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Christoph Alexiou · Roswitha J. Schmid
Roland Jurgons · Marcus Kremer · Gerhard Wanner
Christian Bergemann · Ernst Huenges
Thomas Nawroth · Wolfgang Arnold
Fritz G. Parak

Targeting cancer cells: magnetic nanoparticles as drug carriers

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Abstract Magnetic drug targeting employing nanoparticles as carriers is a promising cancer treatment avoiding side effects of conventional chemotherapy. We used iron oxide nanoparticles covered by starch derivatives with phosphate groups which bound mitoxantrone as chemotherapeutikum. In this letter we show that a strong magnetic field gradient at the tumour location accumulates the nanoparticles. Electron microscope investigations show that the ferrofluids can be enriched in tumour tissue and tumour cells.

Keywords Cell biophysics · Biological tissue · Microscopy

C. Alexiou · R. J. Schmid · R. Jurgons
Klinik und Poliklinik für Hals- Nasen- Ohren- kranke,
Universität Erlangen – Nürnberg, Erlangen, Germany
E-mail: C.Alexiou@web.de

M. Kremer
Institut für Pathologie, Klinikum rechts der Isar,
TUM, Munich, Germany
E-mail: Kremer@lrz.tu-muenchen.de

G. Wanner
Fakultät für Biologie I der LMU, LMU, Munich, Germany
E-mail: wanner@botanik.biologie.uni-muenchen.de

C. Bergemann
chemicell, Berlin, Germany
E-mail: Chemicell@aol.com

E. Huenges · T. Nawroth · F. G. Parak (✉)
Physik - Department E17, TUM, Munich, Germany
E-mail: Fritz.Parak@ph.tum.de

W. Arnold
Hals- Nasen- Ohrenklinik und Polyklinik rechts der Isar,
TUM, Munich, Germany
E-mail: w.arnold@lrz.tu-muenchen.de

Introduction

In cancer therapy a major difficulty is to destroy tumour cells without harming the normal tissue. Radiotherapy attempts to focus irradiation on the tumour, but nevertheless damages healthy tissue which cannot always be protected in the desired way. Chemotherapy involves the use of powerful drugs which are unfortunately rather unspecific, yielding unwanted side effects because of their toxicity. Sufficiently large tumour-toxic doses of the chemotherapeutics are often not tolerated by the patient. The general problem "Drugs on Target" was brought into focus by Langer (2001) several years ago.

In the last years we worked on a method to concentrate a high amount of toxic drugs within cancer tissue, with only a small amount of drug contaminating the normal tissue. we used magnetic nanoparticles as drug carriers that were injected into the blood circulation. They were retained in the region of the tumour by a strong inhomogeneous magnetic field. We used ferrofluids which are commercially available (Bergemann 1998). The iron oxide core was covered by a layer of starch polymers which makes the ferrofluids tolerable to the body, as shown in an earlier "Phase I" clinical trial (Lübbe et al. 1996a, b). The drug mitoxantrone was bound to phosphate groups of starch derivatives. A successful application of the magnetic drug targeting was already demonstrated by experiments on a number of female New Zealand White Rabbits in which VX-2 squamous cell carcinoma was placed at the medial portion of the left hind limb. Typically 35 days after the treatment the tumour disappeared completely (Alexiou et al. 2000). No metastases or negative side effects were observed. The time-dependent distribution of the ferrofluids within the body was already investigated by labelling the nanoparticles with the radioactive isotope ^{59}Fe (Alexiou et al. 2003). The amount of ferrofluids in the organs differs drastically. Only with an externally applied magnetic field, a large amount of the

