Morphological analysis of mouse lungs after treatment with magnetite-based magnetic fluid stabilized with DMSA

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Available online 10 March 2005

Abstract

Mouse lungs injected with magnetic fluids based on magnetite nanoparticles stabilized by 2,3-dimercaptosuccinic acid were studied. We observed clusters of magnetic nanoparticles inside blood vessels, within the organ parenchyma and cells, as well as increased numbers of leukocytes in the organ. Both the particle concentration and organ inflammation diminished in a time-dependent manner.
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Keywords: Nanoparticles; Magnetic fluid; Dimercaptosuccinic acid (DMSA); Morphological analysis; Lung inflammation; Toxicity; Mice; Biocompatibility

Magnetic nanoparticles (MNP) are traditionally ferrite-based materials with the general formula MFe_2O_4, where M is a divalent metal-ion [1]. A solution of magnetic nanoparticles is considered a magnetic fluid (MF) if the solution is composed of monodomain MNP dispersed in an organic or inorganic liquid carrier forming an ultra-stable colloidal suspension [1]. Conventional MF are organic-based colloidal suspensions stabilized by steric repulsion after coating the magnetic nano-grains with surfactant agents. Ionic MF are water-based colloidal suspensions stabilized by coulombic repulsion after adding an electric surface charge density at the MNP [2]. Most biocompatible magnetic fluids instead are highly stable MF in physiological medium (neutral pH and 0.9% sodium chloride), which may hold a variety of biological effectors chemisorbed at the MNP surface [3]. From a general point of view the colloidal stability of the biocompatible MF upon clustering depends on both steric and coulombic repulsion [3]. Depending upon the particular application, nucleotides, oligonucleotides, peptides,

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vitamins, and antibiotics can be bounded at the MNP surface. MF have been used for several biomedical applications, including separation and purification of cells [4], contrast agents in magnetic resonance imaging [5], and in the magnetic hyperthermia of tumor cells [3]. The biological effects of biocompatible MF must be extensively evaluated before medical and clinical applications can be put forward. The present study complements and reinforces, in an extended time window, previous studies that had demonstrated a preferential deposition of MNP stabilized with 2,3-dimercaptosuccinic acid (DMSA-MF) in lung from 5 min up to 24 h after intravenous injection of DMSA-MF in mice [6]. Most of MNP were found within blood vessels, mainly capillaries, in spite of some MNP inside parenchyma cells. Though the amount of MNP in an animal's lung decreases as a function of time, we still found a high amount of magnetic materials 24 h after injection. Yet, some inflammatory cells groups were observed in the organ, mostly associated with the presence of MNP. As any foreign materials MNP could induce an inflammatory process. In addition, lung cells may secrete inflammatory mediators and increase the local inflammation. In a long-term scenario, if the inflammatory process is not controlled, it can lead to pulmonary fibrosis. The aim of this study was to investigate if morphological changes, such as chronic inflammation and/or pulmonary fibrosis, occur in a long-term treatment of DMSA-MF in the lungs of mice.

DMSA-MF was synthesized by chemical co-precipitation of Fe(II) and Fe(III) ions in alkaline medium. After precipitation, MNP were surface-coated with DMSA to obtain a stable MF sample at physiological condition. In order to investigate the effects of the DMSA-MF on the lung morphology of adult female Swiss mice (nine animals per group), 100 μL of 8.6×10^{15} particles/cm^3 of DMSA-MF dissolved in saline solution was given as an intravenous bolus dose through the animal's tail vein. The average particle size was 9.4 ± 0.1 nm. The particle size polydispersity was obtained from a transmission electron microscopy (TEM) micrograph. The particle size histogram obtained from the TEM data was curve-fitted using the log-normal distribution function, as

described elsewhere [7]. The animals were killed 24 h (EG1), 48 h (EG2), 7 days (EG7), 15 days (EG15), 30 days (EG30), and 90 days (EG90) after injection. Control animals received saline solution only (CG). Lungs were dissected en bloc and fixed for light microscopy in buffered 4% paraformaldehyde solution. After 8 h of immersion in fixative, the organs were dehydrated through ethanol (70%, 80%, 90%, and 100%), clarified in xylene, and embedded in paraplast. Serials sections were cut (5 μm), stained with hematoxylin and eosin (H&E) or Perl's methods for histopathological analysis. Perl's method is a histochemical method that stains iron(III).

