Diffusion of Nanoparticles through the Tissues in Experiment

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ABSTRACT

The methods of control of the of nanoparticles distribution (external magnetic field, aggregation with the antibodies to the tumor antigens) are known, but for using these methods it is necessary to study nanoparticles biodistribution in the absence of any external control.

Two models wee used.

1. Nanoparticle formulation was brought onto the intact tongue of rat and remained on it in the period from 7 - 10 minutes to 1 hour.

2. We subcutaneously injected Ra-1 cells into female adult rats femur. After 3 weeks of tumor growth nanoparticle formulation was injected into tumor tissue.

Rats were sacrificed in 1-3 days and histological investigations of tissues involved were performed.

The particles of submicron sizes diffuse with the noticeable speed from the surface of the intact epithelium on the lymphatic vessels and the vascular fascicules into the depths of the tissue under the effect of the concentration gradient.

The particles of submicron sizes do penetrate the cells membranes under a concentration gradient.

Keywords: nanoparticles, biodisribution, tumor tissue

1 AIMS AND INNOVATION

It is very important to consider the special features of nanoparticles (ability to penetrate the pores of capillaries and epitheliums, through the lumens of lymphatic vessels, ability to form conglomerations) in those medical applications, which rely on modulating function of nanoparticles.

The methods of control of the of nanoparticles distribution (external magnetic field, aggregation with the antibodies to the tumor antigens) are known, but for using these methods it is necessary to study nanoparticles biodistribution in the absence of any external control.

2 MATERIALS

We have created nanocrystals of silicon with sizes in the greatest measurement from 50 to 100 nm, and nanocrystals of the oxide of aluminum with the adsorbed molecules of phthalocyanines, the diameters of nanoparticles: 200 - 300 nm. These nanoparticle formulation supposed to be used later to improve the effectiveness of PDT.

3 MODEL

Suspension of particles of both types in dextran were used. Suspension stability proved to be enough to prepare pharmaceutical forms resistant to the coagulation during two hours. For an example Table 1 shows the periods of the sediment formation of studied silicon nanoparticles in “imitators” of biological fluids. All the concentrations were 1 g/L.

Table 1: The Precipitation Times

Fluid	Precipitation Time (min)
Ionosteril	15
Rheopolyglukin	105
Physiological salt solution	15
Polyglukin	0

Surgical procedures were conducted in compliance with ethical principles for animal research, as approved by MROI and European (European Convention for the protection of vertebrate animals used for experimental and other scientific purposes) guidelines.

Laboratory animal (rat) was put into deep narcosis.

Three models wee used.

1. Normal muscular tissue. Nanoparticle formulation was injected subcutaneously into female adult rats femur.
2. Normal epithelium, applicative method: Nanoparticle formulation was brought onto the intact tongue of rat and remained on it in the period from 7 - 10 minutes to 1 hour.
3. Biliary tract cancer tissue, injective method: We subcutaneously injected Ra-1 cells into female adult rats femur. After 3 weeks of tumor growth nanoparticle formulation was injected into tumor tissue.

Rats were sacrificed in 1-3 days and histological investigations of tissues involved were performed.

4 RESULTS

4.1 Results of normal muscular tissue investigations

No penetrations into muscular tissue were noted (Figure 1).

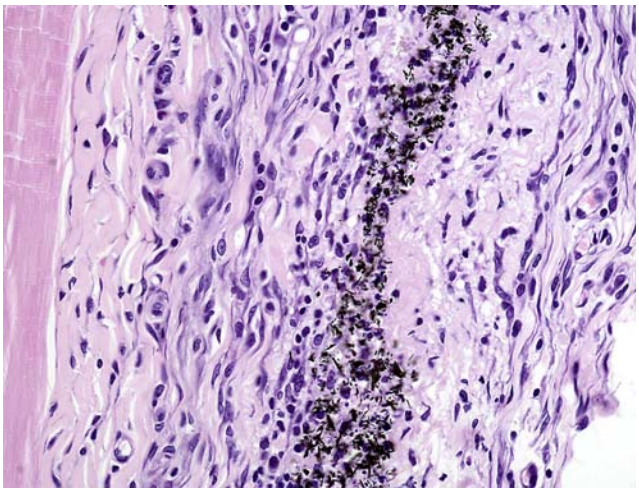

Figure 1. The particle of optically opaque substance can de seen in the cytoplasm of cells. Musculature is not changed, particles are absent.

4.2 Results of normal epithelium morphological investigation

The particles do penetrate through intact epithelium (figure 2)

In the thickness of the muscular layer alongside of the blood vessels and in the lumens of lymphatic vessels optically opaque structures can be seen.

