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In vitro and in vivo investigations of targeted chemotherapy with magnetic nanoparticles

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Abstract

Magnetic drug targeting is a local drug delivery system. Electromicroscopic pictures document the ferrofluid enrichment in the intracellular space in vitro. In vivo experiments were performed in VX2 tumor-bearing rabbits using magnetic nanoparticles bound to mitoxantrone. High-pressure liquid chromatography (HPLC) analyses after magnetic drug targeting showed an increasing concentration of the chemotherapeutic agent in the tumor region compared to regular systemic chemotherapy.

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Keywords: Magnetic drug targeting; Mitoxantrone; Nanoparticles; Cancer treatment; Chemotherapy; VX2 Tumor; Ferrofluids; HeLa cells

1. Introduction

In cancer treatment the use of chemotherapeutic agents is frequently associated with severe negative side effects, due to the systemic distribution. Therefore, during the last 20 years different approaches of regional drug applications were developed to obtain an increasing effectiveness of the applied drugs in the respective tumor and to protect healthy tissue [1–5].

Magnetic Drug Targeting (MDT) is a drug delivery system that can be used in locoregional cancer treatment. Starch-coated magnetic nanoparticles (ferrofluids) are ionically and reversibly bound to a chemotherapeutic agent. After intraarterial injection they are attracted to the tumor

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region by an external magnetic field. The attraction of the magnetic nanoparticles is dependent on the magnetic field strength [6].The chemotherapeutic agent desorbs from the magnetic carrier in a time-dependent manner [7]. After treatment, the ferrite nanoparticles are enriched and metabolized in the spleen and liver [7].

In the present study, we investigated the distribution of the magnetic carrier at the cellular level in vitro and that of the chemotherapeutic agent (i.e. mitoxantrone) in vivo.

2. Materials and methods

For in vitro experiments, HeLa-cells were incubated in a ferrofluid-containing medium (30 μg iron oxide/ml) and placed over an external magnetic field (0.4 T, permanent magnet) in six-well plates for 60 min at room temperature. Furthermore, HeLa-cells were incubated at 4 °C to study if endocytosis is the incorporation mechanism of ferrofluids. At a temperature of 4 °C endocytosis is nearly completely suppressed [8]. Then the HeLa-cells were prepared and embedded for electron microscopy.

2.1. Preparation of HeLa-cells for electron microscopy

After incubating with ferrofluid-containing medium, the HeLa-cells were solved with EDTA-trypsin. After centrifugation the cell centrifugate was embedded in 2% agar (Merck, Darmstadt, Germany) and fixed in 1% glutaraldehyde/PBS. Then the cells were dehydrated with ethanol (Merck) and acetone and embedded in Epon (Roth, Karlsruhe, Germany).

For in vivo experiments, we implanted VX2 squamous-cell carcinomas on the hind limb of rabbits in the supplying field of the femoral artery. We injected the ferrofluids (Chemicell, Berlin Germany) ionically bound to the chemotherapeutic agent mitoxantrone (Novantron, Wyeth Pharma, Germany) into the femoral artery. The ferrofluids used in our experiments consisted of superparamagnetic magnetite nanoparticles with an average size of 100 to 200 nm and a crosslinked starch matrix with terminal cation-exchange phosphate groups for reversible binding of positively charged drugs [6–8].

To document the distribution of mitoxantrone with and without MDT, tissue samples of different body compartments were analyzed by high-pressure liquid chromatography (HPLC) after the following different application modes:

- Intraarterial application of 5 mg/m^2 body surface ff-mitoxantrone (50% of the regular systemic dose, with external magnetic field, $n = 1$).
- Intraarterial application of 2 mg/m^2 body surface ff-mitoxantrone (20% of the regular systemic dose, with external magnetic field, $n = 1$).
- Intravenous application of 10 mg/m^2 body surface mitoxantrone (regular systemic dose, $n = 1$).