Light microscopy analysis of lung revealed that EG1 animals had MNP aggregates inside large blood vessels (Fig. 1B), with MNP clusters dispersed inside their lumen and within leukocyte cytoplasm. MNP clusters were also found inside capillaries and in the parenchyma cells. Two days after injection, aggregates composed of MNP were rarely found in large blood vessels and the clusters were mainly seen inside capillaries (Fig. 1C). MNP aggregates were also observed in the cytoplasm of parenchyma cells surrounding capillaries (Fig. 1D). Seven days after injections MNP clusters were mainly found inside parenchyma cells and some in the cytoplasm of macrophages within bronchiolar lumen (Fig. 2E). At day 15 post-treatment, MNP aggregates were still found within capillaries, but most of them were observed inside cells around blood vessels (Fig. 1F). Bronchiolar cells were also found with MNP inside their cytoplasm (see Fig. 2A). In all the previous observed time windows (up to 15 days), we found just a mild inflammation, mainly close to blood vessels and bronchioles, with vasodilatation, and small inflammatory cell groups. However, the lungs of EG30 mice presented an apparent increased number of inflammatory cells spread all over the parenchyma, when compared to the CG (compare Fig. 1A with Fig. 2E) or else compared to the DMSA-MF treated groups up to 15 days (EG1, EG2, EG7, and EG15). Leukocytes were mainly responsible for this increase. This phenomenon was more evident in areas close to large blood vessels and bronchioles (Fig. 2E). In these regions the occurrence of
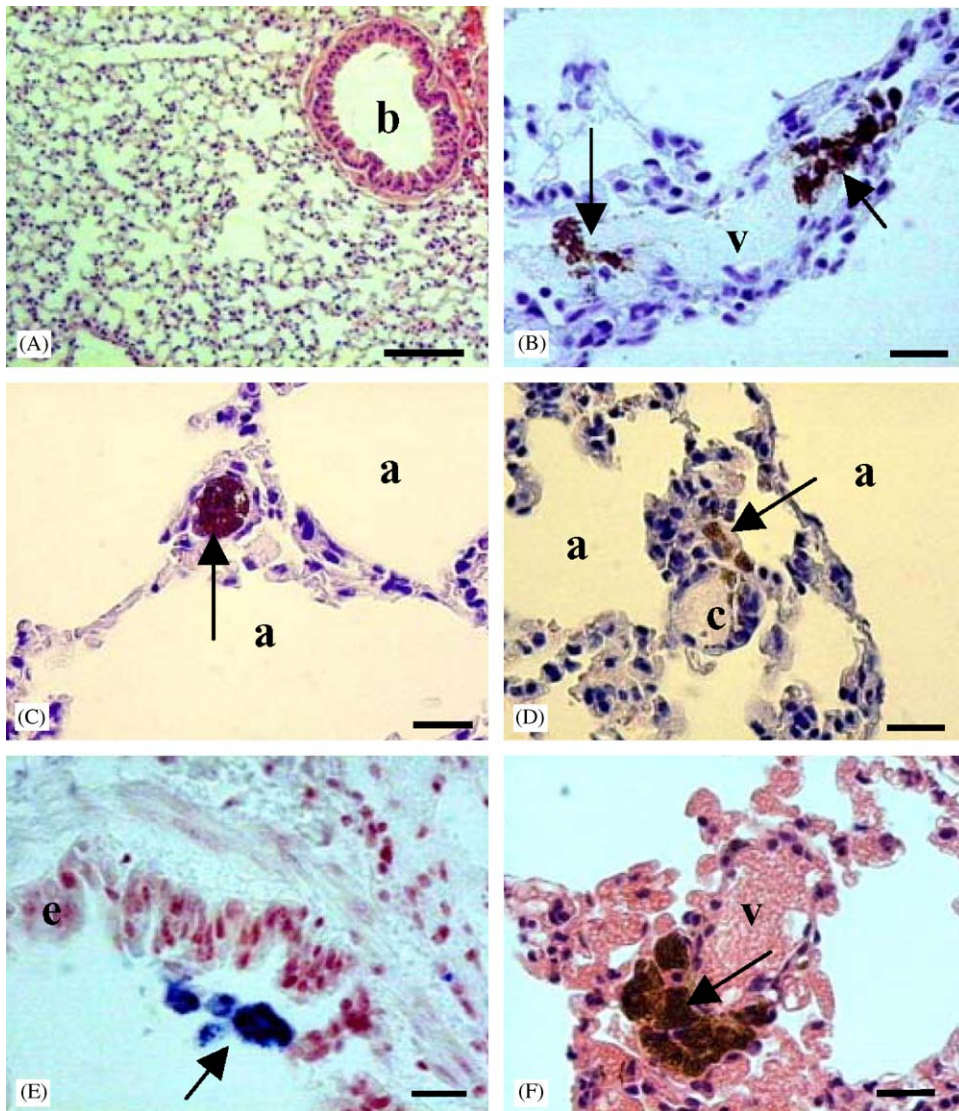
Fig. 1. Lung photomicrography of treated and control animals. (A) CG animal with normal histology. (B) EG1 animal, a venule (v) with clusters of MNPs in its lumen (arrow) and inside of leucocytes. (C) EG2 animal, a capillary lumen completely filled by MFs. (D) EG2 animal with MNPs within the lung parenchyma (outside the blood vessels). (E) EG7 animal, MNPs inside of bronchiolar epithelial cells (arrow). (F) EG7 animal, parenchyma cells with MNPs inside their cytoplasm (arrow) near a venule (v). [a = alveolus, b = bronchiole. (A–D) and (F), HE stain and (E), Perls stain]. Bars = (A) 100 μm; (B), (C), (D), and (E) 20 μm; (F) 10 μm.

