Electron Microscopy of Rat Liver after Intravenous Injection of Nanosized Magnetite Suspension

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Rat liver was examined by transmission electron microscopy after a single intravenous injection of nanosized magnetite suspension (0.1 g (Fe_3O_4)/kg body weight). Magnetite particles were found in Kupffer's cells and hepatocytes. Accumulation of the particles by these two cell types was different. Morphometry of magnetite-containing granules in Kupffer's cells and nanoparticle agglomerations in hepatocytes was carried out. The ultrastructure of Kupffer's cell granules was described and the mechanism of penetration of nanosized magnetite particles into the cells was suggested. Nanosized magnetite particles were not completely eliminated over 40 days after a single injection.

Key Words: *magnetite nanoparticles; hepatocyte; Kupffer's cell (stellate macrophage)*

The use of nanosized magnetite in medicine opens new diagnostic (MRT) and therapeutic potentialities (regulated local hyperthermia, targeted magnet-regulated drug delivery, *etc.*) [8,10].

The liver is one of the key sites in pharmacokinetics of nanomaterials, but ultrastructure of this organ after intravenous injections of nanomaterials received little attention in studies of interactions between inorganic nanoparticles and living bodies [3,4,7,9].

We studied ultrastructural changes in rat liver after a single intravenous injection of nanosized magnetite particles.

MATERIALS AND METHODS

Suspension of nonmodified nanosized magnetite nanoparticles was prepared in aqueous stabilizing saline containing sodium chloride, sodium citrate, and 4-(2-hydroxyethyl)piperazine-1-ethane sulfonic acid (HEPES) disodium salt [5].

Magnetite content in the suspension was evaluated by iron concentration by the X-ray fluorescent method. The size distribution of magnetite particles in suspension was evaluated by the laser diffraction method. The shape and structure of the particles in suspension were studied by transmission electron microscopy.

The study was carried out on outbred male rats (150±30 g; n=50) distributed into 3 groups. Group 1 (n=10) were intact animals. Group 2 (n=20) were controls, injected with a single dose of 2 ml stabilizing solution into the caudal vein. Group 3 (n=20) received a single injection of 2 ml standardized magnetite suspension (0.1 g (Fe_3O_4)/kg) into the caudal vein.

Electron microscopic examination of rat liver was carried out on days 1 and 40 after intravenous injection of magnetite suspension. Liver fragments of about 1 mm^3 were fixed in 4% paraformaldehyde in Hanks' fluid (pH 7.4) for 24 h at 4°C and then in 1% OsO_4 in the same buffer for 3 h at 4°C. The preparations were then dehydrated and embedded in epon-araldite mixture. Semithin sections (1 μ) were stained with 1% toluidine blue in saturated borax solution. Ultrathin sections were mounted on lattices, contrasted by uranyl acetate and lead citrate, after which they were examined in a JEM-100 CX II electron microscope (Jeol) and photographed at ×2900, 3600, and 48,000.

Ultrastructural morphometry of magnetite-containing granules in Kupffer's cells and free agglome-

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rations of nanosized particles in hepatocytes was carried out. The results were presented as the mean number of granules or agglomerations/μ^2 cytoplasm of the corresponding cell. The results were processed by SPSS 11.5 statistical software. Since the distribution conformed to the normal (according to Shapiro–Wilk test), the significance of differences between the means of morphometric parameters was evaluated by the *t* test for related samples.

RESULTS

Magnetite particles in stabilizing solution are mainly spherical and are suspended as individual particles and agglomerations. The mean size of free particles is 10 nm, agglomerations are no larger than 100 nm.

MRT studies after intravenous injection of magnetite suspension to rats showed superparamagnetic particles in the liver and spleen. Subsequent histological and histochemical studies showed magnetite accumulation in the mononuclear phagocytes (MNP) of these organs [2]. It is noteworthy that Perls' histochemical reaction in the hepatocyte cytoplasm of intact, control, and experimental (after magnetite injection) animals was negative on days 1 and 40.

The aim of this study was to detect ultrastructural characteristics of liver cells and mechanisms of absorption of nanosized magnetite by these cells from systemic circulation. No disorders of any kind in the ultrastructure of Kupffer's cells (stellate macrophages) and hepatocytes were detected on days 1 or 40, that is, the destructive effect of nanosized particles on liver cells was not manifest after a single injection. Dilatation of endoplasmic reticulum and numerous mitochondria with dark matrix in the hepatocytes in comparison with the picture in intact and control animals are worthy of note.

The absence of nanosized particles in the hepatic sinusoidal lumen 1 day after a single injection of magnetite can be explained by their active absorption by the MNP system cells and presumably by low content of magnetite in the vascular bed after a single injection. Finely dispersed magnetite disappeared from the blood 1-6 h after intravenous or intra-arterial injection and was redistributed in organs and tissues [1].