Sometimes structures are decomposed into fine dust-like particles. We observed the maximum concentration of silicon nanocrystals at tongues surface with uniform decrease with the increase of distance from surface. Nanocrystals of the oxide of aluminum with the adsorbed molecules of phthalocyanines proved to be evenly distributed throughout the entire thickness of the tongue.

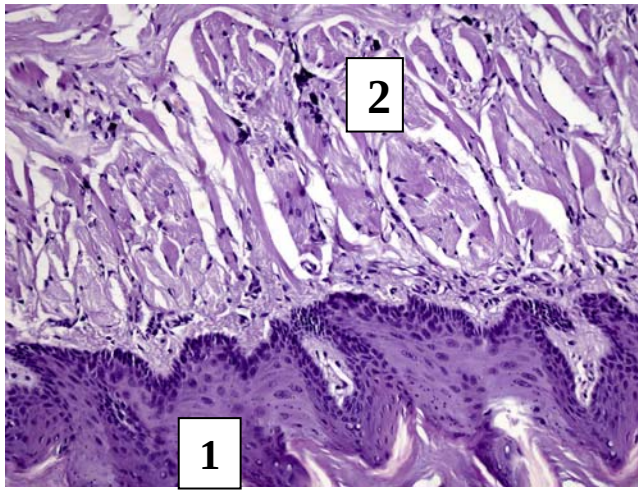

Figure 2. Particles, penetrated through intact epithelium. 1 – surface of rats tongue; 2 – nanoparticles.

The diameter of structures is equivalent 1 – 4 diameters of erithrocite (figure 3).

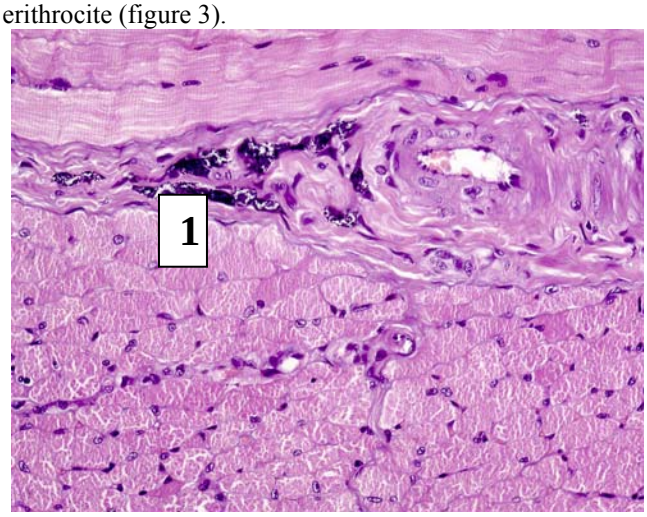

Figure 3. Optically opaque structures (1) can be seen alongside of the blood vessels and in the lumens of lymphatic vessels.

Diffusion speeds through the epithelium are different for different particles. Speeds are determined by the entire totality of the properties of particles, but not only by their sizes. This follows of the clearly larger rate of diffusion of particles with the sizes 200 - 300 nm in comparison with the particles of 50 - 100 nm.

It is important to note that the penetrations inside the cells was not noted

4.3 Results of tumor tissues investigations

During the study of tumor tissues is also discovered the penetration of particles into the tissue, the slower than for the normal epithelium. In contrast to the healthy epithelial

tissue attention should be drawn to the penetration of particles into the cells (figure 4).

Table 1 presents the results of experiments.

We have set value 0 in the column «Particles penetration into cells», if there were no particles inside of cells, visible by optical microscopy, 0,5 – if particles can be seen only "in cytoplasm of some cells", 1 – if particles can be seen in cytoplasm of all the tumor cells.

We can say that in this column the "concentration" of particles in the cells in some arbitrary units are contained.

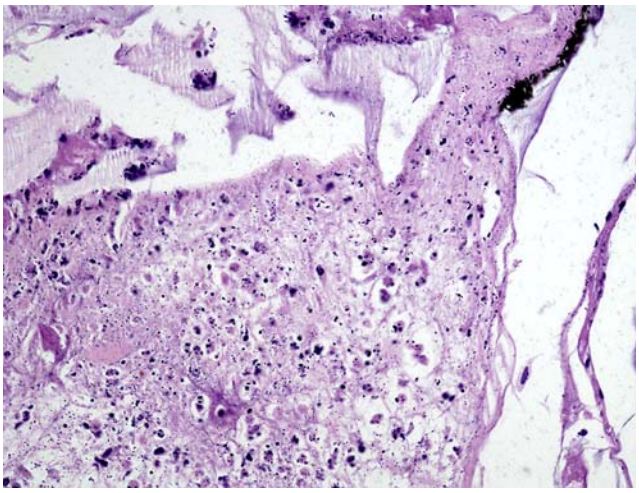

Figure 4. There are dispersed powdered particles of brown color, which form the single conglomerations, in the cytoplasm of tumor cells and in the preserved stroma.

