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NANOTECHNOLOGIES

Elimination of Iron-Containing Magnetic Nanoparticles from the Site of Injection in Mice: a Magnetic-Resonance Imaging Study

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Suspension of magnetic nanoparticles (0.7 g/liter) obtained from *Klebsiella oxytoca* culture was injected intraperitoneally (1 ml), intramuscularly (in the hip; 100 μl), and subcutaneously (200 μl) or administered orally instead of drinking water for 2 days. The presence of magnetic nanoparticles was evaluated detected by MRI in 15 min and 2 h after injections and in 1 and 2 days after the beginning of oral consumption of the suspension. Magnetic nanoparticles were eliminated from the site of intramuscular and intraperitoneal injections and after oral consumption. The period of elimination after intramuscular and intraperitoneal injections did not exceed 2 h, while after oral consumption it corresponded to the time of gastrointestinal tract contents evacuation.

Key Words: *magnetic nanoparticles; Klebsiella oxytoca; magnetic resonance imaging; elimination*

Biomedical application of nanoobjects is a priority trend of modern experimental biology. Nanoparticles (NP) of different nature are suggested to be used for the diagnosis and therapy of various diseases [1,2,5,6]. It is therefore essential to study the distribution of these particles in the body and their elimination after administration via different routes.

Iron-containing NP (2-5 nm) with magnetic properties were obtained at Krasnoyarsk Research Center. They can serve as magnetoregulated drug carriers and magnetic labels. It is therefore essential to study their behavior in the body without exposure to the regulatory magnetic field.

As the studied NP can be detected in the body by NMRI technique [1,3], we use this method in our study.

We studied elimination of iron-containing magnetic NP in mice after their injections via different routes and after oral administration.

MATERIALS AND METHODS

Magnetic ferrihydrite $5Fe_2O_3 \times 9H_2O$-based NP were obtained from *Klebsiella oxytoca* culture at Krasnoyarsk Research Center. The method for obtaining NP and the results of studies of their structure and physical characteristics, including magnetic properties, were described previously [4,7]. According to the small-angular X-ray diffusion method, the size of the NP used in the study was 2-5 nm [7].

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Experiment was carried out on ICR mice from Breeding Center, Vector State Research Center of Virology and Biotechnology. The animals received suspension of magnetic NP (0.7 g/liter): a single intraperitoneal injection (1 ml); a single intramuscular (in the hip) injection (100 μl); and a single subcutaneous injection (200 μl). NMR images were acquired 15 min and 2 h after injection of NP. Water suspension of NP in the same concentration was administered orally instead of drinking water for 2 days. NMR images were acquired 1 and 2 days after the beginning of oral consumption of NP suspension.

The animals were narcotized with ether before NMRI study.

Intact animals and animals receiving isotonic saline intramuscularly or intraperitoneally served as controls.

All NMRI studies were carried out on an NMR microtomograph based on Bruker Avance DPX 200 in a 4.7 T field with Greate 3/60 gradient block on a PH Micro 2.5 pickup (25-mm radiofrequency coil). The images were obtained by ^{1}H nucleus. The sensitivity of the method for the studied NP was 10 μg particles/g tissue.

NMR images were acquired by the Multi Slice Multi Echo technique [4]. Two applied RF pulses were used: 90° (stimulatory) and 180° (generating the spin echo signal). The main advantage, which determined the use of this method, was the possibility to obtain images weighted by different parameters (T_1 time of spin-lattice and T_2 time of spin-spin relaxation).

The pulse sequence presented is determined by a set of parameters: TE (time of echo) – interval between the 90° pulse and echo signal; TR (time of repeat) – interval between each applied pulse sequence (from the first stimulatory 90° pulse till the next). The number of pulse sequence repeats is preset. The signal intensity after 180° pulse can be presented as follows:

$$I_{SE}=k\rho[1-\exp(\frac{TE-TR}{T_1})]\exp(\frac{-TE}{T_2}),$$

where T_1 is the time of spin-lattice relaxation, T_2 time of spin-spin relaxation, ρ is space-dependent distribution of proton density in the sample, and k is the constant. For rather high TR (TRH≈5T_1 often experimentally preset at TR>>TE) the contribution of the brackets can be neglected (relationship between I_{SE} and T_1), as it is close to one. Selecting the TEH≈T_2 (at sufficiently high intensity of the signal) it is possible to make the contribution of the last member in I_{SE} the decisive one, in other words, to obtain the so-called T2-weighted image. Varying the TE parameter is possible to attain the desired depth of T2-weighted image (in this case the contrast of T2-weighted image improves with increase of TE). Selecting TE<<T_2 (at TRH≈5T_1 unchanged), is possible to approximate the exponent to one, which will provide the maximum contribution to relationship between I_{SE} and ρ. A ρ-weighted image is thus obtained (with I_{SE} proportional to ρ). In order to obtain a T1-weighted image, one should reduce TR (at TE<<T_2 unchanged) so that the relationship with T_1 predominated over relationship with ρ. The possibility to obtain an image weighted by a certain parameter leads to the optimal contrast of the tomographic image in the target region [3].