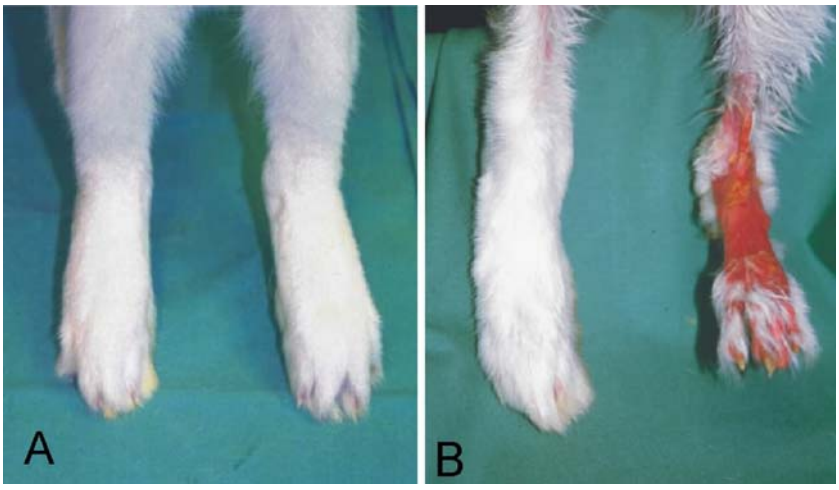

Fig. 1 Rabbits with VX-2 squamous cell carcinoma placed at the medial portion of the hind limb (*left side*). **a** Forty days after treatment by magnetic drug targeting with 20% of the systemic dose of mitoxantrone. **b** Forty days after treatment with 75% of the regular systemic dose of mitoxantrone (intra-arterial application)

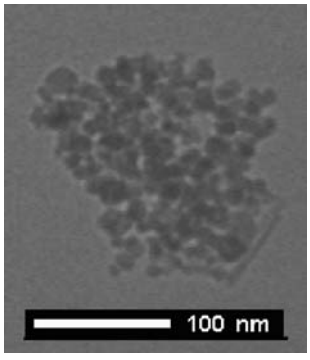

Fig. 2 Electron microscopic picture of the magnetic carrier

nanoparticles was kept in the region of the tumour, emphasizing the importance of application of a magnetic field. In addition to the systematic investigations published by Alexiou et al. (2000), Fig. 1 demonstrates the principal capacity of this method. Both rabbits have been treated with mitoxantrone. The amount of the applied mitoxantrone was calculated according to the usual "systemic dose" (b). One rabbit (Fig. 1a) was treated using magnetic drug targeting. Only 20% of the systemic dose was necessary to achieve complete remission of the tumour after 16 days. We did not register any negative side effects. The second rabbit (Fig. 1b) was treated with conventional local application (femoral artery) of mitoxantrone. Seventy-five per cent of the systemic dose was necessary to obtain a complete remission of the tumour. However, the side effects of this treatment are clearly visible: atrophy of the left hind limb, alopecia, ulcerations and weeping inflammation of the skin.

While the former publications focused on the medical applicability of the method, we show in this letter that the ferrofluids cannot only concentrate in the cancer tissue but also penetrate into the tumour cells.

Material and methods

In order to transport the bulk amount of drugs into the tumour before the carrier is destroyed by the liver, we used an intra-arterial application. A catheter was inserted into the artery and brought as close as possible to the tumour tissue. A strong inhomogeneous magnetic field was applied over the tumour for 1 h. The field gradient connected with the inhomogeneous magnetic field attracts paramagnetic material.

