Practices with the agreement of animal owners. The magnetic fluid was injected in the following tumor types: mammary adenocarcinoma, fibrosarcoma, epithelial tumors. The concentration of nanoparticles injected into tumor was about 5 mg/1cm^3. This dose was established as been efficient by previous experiments. (6, 7).

The surgical removal of the tumors was done at 1 h, 7 days and two months from the magnetic fluid inoculation, with the agreement of animals' owners. The tumors were prepared for cytohistological study by hematoxiline-eosine and Perls methods.

Results and discussions

The cytohistological exam of the guinea pigs intestine at 1 hour after oral administration of the magnetic fluid showed a massive endocytosis of the nanoparticles by enterocytes. After 6 hours the magnetic nanoparticles were not present in the absorbent cells anymore. On the other hand, the cobalt ferrite nanoparticles were present in a small amount in the normal fibroblasts, in epidermal cells, in glandular cells and in higher amount in the extracellular matrix. In normal conditions all these cells possess a specific endocytosis capacity.

The microscopic exam of squamous cell skin tumors injected with magnetic fluid revealed that the epithelial tumor cells were filled with cobalt ferrite nanoparticles. This phenomenon also was observed for the tumoral glandular cells in mammary adenocarcinoma and for tumorale, fibroblasts in fibrosarcoma.

On the other hand, macrophages (cells that have the main role to eliminate foreign bodies from an organism) have a high capacity of endocytosis towards nanoparticles of cobalt ferrite. As the tumor cells lose their specific endocytosis capacity, they become overloaded with magnetic nanoparticles.

The cells that suffered a tumoral development take up massively a lot of substances from extracellular matrix that is necessary for an intense proliferation.

In the first hour after the magnetic fluid injection, most of the tumor cells had endocytosed a large amount of cobalt ferrite nanoparticles that step by step overloaded the cytoplasm and blocked the cell metabolism.

It has been proven that this magnetic fluid, based on cobalt ferrite nanoparticles is very stable in animal tissues and its structure can be maintained more days. Other magnetic fluid types, especially based on dextran are very instable and in animal tissues are very rapidly disintegrated. The biocompatible magnetic fluids with a high stability in the animal tissues have a prolonged antitumoral effect.

After seven days it was observed that the tumor proliferation has stopped. The microscopic exam revealed that the most tumor cells were overloaded with magnetic nanoparticles and presented degenerative phenomena.

The cytohistological study of the tumors after two months pointed out that the majority of tumor cells were destroyed. In some areas, clusters of tumor cells filled with magnetic nanoparticles were noticed. Some that could not be eliminated had become petrified.

These observations confirm that after the treatment of tumors with magnetic fluids, in time the surgical excision of the tumor is necessary.

Conclusions

The cells that suffered a tumoral change lose their specific endocytosis capacity and take over from extracellular matrix a lot of substances that are ncessary to their intense feeding, growth and proliferation.

The overloading of the tumor cells with magnetic nanoparticles blocks the tumoral development and determine the tumor cells lysis.

Macrophages and fibroblasts filled with magnetic nanoparticles that remain in the mammary gland for a long time, as well as cloggy ferrite residua that turn cells into stony cells finally call for a surgical excision.

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