groups of inflammatory cells was also constant (Fig. 2E), always associated with the presence of MNP inside some of them. We also found bronchiolar cells containing MNP. The presence of alveolar macrophages containing MNP was observed as well (Fig. 2B). EG90 animals presented lungs with a relative decrease in inflamma-

tory cells all over the parenchyma, even in areas close to the bronchioles and large vessels (see Fig. 2F). Cells containing MNP were found within the bronchiole lumen (Fig. 2C). The presence of alveolar macrophages containing MNP was uniform in animals of this group (Fig. 2D). It is worthy to note that the number of MNP clusters in
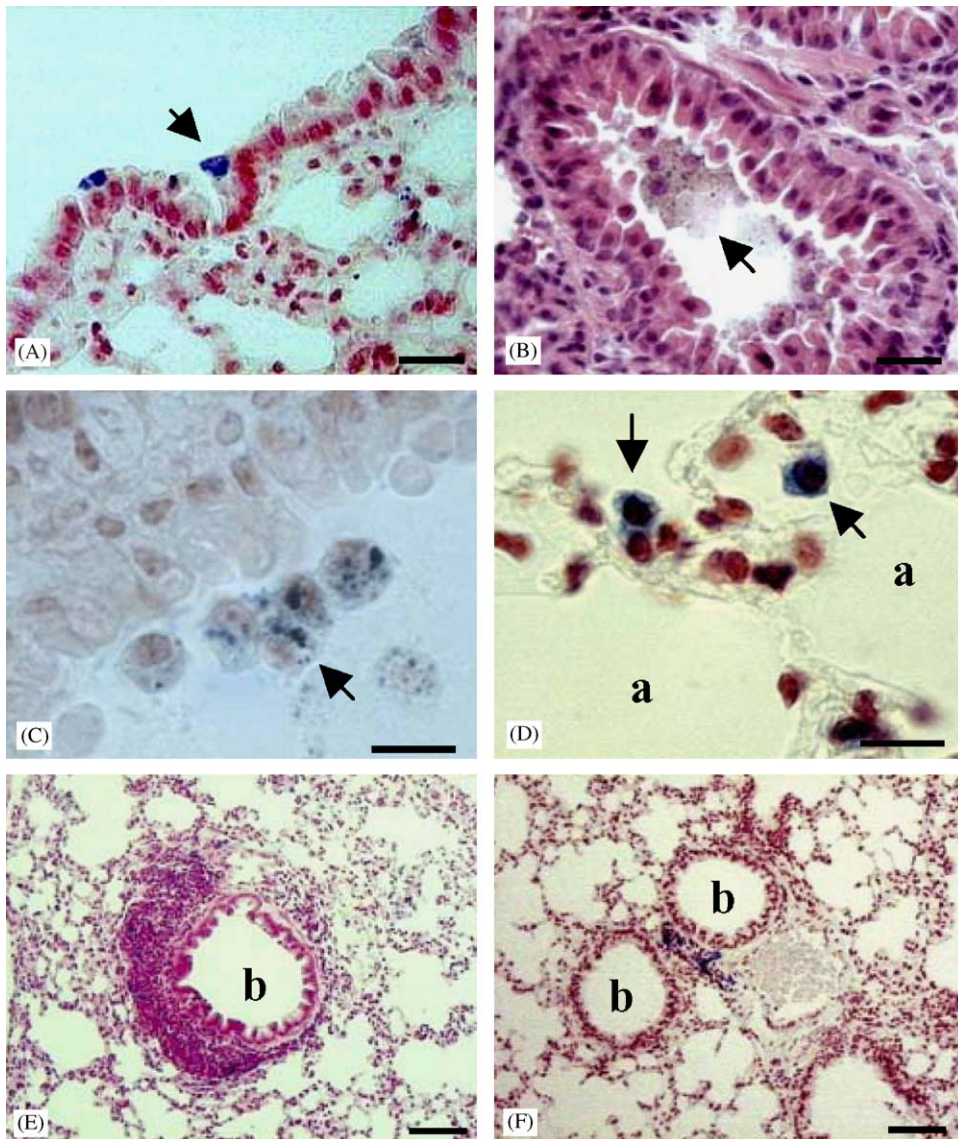
Fig. 2. Lung photomicrography of intravenously injected DMSA-MF animals. (A) EG15 animal. Observe cells containing MNPs attached to the epithelium of a bronchiole (arrow). (B) EG30 animal with macrophages inside a bronchiole lumen (arrow). Note the presence of MNPs inside their cytoplasm. (C) and (D) EG90 animal. Observe macrophages containing MNPs inside bronchiole and alveoli, respectively. (E) EG30 and (F) EG90 animals. Note the stronger inflammatory process close to the bronchiole (b) in (E) as compared to (F). [a = alveolus, b = bronchiole. (B) and (E), HE stain and (A), (C), (D), (F), Perls stain]. Bars = (A) and (B) 20 μm; (C) and (D) 10 μm; (E) and (F) 100 μm.

the lungs was observed to decrease as a function of time. Control animals had normal histological appearance, as shown in Fig. 1A.

At this point, we note that normal lung presents a morphological structure composed of conduct-ing portions, which includes the internal bronchi that undergo extensive branching to give rise to bronchioles, the latter representing the terminal part of the conducting passages. The respiratory portion is that in which gas exchange occurs. It

sequentially includes respiratory bronchioles (thin bronchioles that have sparse alveoli in their wall), alveolar ducts, and alveolar sacs whose wall is composed of only alveoli, and the alveoli itself. Alveoli are surrounded and separated from one another by a very thin connective tissue layer that contains numerous blood capillaries. The tissue between adjacent alveolar air space is called alveolar septum. It is worthy to note that an increase in inflammatory cells in the lung parenchyma reveals that an inflammatory process has taken place in that organ.