Granules of up to 1.5 μ in diameter enveloped in biological membrane and containing nanosized particles were found in the cytoplasm of Kupffer's cells located in the sinusoidal wall. This indicated penetration of nanosized magnetite into the cells by phagocytosis with the formation of phagolysosomes (Fig. 1). The number of phagolysosomes in different Kupffer's cells varied from just several to several tens per cytoplasm section even during the same period. This observation was in good agreement with light microscopy findings:

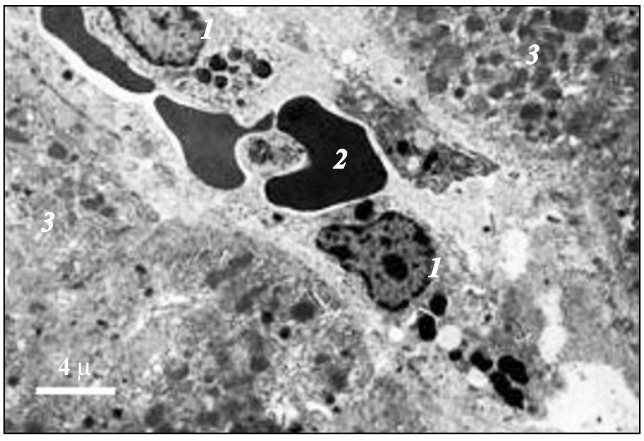

Fig. 1. Rat liver on day 1 after a single intravenous injection of magnetite suspension. Kupffer's cells with granules (*1*). Erythrocyte in sinusoidal capillary (*2*). Hepatocytes (*3*). Transmission electron microscopy, ×2900.

the cytoplasm of different Kupffer's cells reacted with different intensity in Perls' histochemical reaction [2]. No free magnetite particles or their agglomerations were found in Kupffer's cell cytoplasm. The specific content of magnetite-containing granules in Kupffer's cells during this period was 0.4 per μ^2.

The morphology of the cytoplasmic granules in Kupffer's cells was different on days 1 and 40 after intravenous injection of magnetite. Three types of granules could be distinguished by the numbers of nanosized magnetite particles present in them and their distribution:

1) dark granules filled with nanosized particles and agglomerations thereof, tightly adhering to each other;
2) granules with moderate amounts of nanosized magnetite, with particles and agglomerations lying more loosely than in the dark granules; some places were quite free from magnetite particles;

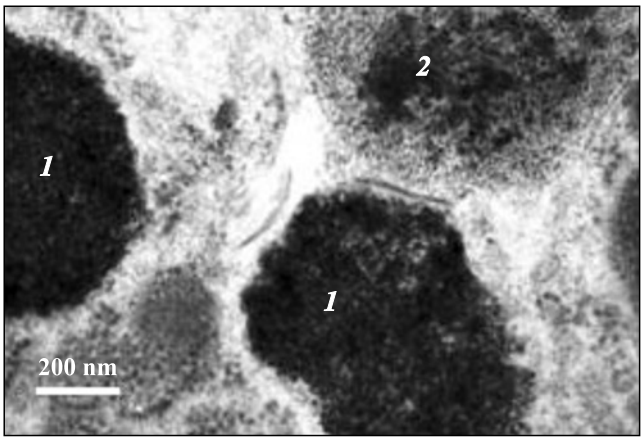

Fig. 2. Rat liver on day 1 after a single intravenous injection of magnetite suspension. Kupffer's cell granules, dark (*1*) and containing a moderate amount of magnetite (*2*) enveloped in membrane. Transmission electron microscopy, ×48,000.

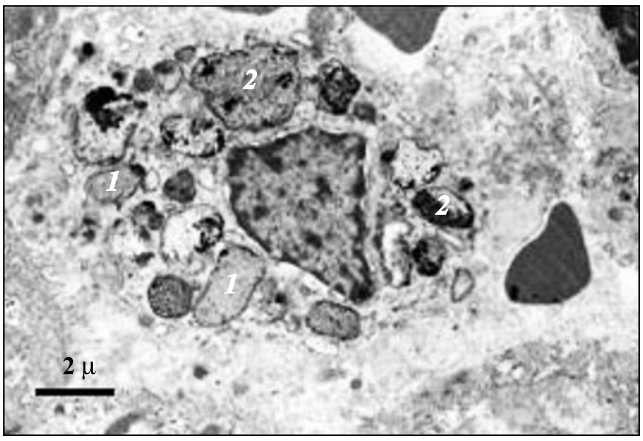

Fig. 3. Rat liver on day 40 after a single intravenous injection of magnetite suspension. Kupffer's cell granules, clear (*1*) and containing a moderate amount of magnetite (*2*), enveloped in membrane. Transmission electron microscopy, ×5800.

the morphology of individual particles was discernible;

3) clear granules containing a negligible number of nanosized particles and agglomerations thereof; the greater part of the inner space of these granules was free.