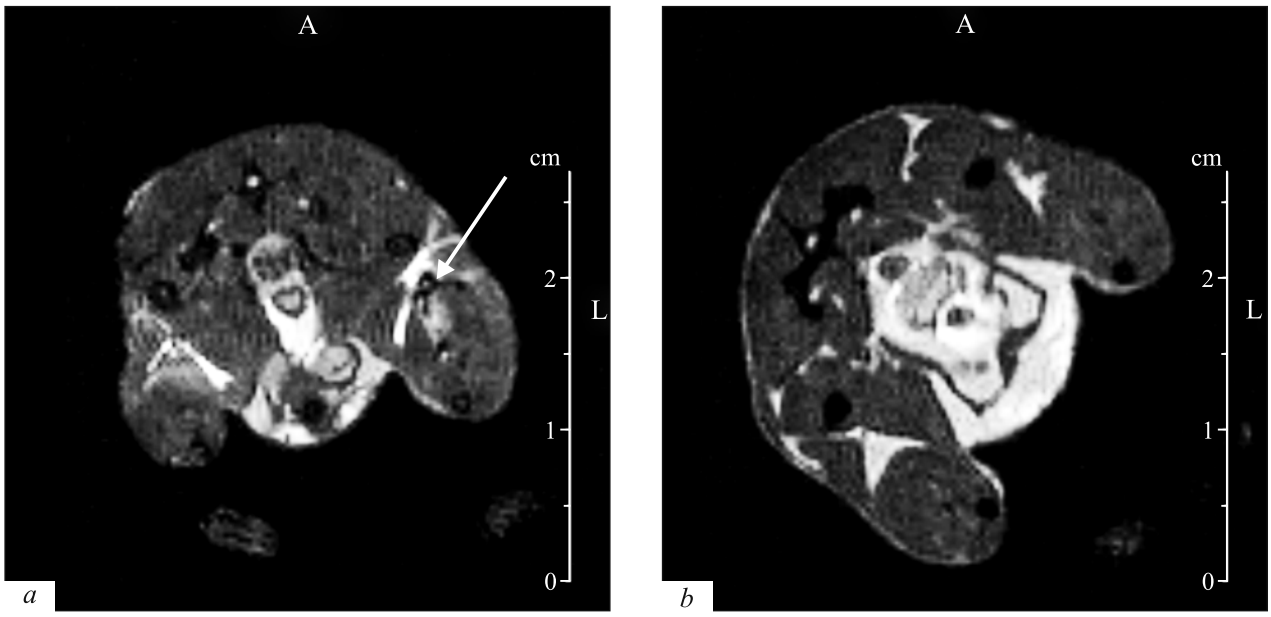

Fig. 1. Femoral transverse section images 15 min (*a*) and 2 h (*b*) after intramuscular injections of suspension of iron-containing magnetic NP. Here and in Figs. 2, 3: arrows show zones with more intense signal due to the presence of NP. Image parameters: section thickness 1 mm, 128×128 matrix, scanning area 40 mm, TR=4000 msec, TE=60 msec.