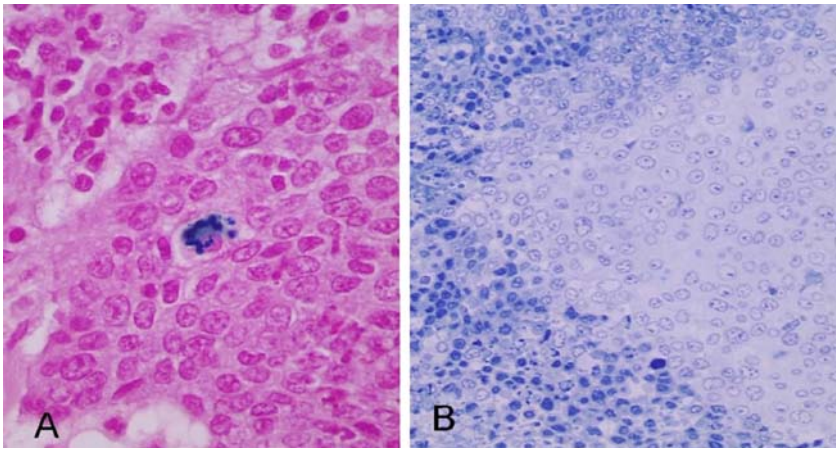

Fig. 3 Light microscopy microphotos. **a** Picture of the tumour tissue, with large tumour cell nuclei surrounded by non-tumourous small lymphocytes; positive iron staining of incorporated ferrofluids in the tumour tissue (*blue*). **b** Serial section of the same tissue as in (**a**), ultra-thin cut ready for EM investigation

