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Clinical Experiences with Magnetic Drug Targeting: A Phase I Study with 4′-Epidoxorubicin in 14 Patients with Advanced Solid Tumors[1]

Andreas Stephan Lübbe,[2] Christian Bergemann, Hanno Riess, Folke Schriever, Peter Reichardt, Kurt Possinger, Michael Matthias, Bernd Dörken, Friedhelm Herrmann, Renate Gürtler, Peter Hohenberger, Norbert Haas, Reinhard Sohr, Bernhard Sander, Arne-Jürgen Lemke, Dieter Ohlendorf, Winfried Huhnt, and Dieter Huhn

Departments of Medicine (Hematology and Oncology) [A. S. L., C. B., H. R., F. S., W. H., D. H.], Traumatology and Plastic Surgery [N. H.], Radiology [B. S., A-J. L.], and Pharmacy [D. O.], Virchow Medical School, Humboldt-Universität zu Berlin, Augustenburger Platz 1, 13353 Berlin; Departments of Medical Oncology and Tumor Immunology [P. R., B. D.], Medical Oncology and Molecular Biology [F. H., R. G.], and Surgical Oncology [P. H.], Robert-Rössle-Klinik, Virchow Medical School, Humboldt-Universität zu Berlin, Lindenberger Weg 80, 13125 Berlin; Department of Medical Oncology, Charite Medical School, Humboldt-Universität zu Berlin, Schumannstraße 20/21, 10117 Berlin [K. P., M. M.]; and Department of Pharmacology and Toxicology, Charite Medical School, Humboldt-Universität zu Berlin, Dorotheenstraße 94, 10117 Berlin [R. S.], Germany

ABSTRACT

Anticancer drugs reversibly bound to magnetic fluids (ferrofluids) could be concentrated in locally advanced tumors by magnetic fields that are arranged at the tumor surface outside of the organism. If certain requirements are met, systemic toxicity might be minimized, and local tumor efficacy might be increased. We have conducted a Phase I clinical trial using this approach in patients with advanced and unsuccessfully pretreated cancers or sarcomas. Nine such patients received two treatment courses, 3 patients received one course, and 2 patients received three courses of magnetic drug targeting consisting of the infusion of epirubicin in increasing doses (from 5 to 100 mg/m^2) that had been chemically bound to a magnetic fluid and the application of magnetic fields to the tumors for 60–120 min. In 2 of 14 patients, the same dose of epirubicin not bound to a magnetic fluid was administered systemically 3 weeks after drug targeting for intraindividual comparisons. Magnetic drug targeting with epirubicin was well tolerated. In one case, a planned second treatment was withdrawn, because of an episode of chills 130 min after infusion of the magnetic drug. Two patients received a third treatment because of good responses after the first two therapies. Based on magnetic resonance tomographic techniques, pharmacokinetics, and the histological detection of magnetites, it was shown that the ferrofluid could be successfully directed to the tumors in about one-half of the patients. Organ toxicity did not increase with the treatment, but epirubicin-associated toxicity appeared at doses greater than 50 mg/m^2. Although treatment with magnetic drug targeting seems safe, improvements are necessary to make it more effective and independent of patient- or disease-related problems. A study design to compare conventional treatments with the new treatment form within one patient seems crucial to eliminate interindividual differences.

INTRODUCTION

Theoretically, disease-affected body compartments can be treated as biological units and should be treated solely (1). Yet, conventional treatment regiments are not able to achieve significant drug concentrations in diseased compartments without distributing drugs throughout most other (healthy) body parts (1, 2). Not only does this only mean that larger amounts of drugs than necessary have to be applied, but also that healthy tissues get exposed to the potential harmful effects of the cytotoxic drugs. This is particularly critical in situations in which drugs with low therapeutic indices must be administered, and it holds particularly true for most conventional anticancer drugs.

In cases in which there are locally advanced tumors that need systemic medical anticancer treatment, such as thoracic wall recurrences after radiation therapy in patients with breast cancer, a locoregional drug application resulting in prolonged exposure of the tumor to high drug concentrations may be considered (1–4).

