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Mitoxantrone-Iron Oxide Biodistribution in Blood, Tumor, Spleen, and Liver—Magnetic Nanoparticles in Cancer Treatment

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Background. **Magnetic drug targeting is a new treatment principle for tumors using cytostatics coupled to ferrogmagnetic nanoparticles and extracorporeal magnets. Higher concentrations in tumor tissue with lower systemic concentrations and without damage of healthy organs should be achieved.**

Materials and Methods. **$n = 42$ adult Wag/Rij rats were transfected with rhabdomyosarcoma R_1H in their right gastrocnemius muscle. In the biodistribution trial ($n = 36$) concentrations of mitoxantrone-iron oxide with and without an extracorporeal 0.6 tesla magnet and regular mitoxantrone were measured in plasma and tumor tissue for one- and two-dose administration. In the plasma iron trial ($n = 6$) iron concentrations were measured in plasma before, during, and up to 30 min after drug administration. Seven days after the trial liver, spleen and tumor samples were obtained and histologically assessed.**

Results. **Mitoxantrone iron-oxide concentration in plasma was significantly ($P < 0.05$) lower when a magnet was placed over the tumor area and as low as uncoupled mitoxantrone. Mitoxantrone concentration in tumor tissue was always significantly higher with magnetic drug targeting when compared with uncoupled mitoxantrone. Two doses resulted in drug accumulation in tumor tissue. Plasma iron concentrations rose when the drug was first administered. Plasma levels fell below the starting level with a magnet applied. A rebound phenomenon with rising iron concentrations was observed after the magnet was removed. Tumors showed fresh necrosis and liver and spleen had detectable iron depositions but no necrosis 7 d after treatment. No allergies or toxic reactions were observed.**

Conclusions. **We showed that magnetic drug targeting achieves higher concentrations of cytostatics in tumor tissue compared with blood. During magnetic drug targeting, iron particles are quickly sluiced and kept in the tumor area. Organs of the reticuloendothelial system are not affected by cytostatic damage.**

Key Words: **iron oxides; magnet; magnetic drug targeting; nanoparticles; sarcoma; reticuloendothelial system.**

INTRODUCTION

Surgical treatment of tumors has undergone rapid developments and included several new treatment principles over the last decade. Adjuvant and neoadjuvant chemotherapy has evolved from systemic treatment basically affecting all cells of the body to target specific therapies (i.e., antibodies, kinase-inhibitors) sparing healthy tissue [1, 2]. However, target-specific drugs are only available for a limited number of tumor entities and a need for locoregional chemotherapy has risen.

Magnetic drug targeting (MDT) is a new treatment concept consisting of coupling different types of bioactive substances to iron oxide [Fe_3O_4] nanoparticles as carrier molecules [3]. These substances can be drugs [4] such as cytostatics, chemotherapeutics, or enzymes. Active MDT uses a magnet to guide the substances to the desired target area [5], whereas passive MDT spares the magnet so nanoparticles accumulate in the reticuloendothelial system (RES). If it was possible to transport cytostatics [6] in a dosage specific for the particular entity to a specific location, without causing systemic flooding, a milestone in tumor therapy would be reached.

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This study's aim was to prove the following hypotheses: (1) Cytostatics coupled to iron oxide particles [Fe_3O_4] can be guided into a tumor by means of a magnetic field focused on the desired area with the cytostatic's concentration being measurable there [7] (see Fig. 1). (2) The concentration of the cytostatics administered should be lower in the peripheral blood than in the tumor. (3) MDT should result in histologically detectable effects in tumor area whereas organs of the RES should not be affected.

Limitation to this principle is the constricted penetration depth of roughly 0.5 cm according to the manufacturer when using a 0.6 tesla Neodyn magnet (Siemens AG, Erlangen, Germany).

MATERIALS AND METHODS

In this trial we used Wag/Rij rats transfected with rhabdomyosarcoma to assess biodistribution of mitoxantrone-iron oxide targeted to the tumor area with a 0.6 tesla extracorporeal magnet. Mitoxantrone concentrations were measured in tumor and blood. Plasma iron concentrations during and after MDT and the effect on tumor and RES tissue were assessed.