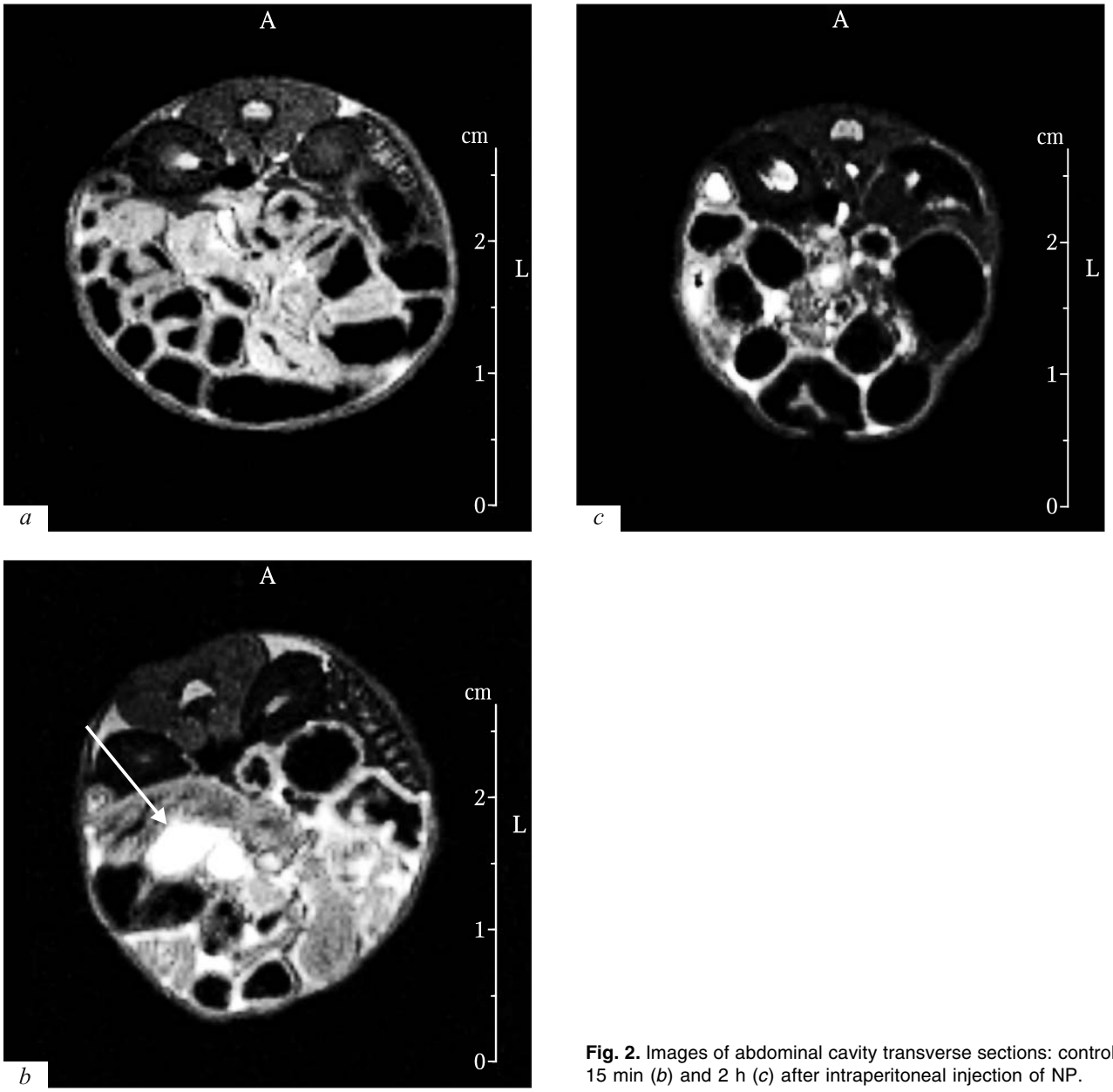

Fig. 2. Images of abdominal cavity transverse sections: control (*a*), 15 min (*b*) and 2 h (*c*) after intraperitoneal injection of NP.

RESULTS

The T2-weighted image of the cross-section of the femoral bone acquired 15 min after intramuscular injection of magnetic NP suspension showed higher intensity of the signal (Fig. 1, *a*), which could be attributed to the effects of the NP. The T2-weighted image acquired after 2 h showed no changes in the signal intensity or tissue morphology at the site of the suspension injection (Fig. 1, *b*), which could mean that the NP concentration in this region decreased to the level undetectable by the instrument used in the study. Hence, NP were eliminated from the site of intramuscular injection within 2 h after injection.

The T2-weighted images of the transverse sections after intraperitoneal injection of magnetic NP suspension showed differences in signal intensity in certain areas located between the abdominal organs (Fig. 2, *a*, *b*), which indicated the presence of NP in the abdominal cavity 15 min after injection. The signal intensity did not differ from the results of control measurements 2 h after intraperitoneal injection of NP. Importantly that it is much more difficult to reproduce the geometry of the sections in studies of the intraperitoneal space than of muscle and organ sections, but the conclusions are based on the sum of the adjacent tomographic sections.