Thus, during the last 20 years, impressive efforts have been undertaken to introduce drug targeting into medical practice (1–6). However, very few approaches are technically feasible at this time. Examples are tumor antigen-directed drug targeting (*e.g.*, antibodies are attached to anticancer drugs or immunotoxins; Refs. 1, 7, and 8) and liposome-encapsulated drugs (anthracyclines being the most used drug group, because they are eliminated by cells of the reticuloendothelial or macrophage-monocyte system; Refs. 9 and 10). These two forms can be classified as passive drug targeting, because drugs are physiologically distributed within the organism but remain at locations at which the "formulated" drugs are captured (*e.g.*, liver and spleen).

Active drug targeting that resists normal distribution patterns and depends solely on forces other than those that lie directly within the organism is very attractive from a theoretical point of view. One way to influence a drug within an organism is to couple it to magnetic particles and to concentrate it in areas of strong magnetic fields. Several theoretical assumptions must be resolved before this procedure can be tested in the clinic (1, 2, 11).

During the last 20 years, a small number of groups have tried to use magnetic fluids for active drug targeting (1, 12–14). However, they failed for different reasons, which were summarized in an article by Gupta and Hung (1).

Thus, at the beginning of the 1990s, there were some valuable preclinical experiments with magnetic fluids in general, but those experiments could not be scaled up. We developed a new, specially designed ferrofluid, the main characteristic of which was the ability to bind the drug of choice directly, but reversibly, by adsorption. We showed that it was possible to direct the ferrofluid as well as the drug-ferrofluid complex (magnetic drug) under the influence of a magnetic field *in vitro* and *in vivo* successfully, and in a quantitatively relevant way, given a certain time frame. The magnetic drug was tolerated well in animals when administered by i.v. injection, and it was able to cause tumor remission when the magnetic fluid was directed to the tumor and attached to it for a long enough time (20 min; Ref. 15). Briefly, the combination of a ferrofluid (0.5% of the estimated blood volume) that had been bound to epirubicin (1 mg/kg body weight) and was directed to the tumor (a human xenotransplanted colon adenocarcinoma in nude mice or a xenotransplanted renal cell carcinoma) for 20 min was sufficient to reach complete and lasting tumor remission. Control studies without a magnetic field, without the ferrofluid, or without the drug did not change normal tumor growth (15).

We started a Phase I clinical trial to find the dose and assess the tolerance of magnetized epirubicin in the treatment of patients with advanced cancer and sarcomas in which magnetic drug targeting was believed to be practically possible. Here, we describe our first expe-

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[2] To whom requests for reprints should be addressed, at Cecilien-Klinik, Cecilienallee 6-8, 33175 Bad Lippspringe, Germany.

riences with the treatment of 14 patients in which active magnetic drug targeting has been used.

PATIENTS AND METHODS

Eligibility Criteria. Seven patients with metastatic breast cancer, two with chondrosarcoma, two with squamous cell carcinoma of the parotis and hypopharynx, respectively, one with Ewing sarcoma, and one with malignant histiocytoma, who had all failed standard chemotherapy were eligible for this Phase I clinical trial. Patients were required to have a Karnofsky performance status of at least 40%, a life expectancy of at least 3 months, and preserved renal (serum creatinine <2.0 mg/100 ml) and hepatic (serum bilirubin <3 mg/100 ml) function, hematopoietic function of at least a hemoglobin level of >8 g/100 ml, WBC of $>1000 \times 10^9$/liter, and platelets of $>100 \times 10^9$/liter. Patients were excluded if they had other acute significant diseases (*e.g.*, uncontrolled diabetes mellitus and fever due to an infectious disease), were not compliant or wished to be excluded from the study at any time, or had any other form of antineoplastic treatment. A treatment-free interval of at least 3 weeks was required, extended to 6 weeks for mitomycin C or nitrosurea and to 8 weeks for large-field radiotherapy. The study was approved by the ethics committee of the Virchow Medical School at the Humboldt-Universität zu Berlin. Written informed consent was obtained from all patients.