2.2. Preparation of tissue samples for HPLC analysis

Tissue samples were removed under sterile conditions and subsequently weighed. The weighing of the entire organ/tissue ratio ensured an exact conversion of the analysis, which indicated the concentration of mitoxantrone (in micrograms) per gram of examined material. Consequently, all of the samples were divided into two parts and weighed again, permitting us to repeat the analysis on each excised sample, thereby confirming our results. Thus, all of the samples were immediately separated from one another and frozen in sterile containers at −70 °C until the analyses were performed. The homogenized organs were extracted with a methanol–HCL solution. The remaining homogenate was poured into an Eppendorf tube via a pipette. The sediment was washed twice more with the methanol–HCL solution and combined with that in the Eppendorf tube. The concentrate was placed in a Speed-Vac. The sample was then resuspended into a solution of 0.01 M ammonium dihydrogen phosphate buffer/iso-propanol (7:3). The filtration occurred over a GHG Nanosep. Centr. Device (45 μm). The centrifugate was poured into a vial using a pipette for the HPLC analysis. Quantitative determination of mitoxantrone was carried out using HPLC

(Fa. SHIMADZU) with a MERCK LiChrosphere 60 RP-select B separating column and the same pre-column. A mixture of 16 vol% acetonitrile, 1.5 vol% triethylamine and 82.5 vol% of 0.01 M ammonium dihydrogen phosphate buffer was used as the eluate. The detection was done at 660 nm. The flow rate was 1.2 ml/min. These analyses were performed by G.O.T. GmbH, Berlin, Germany.

3. Results

The electromicroscopic investigations of the HeLa-cells revealed that after incubation with ferrofluid-containing medium at room temperature for 60 min and focusing by an external magnetic field, the ferrofluids disseminated throughout the intracellular space (Figs. 1–3). HeLa-cells incubated at 4 °C did not show any intracellular uptake of ferrofluids after MDT.

HPLC analyses of tissue samples of different body compartments show after intraarterial injection of ferrofluids bound to mitoxantrone in a dosage of 50% and 20% of the regular systemic dose (10 mg/m^2) and with MDT an amount of

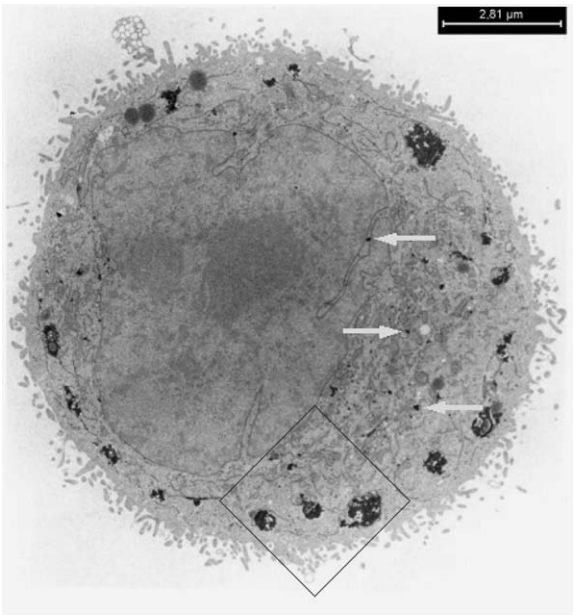


Fig. 1. Electron microscopic picture of a HeLa-cell after ferrofluid incubation under the influence of an external magnetic field (0.4 T). The ferrofluids are visible as black spots in the peripheral area (frame) and disseminated in the intracellular space (marked with arrows).

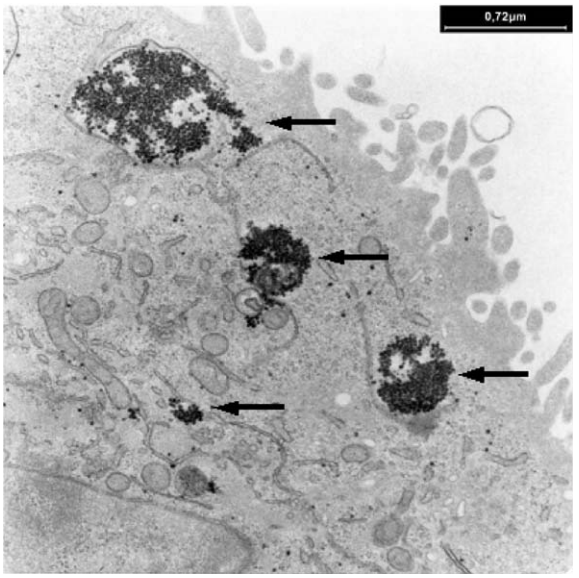


Fig. 2. Magnification of the frame shown in Fig. 1—ferrofluids in the peripheral area of a HeLa-cell (marked with arrows).