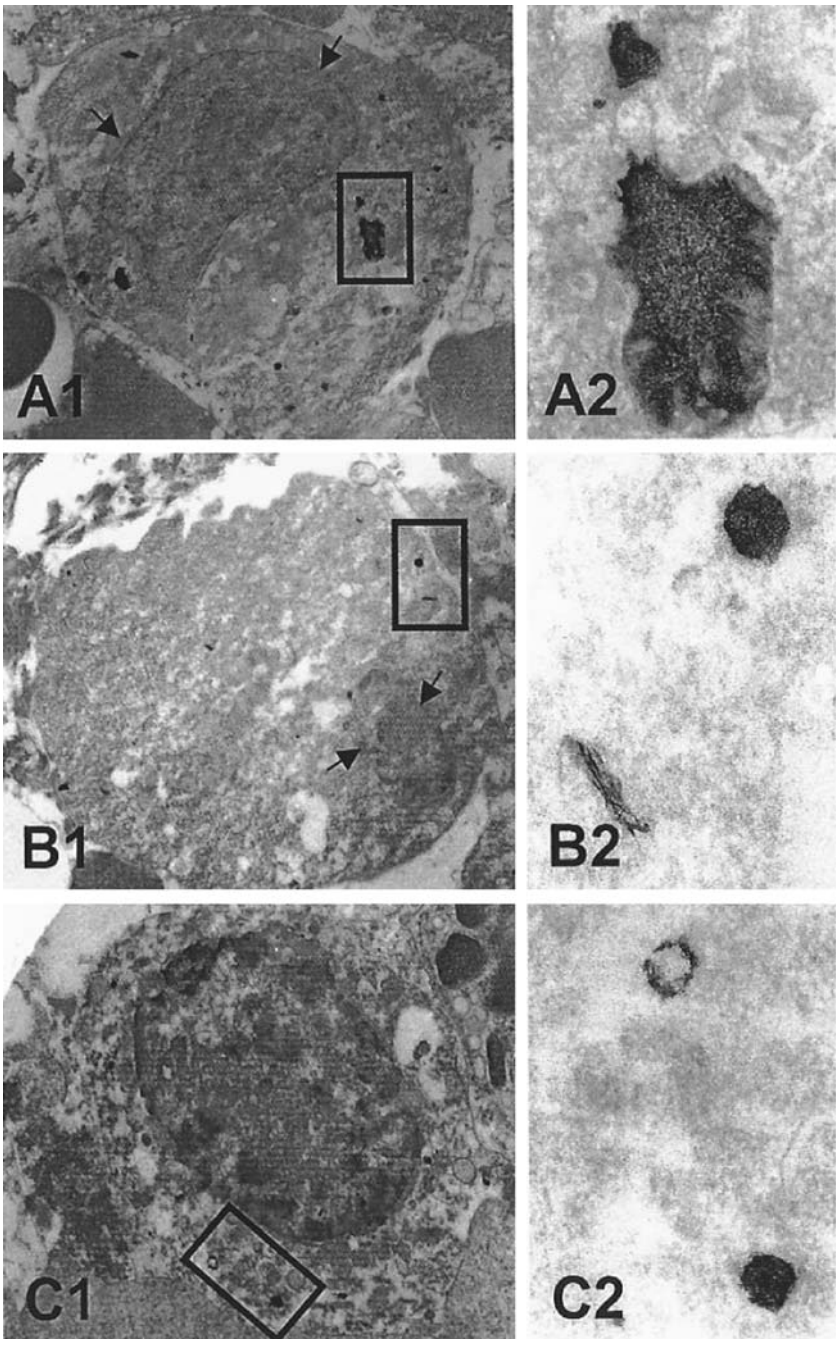

Fig. 4 The electron microscopic image of different tumour cells of squamous cell differentiation, with clearly identifiable nuclei (*arrows*) with irregular nuclear shape and faintly visible nucleoli (*A1*, *B1*) (original magnification ×9.400). *C1* Photograph of a peritumoral macrophage (original magnification ×9.400). *A2*, *B2* and *C2* Higher magnification of the marked fields (*squares*) (original magnification ×52.400, ×82.600, ×52.400)

For the magnetic drug targeting the magnetic nanoparticles are of central importance. Figure 2 shows the picture of a typical magnetic transport vesicle taken by an electron microscope. It is an aggregation of several super paramagnetic nanoparticles of about 5 nm diameter composed of iron oxides. These particles cluster to larger units of about 100 nm in size, which are strongly attracted in a magnetic field gradient. While the magnetic moment of 5 nm particles is too weak, a substantially larger aggregate may cause embolism. The core is covered by a layer of starch polymers. Many of the outer groups of the surrounding polymer can be activated to bind phosphate groups, which in turn bind the drug. In our experiments, one particle could carry at least 10^5 molecules of mitoxantrone (a), used as chemotherapeutic agent. The chemo-adsorptive binding is strong enough to be stable during the time of transport. Desorption occurs with a half-life of about 30 min and releases the drug in its active form.

Results and discussion

In order to investigate the enrichment of ferrofluids in the cancer tissue, we used the light and the electron microscope. For the treatment of the rabbit a magnetic

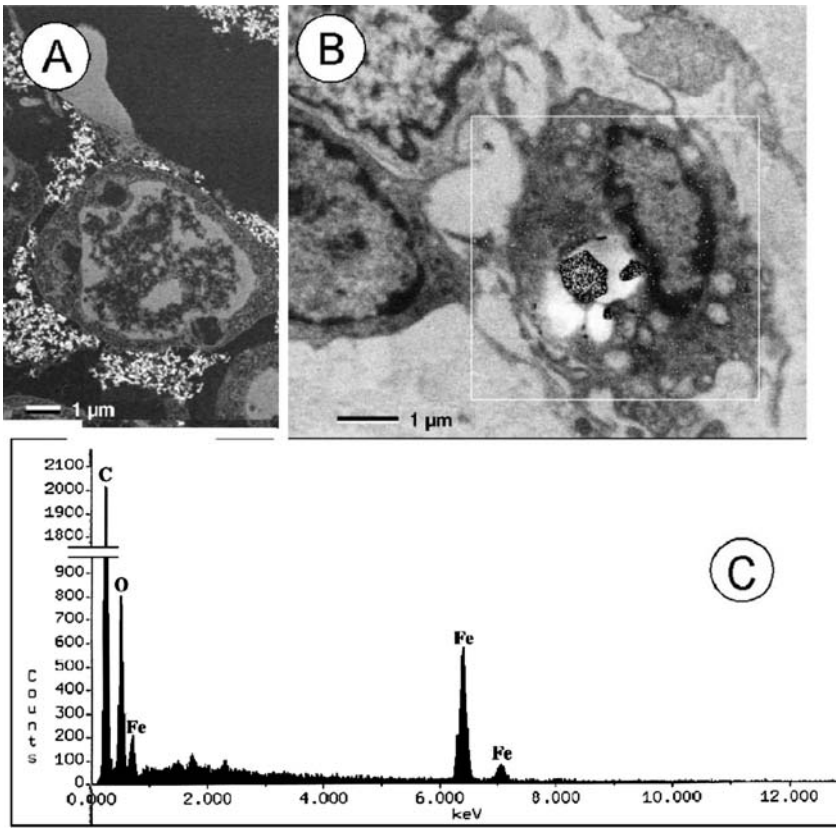

Fig. 5 **a** Scanning electron micrographs of ultra-thin sections (thickness approx. 70–100 nm) of tumour tissue after magnetic drug targeting. **b** Areas of interest (*framed*) mapped using the Fe K_α -signal (tumour cell). **c** Fe-EDX spectrum of ferrofluid aggregates within tumour cells