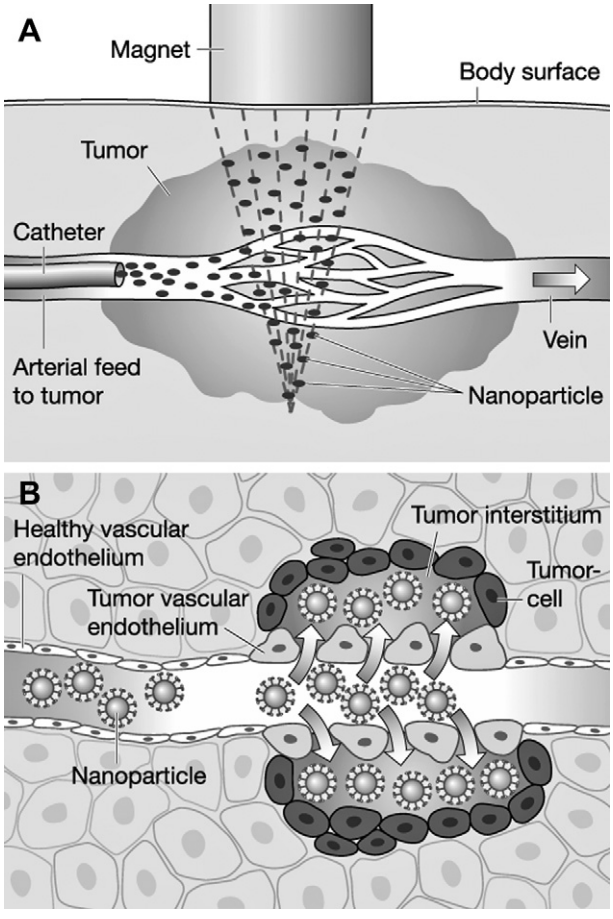

FIG. 1. (A) Principle of magnetic drug targeting. Nanodurgs are guided to the tumor tissue by an extracorporeal magnet. (B) Extravasation of nanodrugs through pathologic endothelium and uncoupling of cytostatics from iron-oxide particles in the tumor interstitium.

Study Animals

A total number of 42 adult Wag/Rij rats (Charles River, Sulzfeld, Germany) were used as study animals. Animals weighed 220–250g to achieve maximum vessel diameter and were kept at room temperature with illumination intervals from 0700 to 1900 h. Altromin 1324 (Altromin, Lage, Germany) was fed as a maintenance diet.

Tumor Model

The study animals were transfected with rhabdomyosarcoma R_1H [8–11] cells supplied by Deutsches Krebsforschungszentrum (DKFZ, Heidelberg, Germany). 50 μL of R_1H tumor cell suspension with a number of 2×10^7 cells were injected into the right gastrocnemius muscle. Tumor growth period was determined to be 7 d. Median tumor diameter at d 7 was 10 mm.

Drug

Standard cytostatic therapies of rhabdomyosarcoma are anthracyclines. Mitoxantrone (Wyeth Pharma, Münster, Germany) is a synthetic anthracenedione and unfolds its cytostatic traits through DNA intercalation and DNA strand crosslinking. Proliferation assays (Tumor Cell Trend, Berlin, Germany) were used to assess susceptibility of R_1H rhabdomyosarcoma to mitoxantrone. Tumor cells were treated with 200 pg/mL or 200 ng/mL of mitoxantrone and cell division rate was measured 72 h after administration. At 200 pg/mL, a 40% reduction, and at 200 ng/mL, a 90% reduction of proliferation was observed. Therefore, we concluded that rhabdomyosarcoma R_1H is sensitive to mitoxantrone.

For MDT iron oxides [Fe_3O_4] were coupled to mitoxantrone (for specifications see Table 1) at physiologic pH of 7.4. Electron transfer from the electropositive substance to a more electronegative element causes the ionic interaction. Two oppositely charged ions with a noble

TABLE 1

Laboratory Data [Micromod*] of the used Mitoxantrone Iron Oxides

Product no.	05-02-252S
Product name	Nanomag-CLD
Product description	Magnetite-dextran composite particles, crosslinked, COOH-modified
Surface	Mitoxantrone (10 μg/mg)
Size	250 nm
Solid content	10 mg/mL
Iron content	>57% (wt/wt), corresponds to > 79% (wt/wt) magnetite
Quantity	10 mL
Polydispersity index	<0.2
Shape	Cluster-type
Density	2.5 g/ccm
Magnetization	43 emu/g particles (H = 1000 Oe)
Saturation magnetization	>67 emu/g particles (H > 10,000 Oe)
Stable in	Aqueous buffers pH > 4
Not stable in	Organic solvents, acidic solutions pH < 4
Product form	Suspension in 0.9% saline
Particles per mL	3.0*10E11
Particles per mg	3.0*10E10
Additional remarks	Storage at 4°C for 3 months, do not freeze

*Micromod, PD Dr. Teller, Rostock, Germany.