Dark granules completely filled with nanosized particles predominated in Kupffer's cells 1 day after a single intravenous injection of nanosized magnetite suspension (Fig. 2). Predominance of the dark granules on day 1 after intravenous injection indicated that the absorption of the particles from the blood stream was the most intense during the first 24 h after injection.

Individual polymorphic electron-dense structures (48.3 per μ^2) 60-90 nm in size were detected in the hepatocyte cytoplasm during this period; no structures of this kind were found in the hepatocytes of intact and control animals. By morphology they corresponded to the agglomerations of nanosized magnetite particles.

No magnetite particles or their agglomerations were detected in the hepatic sinusoidal lumen on day 40 after a single intravenous injection of magnetite particles suspension. The particles did not adsorb on the blood cell surface. Kupffer's cells looked as usual, contained common organelles and granules with just moderate or negligible quantities of nanosized particles (Fig. 3). The granules were enveloped in biological membrane and were 0.3-1.5 μ in size. The specific quantity of magnetite-containing granules on day 40 was 5.3 per mm^2 ($p<0.05$ *vs.* day 1).

Polymorphic agglomerations of nanoparticles (36.4 per mm^2) 60-90 nm in size without biological membranes were regularly found in the hepatocyte cytoplasms (Fig. 4). The quantity of magnetite nanoparticle agglomerations in hepatocytes reduced by day 40 ($p<0.05$).

The presence of these structures in the animal hepatocytes after intravenous injection of magnetite suspension and their absence in intact and control groups suggested identifying these structures as agglomerations of nanosized magnetite particles. In hepatocytes

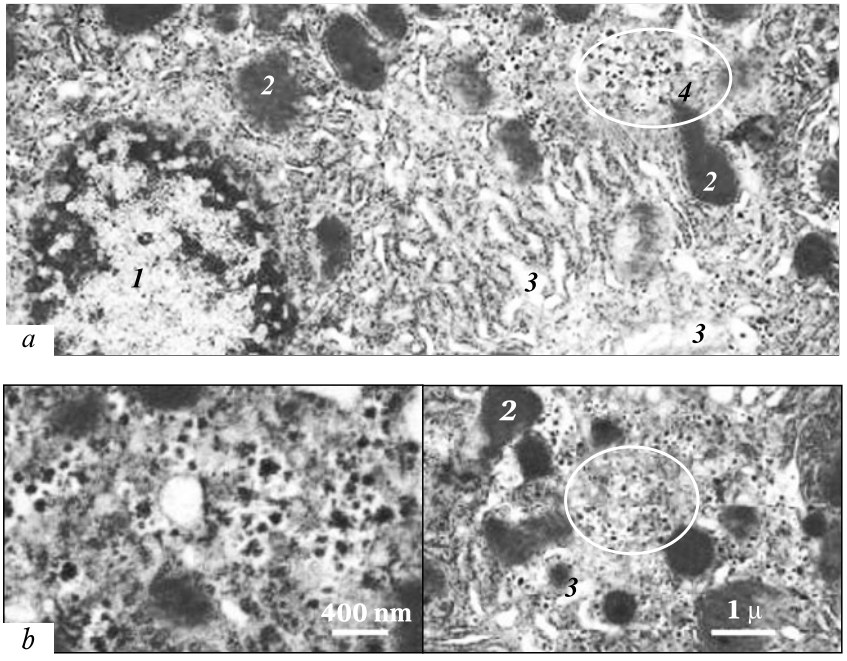

Fig. 4. Rat liver on day 1 after a single intravenous injection of magnetite suspension. *a*) hepatocyte: nucleus (*1*), mitochondria with compact matrix (*2*), dilated lumen of endoplasmic reticulum (*3*), agglomeration of magnetite nanoparticles in cytosol (*4*). *b*) hepatocyte cytoplasm site with agglomerations of nanosized magnetite particles. Transmission electron microscopy, ×12,000 (*a*), ×25,000 (*b*).

the presence of structures, morphologically similar to the particles in granules of Kupffer's cells, indicated the involvement of hepatocytes in elimination of nanosized particles.

The absence of membrane in the particles found in the hepatocyte cytoplasm indicated penetration of magnetite inside the cells by mechanisms other than phagocytosis, for example, through pores or channels, by transmitters, or other mechanisms of transmembrane transport.

Nanosized magnetite particles were not completely eliminated 40 days after their single intravenous injection. The aftereffects of magnetite persistence in the body in more remote periods are difficult to predict at this stage of the study. It is noteworthy that these magnetite nanoparticles are characterized by slow elimination kinetics and are liable to accumulate in the body [6,7,11].

Hence, a single intravenous injection of stabilized suspension of nanosized magnetite particles (0.1 (Fe_3O_4)/kg) led to their accumulation mainly in Kupffer's cell phagosomes. Only free agglomerations of nanosized magnetite particles were found in the hepatocyte cytoplasm. Magnetite content in these cells decreased 40 days after injection, but the nanomaterial was not completely eliminated by this time.

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