Study Drugs. Epirubicin (4′-epidoxorubicin; Farmorubicin) was supplied by Pharmacia. The lyophilized powder was reconstituted with the ferrofluid (all treatments but the last two) or with isotonic saline solution (the last two treatments in patients 13 and 14) according to standard procedures within 15 min prior to administration.

The ferrofluid was obtained from Nano-Technologies [Gesellschaft Bürgerlichen Rechts (GBR), Berlin, Germany; German patent 19624426.9]. The final pyrogen and sterile tests were performed in the Department Pharmacy, Virchow Medical School, according to GMP (Good Medical Practice) guidelines. Before use, the fluid had been autoclaved and filtered through commercially obtained 200-nm filters. The ferrofluid was a colloidal dispersion, made by wet chemical methods out of iron oxides and hydroxides into specially arranged multidomain particles. Those particles were surrounded with anhydroglucose polymers to stabilize the magnetic particles under various conditions. In addition, the surrounding polymer was used for chemoadsorptive binding of numerous molecules, among those epirubicin. The characteristics of the fluid are depicted in Table 1.

The Magnetic Field. High-energy permanent magnets were used in this patient trial. The magnets consisted of rare earths, the majority being neodymium. There were large ($8 \times 4 \times 2$ cm) and small ($3 \times 3 \times 1$ cm) blocks, and these blocks could be arranged according to the individually shaped tumor of the patient. Magnetic field strengths of at least 0.5 tesla and in general 0.8 tesla could be reached and were confirmed at the patient's bed. The distance between the tumor surface and the magnet was assured to be less than 0.5 cm. In the first few patients, a cooling spray was applied to the skin to avoid possible local toxicities in the region next to the magnet, but this turned out be unnecessary in the remaining patients.

Study Design and Treatment Plan. Before treatment, each patient was evaluated with a complete medical history. Baseline diagnostic studies included a physical examination, non-contrast-enhanced MRT[3] of the region to be treated, a blood count, prothrombin time, partial thromboplastin time, fibrinogen levels, serum chemistries, iron and ferritin contents, a urinanalysis, a two-view chest X-ray, and an electrocardiogram. Blood tests were repeated twice daily for 3 days after each treatment and in at least weekly intervals for up to 6 weeks. Tumor response was monitored weekly by physical examination and at the end of the treatment. In patients with measurable disease, tumor responses were described, but not further evaluated, because this was not the primary objective of this study.

A test dose of the magnetic fluid (0.2% of the estimated blood volume) without the cytotoxic drug was administered i.v. over 5 min 2 days prior to the first of two treatments in each patient to test for possible intolerances. The blood volume was estimated to be 7% of the body weight in females and 8% of that in males. Two days later, after preparation of the magnetic epirubicin

Table 1 *Characteristics of the magnetic fluid*

Particle size	100 nm
Magnetites	1.5% of total weight of ferrofluid
Iron content (wt/wt)	60% (15 mg iron/25 mg magnetites)
Stabilizer (anhydroglucose polymer)	0.5% of weight
pH	7.4
Color	Black
Odor	Neutral
Iron content	~6 mg/ml (0.108 mM)
Carbohydrate content	~5 mg/ml
No. of particles	$\sim 10^8$/ml
Wt/v	10 mg/ml

(0.5% of the estimated blood volume), the substance was infused i.v. over 15 min into a vein located contralaterally to the tumor. During the time of infusion and for at least the next 45 min (patient 6; patients 8–14, total of 120 min), a magnetic field was built up as close as possible to the tumor. Prior to treatment and in regular intervals thereafter, blood samples (5 ml) were taken from the patients (vein contralateral to application of magnetic epirubicin) for pharmacokinetic evaluations. No antiemetic or other therapy-related drug was given at any time during the trial. A second treatment with magnetic drug targeting followed 7 days later, provided that there was no significant toxicity associated with the first treatment and that the disease had not overtly progressed. Toxicity was assessed according to standard WHO guidelines. Then, 24 h and 5 weeks after the second treatment, follow-up MRT was done to test for accumulation and degradation of the ferrofluid in the tumor and sometimes in the liver. In one patient, histological examinations for iron content were performed 6 weeks after the second treatment.