field with 1.7 T in its maximum was applied (diameter 2 cm adapted to the tumour size). The rabbit was sacrificed 1.5 h after the application of the drug. Tumours were formalin-fixed and paraffin-embedded. Serial sections showed partial necrotic tumour tissue with surrounding non-tumourous lymphocytes (Fig. 3). To accentuate high concentration of ferrofluids, a Berlin blue staining of the histological specimen was added. To ensure the correct position for the subsequently performed electron microscopy (EM), areas with a positive iron staining were identified and marked on the paraffin block. These areas were extracted and investigated by electron microscopy. Utmost care was taken to prepare the most parallel section to the iron-stained area. Figure 3b shows a semi-thin cut of the same tumour as shown in Fig. 3a ready for the EM. With this sample higher magnification of single tumour cells and macrophages by EM were obtained (Fig. 4). The electron microscopic image shows different tumour cells of squamous cell differentiation, with clearly identifiable nuclei (arrows) with irregular nuclear shape and faintly visible nucleoli (Fig. 4A1, B1). The cytoplasmic structures are poorly visible due to the formalin fixation and paraffin embedding procedure. Note numerous intracytoplasmic irregular granules, representing ferrofluids, which were exclusively found in the cytoplasm. (original magnification ×9.400). Figure 4C1 is a photograph of a peritumoural macrophage, which shows a large nucleolus with homogenous chromatin and several cytoplasmatic vacuoles and granules (original magnification ×9.400). Higher magnification of the marked fields (rectangles) reveals the irregular structure of the granules, consisting of needle-shaped material (Fig. 4A2, B2 and C2) (original magnification ×52.400, ×82.600, ×52.400). The irregular cytoplasmatic granules, representing ferrofluids are clearly visible within the cells.

To firmly demonstrate that tumour cells have really incorporated ferrofluids we used an additional technique. With high-resolution scanning electron microscopy it is possible to detect Fe and its distribution in cells (Fig. 5). Ultra-thin sections of tumour tissue penetrated with ferrofluids were observed with a high-resolution backscattered electron (BSE) detector of the YAG type. Elements of higher atomic number give bright signal spots (Fig. 5a). To prove that the BSE signal derives from iron, EDX mapping of the Fe-K_α line has been performed at regions of interest (Fig. 5b, c). The electron micrographs demonstrate clearly that ferrofluids are not only observed between tumour cells (Fig. 5a) but are also found in considerable amounts within the tumour cells (Fig. 5b).

What are the prospects for applying magnetic drug targeting to humans? The starch layer makes the magnetic nanoparticles biocompatible and able to react with various end standing functional groups for binding other molecules (Lübbe et al.1996b). The large amount of iron oxide of the ferrofluids injected to a patient is not

toxic. It is mainly metabolized in the hepato-renal system and also used in the synthesis of haemoglobin (Weissleder et al. 1989). Magnetic drug targeting offers an opportunity to treat malignant tumours locoregionally without systemic toxicity. Furthermore, magnetic nanoparticles could be used as a carrier system for a variety of anticancer agents, e.g. radio nuclides, cancer-specific antibodies and genes. A modification of the nanoparticles by adding ^{10}B into the core may cause a revival of the Boron Neutron Capture Therapy.

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Notes

(a) More precisely, Mitoxantronehydrochloride, commercially Novantron from Lederle, Wolfratshausen, Germany, inhibits the DNA and the RNA syntheses by intercalating in the DNA-causing funicular fractures.
(b) The systemic dose for mitoxantrone equals 10 mg/m^2 body surface. Tables are available to determine this surface.

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