The distribution of magnetic NP in the body after their oral consumption was particularly interesting,

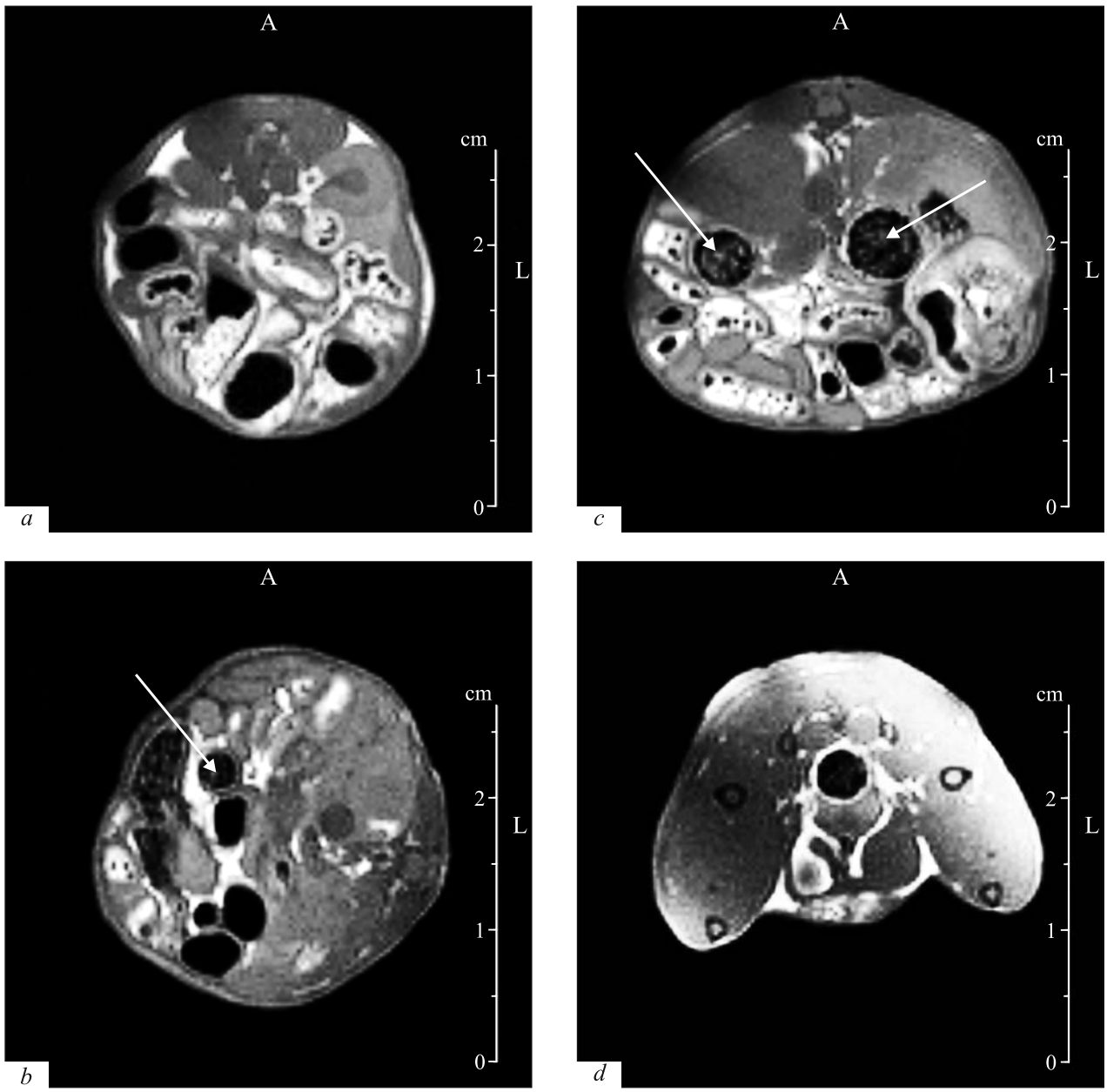

Fig. 3. Abdominal cavity transverse section images after oral intake of iron-containing magnetic NP. *a*) Control; *b*) oral consumption of NP during 24 h; *c*, *d*) during 48 h. Image parameters: TR=650 msec, TE=3.2 msec.

as this route could be one of the major ones for NP entry. However, T2-weighted images were not sufficiently contrast, because the analyzed medium differed significantly from compact tissue. This problem was solved by using T1-weighted images, which was in agreement with modern data [3].

The T1-weighted images of abdominal cavity cross-sections after oral intake of NP suspension showed a significant increase of the signal intensity in the intestine in comparison with the control (Fig. 3). This could be due to oral NP. Hence, oral administration of NP was followed by their elimination through the intestine. Anyway, their effects were detected at a considerable length of the large intestine, including the rectum (Fig. 3, *d*).

No signs of magnetic NP were detected in the tomogram after their subcutaneous injection, presumably because of insufficient volume of the suspension and rapid distribution in a thin layer under the skin, which was not discernible in the tomogram.

Iron-containing magnetic NP used in our experiment were eliminated from the site of injection after their intramuscular and intraperitoneal injections and after oral intake. The duration of elimination was no

longer than 2 h after intramuscular and intraperitoneal injections; after oral intake, the elimination length corresponded to the duration of the gastrointestinal tract contents evacuation.

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