Abstract. Background: Superparamagnetic nanoparticles are currently used as contrast agents for magnetic resonance imaging. These particles can also be used as drug carriers for local chemotherapy, called magnetic drug targeting. Using an external magnetic field, colloidal nanoparticles can be directed to a specific body compartment (i.e. tumor). Materials and Methods: After magnetic drug targeting in an experimental rabbit model with a VX2 squamous cell carcinoma, tumor tissue was extracted and embedded in paraffin for histology and X-ray imaging. Results: Distribution of magnetic nanoparticles was detected holistically with X-ray imaging and in detail using Prussian blue staining of histological cross sections. Conclusion: The biodistribution of magnetic nanoparticles can be visualized with X-ray imaging and histologically confirmed.

Superparamagnetic nanoparticles are used in medicine in vitro (1) and in vivo. For one of the first in vivo applications of magnetic particles in humans, Alksne et al. (2) performed experiments with carbon-coated iron combined with an external magnetic field for occluding intracranial aneurysms. The therapeutic efficacy was confirmed by X-ray investigations. Furthermore, superparamagnetic particles are used as contrast agents for magnetic resonance imaging (3). A new approach in local cancer therapy is magnetic drug targeting (MDT). Starch-coated magnetic nanoparticles labelled with the chemotherapeutic agent mitoxantrone were given intraarterially into the tumor-supplying artery of tumor-bearing rabbits (VX2 squamous cell carcinoma) and focused in the tumor region with an external magnetic field. With this delivery system, total tumor remission without negative side-effects could be accomplished using only 20% and 50% of the regular systemic chemotherapeutic dosage (4). Radioactive $^{59}$Fe-nanoparticles showed 114 times more activity in the tumor region after MDT compared to the control without a magnetic field (5). Furthermore, it was shown that with this system a high and specific enrichment of the bound chemotherapeutic agent in a desired body compartment (i.e. the tumor) was possible. HPLC-analysis of the chemotherapeutic agent after MDT revealed a 75-fold higher concentration of the administered dose in the tumor region compared to the regular systemic administration (5, 6). The aim of the present study was to non-invasively investigate the distribution of the particles with a common imaging technique (X-ray).

Materials and Methods

Tumor model. Experiments were performed on 8 tumor-bearing rabbits. A VX2 squamous cell carcinoma was placed at the medial portion of the hind limb. Two weeks after implantation, the tumors reached a size of about 2 cm$^3$ and the nanoparticles were administered intraarterially (i.a.) into the femoral artery.

Magnetic field. For the experiments, a powerful electromagnet with a magnetic field strength of 1.7 Tesla was used. The pole shoe of the magnet was focused over the tumor region during the infusion of the nanoparticles.

Magnetic nanoparticles. The nanoparticles in this study consisted of iron oxides covered by starch polymers for colloidal stabilization. The hydrodynamic diameter was approximately 250 nm.

Histology. Histological investigations were performed to visualize the distribution of the ferrofluids in the tumor tissue.

Light-microscopy. The excised tissue samples, taken 1 h after ferrofluid administration were fixed for 24 h in 4% PBS formaldehyde. After dehydration through increasing alcohol
concentrations and a xylol-step they were embedded in paraffin and slides of 5 μm-thick slices were made.

**Prussian blue stain.** The Prussian blue stain provides the histochemical evidence of the presence of trivalent iron according to the following reaction: trivalent iron is visible as blue pigment while the remaining structures appear red in color.

**X-ray-imaging.** For the visualisation of the particle distribution, paraffin-embedded tumor tissue was investigated with x-rays in 221 pictures around 360 degrees. The x-ray machine (Institute of Fluid Mechanics, Technical University of Dresden, Germany) consisted of a passive-cooled integral X-ray tube with an acceleration voltage of up to 50 kV at an electron emission current of 1 mA. The limit of resolution with these parameters was 40 μm. X-ray visualisation was performed with a thermoelectrically-cooled slow-scan CCD camera (1024 x 1024 pixels).

**Results**

X-ray-tomography images show that the whole vascular system of the tumor can be reached by the nanoparticles with the influence of a focused external magnetic field (Figure 1) and this was confirmed with the corresponding macroscopic and histological examination of the tumor (Figures 2, 3). Histological cross-sections show the nanoparticles after intraarterial application in the vascular system of the tumor (Figure 3a). Sporadic occurrence of ferrofluids in macrophages was detected (Figure 3b).

**Discussion**

For 30 years, several approaches and carrier systems for the site-specific transport of therapeutics were developed in medicine (7, 8). Magnetic albumin microspheres containing chemotherapeutic agent (Adriamycin) were injected into the ventral caudal artery of rats in the absence and presence of an external magnetic field. These studies showed that the application of the magnet increased the targeting efficacy of the carrier by a factor of 6, as well as that of the drug exposure (9). In our experiments, the particles consist of magnetite (γ-Fe₃O₄) coated with phosphated starch molecules of defined polymer size. For magnetic drug targeting, the chemotherapeutic agent mitoxantrone has been reversibly bound to the polymer. Previous studies revealed that in HeLa cell culture, particles which were targeted by a magnetic force placed under the cell culture well for one hour were internalised by an endocytotic pathway (10). Kohler et al. (11) used particles with the same core but a different coating. The particles were coated with a self-assembled monolayer of (3-aminopropyl) trimethylsiloxane conjugated by amidation with the cytostatic agent methotrexate, resulting in a covalent drug bond on the surface. Despite the different particle characteristics, both described the same manner of particle internalisation in the cell by endocytosis. This leads to the indication that this is a general pathway for particle internalisation in HeLa cell culture. In contrast to the in vitro observations in tumor cell culture (10), the present in vivo data show a different pattern. After intraarterial infusion of magnetic nanoparticles in tumor-bearing rabbits, the ferrofluids are accumulated mainly in the vascular system of the tumor. Histological cross-sections of the tumor tissue showed the nanoparticles in the tumor vessels. Infrequently, nanoparticles were detected in the intracellular space of the cells. The proof of iron presence was seen in macrophages and endothelial cells of the tumor tissue (5) (Figure 3a). X-ray images confirm the ferrofluid delivery shown by histology. The vessel system of the examined tissue samples are detectable by x-ray examination. Due to the radiographic technique of consecutive imaging over 360° of the whole tumor, it is possible to reconstitute a three-dimensional picture of the tumor and the distribution of the particles.

An important parameter for the effectiveness of a local chemotherapy is the resulting distribution of the respective therapeutic agent in the tumor region (12, 13). Using drug loaded nanoparticles, the biodistribution is usually studied via histological cross-sections of the examined tissue samples, a technique which provides only very local information about the overall distribution. X-ray microtomography is a promising tool for gaining
information about the overall distribution of the particles in the tumor region. It is a strong and non-invasive procedure and could also be very useful to control the effectiveness of magnetic drug targeting in vivo.

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References


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