Table 1: Formalized results of experiments with the interwoven tumors of the rats

	Time from injection to animal sacrifice (hours)	Necrosis percentage	Particles penetration into cells
1	2	3	4
1	26,25	50	0
2	5	50	0
3	24,75	55	0,5
4	52	70	1
5	24,5	60	1
6	24,5	30	0
7	24,5	80	0
8	24,5	70	0
9	72,2	60	0,5
10	72,4	60	0,5

4.4 Statistical processing of the results of investigating of the nanoparticles behavior in the tumor tissue

The correlation coefficients between the parameters were calculated for the development of possible interdependences between the values of parameters of the experiment.

If we have a series of n measurements of X and Y written as xi and yi where i = 1, 2, ..., n, then the Pearson product-moment correlation coefficient can be used to estimate the correlation of X and Y. The Pearson coefficient is also known as the "sample correlation coefficient". The Pearson correlation coefficient is then the best estimate of the correlation of X and Y. The Pearson correlation coefficient is written:

$$r = \frac{(\Sigma XY) - n\bar{X}\bar{Y}}{(n-1)s_X s_Y},$$

where $\bar{x}$ and $\bar{y}$ are the sample means of x_i and y_i, Sx and Sy are the sample standard deviations of x_i and y_i and the sum is from i = 1 to n. Cohen [1] has suggested the interpretations for correlations coefficient showed in table 2.

Table 2: Interpretations for correlations coefficient

Correlations	Negative	Positive
Small	-0,29 to -0,10	0,10 to 0,29
Medium	-0,49 to -0,30	0.30 to 0.49
Large	−1.00 to −0.50	0.50 to 1.00

Does the necrosis percentage depend upon the time from injection to animal sacrifice?

We have calculated the Pearson correlation coefficient between columns 2 and 3. This coefficient proved to be equal to 0,12, which means very small.

Does the penetrations rate depend upon the time from injection to animal sacrifice?

We have calculated the Pearson correlation coefficient between columns 2 and 4. This coefficient proved to be equal to 0,46, which means medium.

The noticeable difference between the degrees of necrosis in different experiences can be explained by the difference in the rates of the development (and, which means, the achievement of the phase of disintegration) of tumors in different animals, for example because of the special features of immunity.

5 CONCLUSIONS

The particles of sub-micron sizes diffuse with the noticeable speed (not less than 1 mm per minute) from the surface of the intact epithelium on the lymphatic vessels and the vascular beams into the depths of the tissue under the effect of the concentration gradient. The penetrations degree is function of time.

No disturbances of the behavioral features of animals was noted. Physiological functions and behavior conformed to severity and terms of surgical intervention. During the

experiment only one animal perished from the progression of tumor process, before the nanoparticles injection. So, it is possible to make a conclusion about the absence of the general toxicity of the nanoparticles investigated

The particle concentration in the cells average observed by the optical microscope depends on time between injection and sacriface of animal (correlation coefficient 0,46). A certain decrease of correlation coefficient is connected with the decrease of the observed concentration in 3 days (experiences 9 and 10). It is possible to make a careful assumption about the fact that this decrease is connected not with absence or weakness of dependence, but with its nonlinearity. Obviously the dependence can be linear only in the limited time interval, and otherwise it is necessary to allow the infinite capacity of cell for the particles and a constant inflow of particles from outside.

The main observation, which was possible to make from experiments in -vivo - it was discovered the phenomenon of the nanoparticles diffusion through different tissues. The diffusion rate changes depending on the type of tissue. It is maximum (for the studied tissues) for the epithelium of oral cavity, somewhat less for the tumor tissues and was not observed (absent or very small) for the healthy muscular tissues.

In the solutions, close to the physiological liquids (physiological salt solution, ionosteril, reopoliglukin) the optimum index of the stability of the suspension of nanoparticles was reached in the low-molecular dextran (100 minutes). Similar solutions could be the carriers of nanoparticles for their application in the living tissues.

REFERENCES

[1] Cohen, J. (1988). Statistical power analysis for the behavioral sciences (2nd ed.) Hillsdale, NJ: Lawrence Erlbaum Associates. ISBN 0-8058-0283-5.