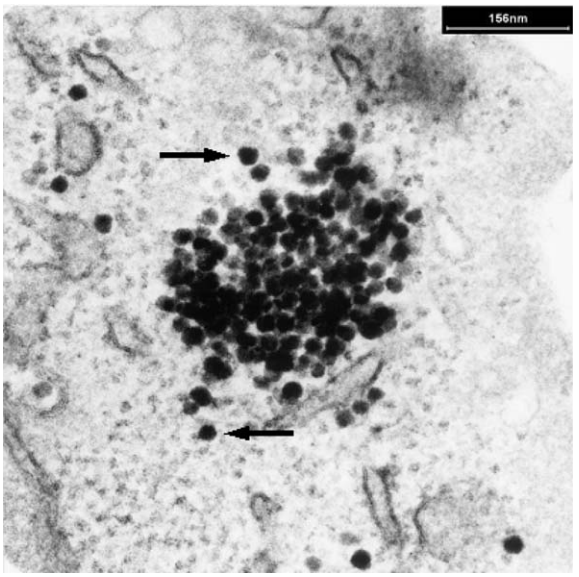
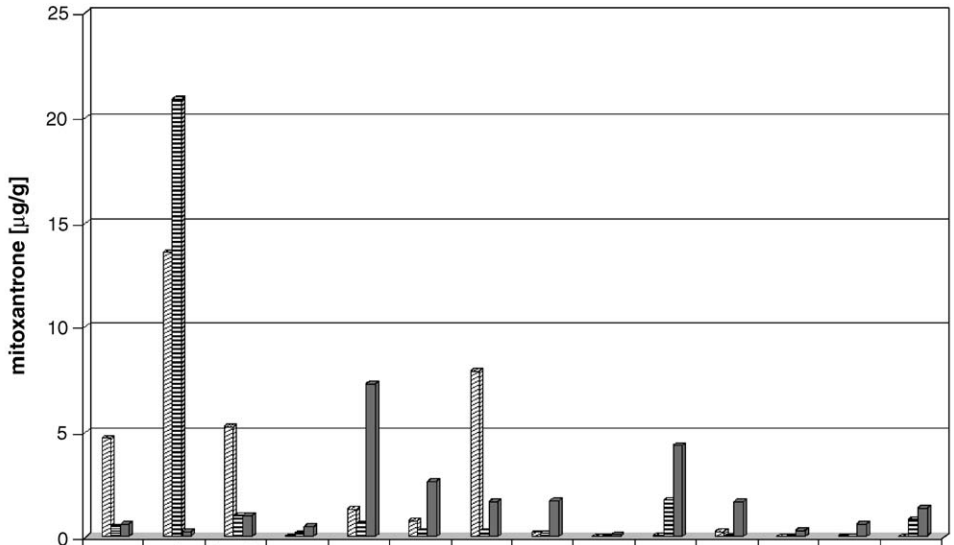


Fig. 3. Magnetic particles in the intracellular space. Single particles (marked with arrows) are surrounded by starch polymers (not visible in the electromicroscopic picture).



	tumor	peri-tumoral region	lymph node ts.	liver	kidney	spleen	lung	heart	brain	bile/g all bladder	lymph node opp.	tissue (muscle) opp.	bone marrow ts.	bone marrow opp.
▨	4.71	13.56	5.25	0.04	1.3	0.73	7.89	0.22	0.02	0.08	0.27	0	0.04	0
▤	0.5	20.93	1.05	0.22	0.62	0.28	0.26	0.2	0	1.75	0	0	0	0.82
■	0.58	0.25	1.03	0.47	7.29	2.65	1.66	1.75	0.12	4.38	1.69	0.32	0.62	1.39

Fig. 4. Results of HPLC analyses of different body compartments in dependence on application modes: ▨—intraarterial application of 5 mg/m^2 ff-mitoxantrone with external magnetic field (MDT, $n = 1$); ▤—intraarterial application of 2 mg/m^2 ff-mitoxantrone with external magnetic field (MDT, $n = 1$) and ■—intravenous application of 10 mg/m^2 mitoxantrone (regular systemic dose, $n = 1$).