DMSA is a well-known molecule, often used as a chelating agent to remove heavy metals from an organism, and therefore considered as a biocompatible agent [8]. In part, this fact has been used to support its application as a biocompatible stabilizing coating for MNP, once DMSA alone shows low toxicity in various biological systems already studied [9–11]. Moreover, effector molecules can be bound to the DMSA structure. Quantitative iron determinations and microscopy studies with intravenous administration of ferrite particles, useful as contrast agents for magnetic resonance imaging, demonstrated that particles are partially degraded and that iron is cleared from the organism during a 3-month period without toxicity response [12]. Since most of the iron-based MNP and DMSA are not toxic, we would not expect to have a strong inflammatory process after DMSA-MF injection in the animals. However, the present study showed that in the time window of 1–30 days there was an enhancement of inflammatory cells in the lung parenchyma. Besides, such an increase was always correlated to the areas where the MNP were found. The specific cause to this inflammation was not determined in this study. Nevertheless, some hypothesis could be made. In spite of the fact that iron is the most abundant metal in the animal's body, when in excess, it can lead to several pathologies. The basic mechanism related with the iron capacity to induce pathologies in the organism is the increase of superoxide radicals. This increase involves the reduction of free oxygen induced by iron. Superoxide radicals can react with cellular membranes promoting its peroxidation, as well as peroxidation of proteins and nucleic acids. All these cellular

disturbances could lead different cell types to signal leukocytes and attract them to the location of injury. Another possibility to explain the increase of cell number in the lung parenchyma is that leukocytes, specifically monocytes and neutrophils, were directly activated by MNP presence. In this case these cells may incorporate MNP first and then produce pro-inflammatory mediators that attracted more leukocytes to the site. This scheme would enhance the inflammatory process. As a matter of fact, from 12 h after the treatment Chaves et al. [6] observed neutrophils and monocytes containing DMSA-MF nanoparticles within blood vessels, showing that the MNP directly activate leukocytes. This explanation does not exclude the possibility that peroxidation also occurs, since free oxygen is abundant in the lung and could react with iron. Indeed, further experiments are necessary to answer this question.

Morphological analysis showed that 90 days after DMSA-MF treatment, animals had their lung parenchyma with similar morphology as the CG, except for a few cells that contained MNP within the parenchyma and bronchioles lumen, alveolar macrophages with MNP aggregates in their cytoplasm, and few areas with small groups of inflammatory cells. In general, all animals of EG90 had a smaller amount of MNP in their lung than all the other treated groups. The reduction of MNP in the lung is more pronounced during the first two days, though it kept decreasing during the time window of our experiment. Results obtained by magnetic resonance showed that some MNP migrate from the lung to other organs such as liver and spleen, where they are incorporated by the local macrophages [5]. This could explain the accentuated decrease in MNP within the lungs. However, the presence of cells containing MNP inside bronchiole lumen could be a good pathway to take the nanoparticles out, since cilia of respiratory cells in the bronchioles and bronchi beat mucus with dust toward the esophagus. Catabolism of iron by the macrophages, including alveolar macrophages, is also a possibility to explain the decrease of MNP in the lung. These two possibilities are most likely to be the only ones responsible for the decrease of MNP in the late phase of this study. The decrease of inflammation

in the lung of EG30 animals along with the decrease in MNP in this organ strongly suggest that DMSA-MF nanoparticles are mainly responsible for this inflammatory process.

In conclusion, when DMSA-MF is injected intravenously, the MNP are preferentially driven towards the lung, where inflammation is induced. However, the inflammatory process reduces as a function of time, and it is not able to promote further pathologies such as pulmonary fibrosis.

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