4.71 μg/g tissue and 0.5 μg/g tissue, respectively, in the tumor vs. 0.58 μg/g tissue after intravenous application of the regular systemic dose. In the peritumoral region ($\leqslant 1$ cm) after MDT mitoxantrone concentrations of 13.56 and 20.93 μg/g tissue, respectively, were measured. After systemic application of the total regular dose of 10 mg/m^2 the amount in the peritumoral region was 0.25 μg/g tissue (Fig. 4).

4. Discussion

Systemic chemotherapy (intravenous application) usually causes severe negative side effects. These unwanted side effects are leucocytopenia, alopecia, ulcers or nausea due to the toxic effects of the chemotherapeutic agent on healthy body compartments. Because of that the application of the regular systemic chemotherapy is limited by the dose, which is tolerated by the patient and not by the effective dose, which is necessary to treat the tumor sufficiently. Local chemotherapy tries to reduce these negative side effects by reducing the total applied dose and concentrating the anticancer agent in the tumor region. Locoregional and intraarterial chemotherapy can avoid the first pass effect and permits a higher local concentration [9]. To enhance local concentration of the chemotherapeutic agent in the tumor, Widder et al. [10] injected intraarterially magnetic albumin microspheres with an incorporated chemotherapeutic agent and tried to focus this compound with an external magnetic field in tumor bearing rats [10]. Due to their size, however, albumin microspheres often tend to embolize vessels and capillaries [11]. In the present study, we used magnetic nanoparticles with a hydrodynamic diameter of approximately 100 nm, which are surrounded by starch polymers to avoid agglomeration and to enable the biocompatibility. In a time-dependent manner the chemotherapeutic agent desorbs from the particles and can freely act in the tumor region after focusing with an

external magnetic field. With this system, called magnetic drug targeting (MDT), we achieved complete tumor remissions with less chemotherapeutic dose (20% and 50% of the regular systemic dose) and without any negative side effects [7,12]. MDT delivers the nanoparticles into the intracellular space of tumor cells (Fig. 1–3) close to the nucleus, which is the weak point of chemotherapy. Furthermore, high-pressure liquid chromatography (HPLC) analysis of mitoxantrone in different body compartments after MDT with an amount of 50% of the regular systemic dose showed in the tumor region a concentration of 18.27 μg mitoxantrone per gram tissue compared to 0.83 μg mitoxantrone per gram tissue after given the regular dose systemically. The concentration of mitoxantrone after MDT with 20% of the regular systemic dose revealed 0.5 μg/g mitoxantrone in the tumor and 20.93 μg/g in the peritumoral area, respectively. Histological investigations showed that this tumor had a poor vascular supply compared to the peritumoral area and gives the evidence that MDT is dependent on the blood supply of the target area. This high concentration of mitoxantrone in the tumor tissue and the intracellular distribution are possible reasons for the high effectiveness of intraarterial MDT.

Acknowledgements

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References

[1] J.M. Collins, J. Clin. Oncol. 2 (1984) 498.
[2] P.K. Gupta, C.T. Hung, Life Sci 46 (1990) 471.
[3] P.K. Gupta, C.T. Hung, J. Microencap. 7 (1990) 85.
[4] P.K. Gupta, J. Pharm. Sci. 79 (1990) 949.
[5] V.P. Torchilin, Eur. J. Pharm. Sci. 11 (2000) 81.
[6] Ch. Alexiou, A. Schmidt, P. Hulin, et al., J. Magn. Magn. Mater. 252 (2002) 363.
[7] Ch. Alexiou, W. Arnold, R. Klein, et al., Cancer Res. 60 (2000) 6641.
[8] Ch. Alexiou, R. Jurgons, R. Schmid, et al., Eur. Arch. Oto-Rhino-Laryngology 261 (2004) 148.
[9] J. Preiss, K.H. Link, E. Schmoll, Regionale Chemotherapie, in: H.J. Schmoll, K. Höffken, K. Possinger (Eds.), Kompendium Internistische Onkologie, part 1, Springer, Berlin, Heidelberg, New York, 1999.
[10] K.J. Widder, R.M. Morris, G.A. Poore, et al., Eur. Cancer Clin. Oncol. 19 (1983) 135.
[11] S.C. Goodwin, C.A. Bittner, C.L. Peterson, et al., Toxicol. Sci. 60 (2001) 177.
[12] Ch. Alexiou, W. Arnold, P. Hulin, et al., J. Magn. Magn. Mater. 225 (2001) 187.