According to Table 2, four different epirubicin doses were administered to the patients. Three patients received 5 mg/m^2 magnetic epirubicin, 50 mg/m^2 were given to five patients, 75 mg/m^2 were administered to four patients, and 100 mg/m^2 were given to two patients. This was done to test whether increasing concentrations of magnetic epirubicin also caused increasing toxicities. In the last two patients, who had received 100 mg/m^2 epirubicin, magnetic drug targeting with epirubicin was done only once. Instead of the second treatment, 3 weeks later, the same dose of epirubicin was infused over 15 min to compare intraindividually pharmacokinetic data of epirubicin in the two forms of application.

All patients were closely monitored during and for 2 h after the end of the therapy. Specifically, the patients were put on an electrocardiographic monitor, and blood pressure as well as respiratory rate and oral body temperature were measured and documented at regular 15-min intervals.

A major objective of this study was to determine a potential toxicity of the treatment with increasing concentrations of epirubicin while the amount of the ferrofluid that had been administered to the patients remained constant (0.5% of the estimated blood volume). Three patients were initially treated at each dose level. If more than 2 patients at one dose level had developed significant (grade 3 or 4) toxicity, this concentration would have been declared toxic, and the dose prior to that one would have been accepted. If one of three patients had developed significant toxicity, up to six patients would have received that dose. In a case of no further such event, one would have progressed. In a case of a second patient with toxicity, the dose below would have been determined the "maximum tolerable dose" with magnetic epirubicin. Another way to end the study was a consensus decision by the involved physicians.

Pharmacokinetic Analysis. Blood samples were taken prior to and at 5, 10, 15, 17, 20, 25, 30, 60, 100, 120, 150, 360, 600, 1440, and 1920 min after administration of the substance. After centrifugation, the plasma was examined for epirubicin with high-performance liquid chromatography. The analytical column was reconditioned and rinsed before chromatographic separation and detection of the anthracyclines by a fluorescence detector (RF-551; Shimadzu, Tokyo, Japan) with excitation at 467 nm and detection at 550 nm. Epirubicin concentrations were calculated by determining the peak areas of the probes. In some probes, daunorubicin had been added as an internal standard, and the final evaluation considered the epirubicin-daunorubicin peak area relation. Prior to each measurement, the high-performance liquid chromatographic system was tested for contamination by using pure plasma.

For the pharmacokinetic data, the parameters were calculated from serum concentration *versus* time data using a custom-made computer program (MW/PHARM; Byk Goulden, Konstanz, Germany) and fitted according to a three-

[3] The abbreviations used are: MRT, magnetic resonance tomography; AUC, area under the concentration curve.

Table 2 *Patient characteristics and study design*

Age (y)	Gender	Tumor	Volume (ml)	Location	Epirubicin (mg/m^2)	Epirubicin (total mg)	Ferrofluid volume (ml)
37	Female	Schwannoma	300	Left arm	5	20	52
30	Female	Histiocytoma	400	Right thorax	5	16	48
53	Female	Breast	250	Right axilla	5	18	52
67	Female	Breast	400	Right thorax	50	160	50
73	Female	Chondrosarcoma	1,500	Right thigh	50	150	48
15	Male	Ewing sarcoma	500	Left scapula	50	310	78
55	Female	Breast	500	Right thorax	50	170	48
56	Male	Parotis	150	Left parotis	50	220	48
58	Female	Breast	200	Right thorax	75	280	52
56	Female	Breast	300	Left scapula	75	250	29
55	Female	Breast	300	Right thorax	75	250	52
68	Female	Breast	400	Right thorax	75	260	52
65	Female	Chondrosarcoma	500	Right axilla	100	200	25
45	Male	Hypopharynx	250	Left neck	100	150	22

compartment model. The highest observed serum concentration was defined as the maximum concentration. The AUC from 0 to 1920 min after the start of magnetic drug targeting was estimated by the trapezoidal rule. Because different epirubicin concentrations had been applied, the AUC was divided by the epirubicin doses. The terminal elimination rate constant was computed as the absolute value of the slope of a least square regression of the natural logarithm of serum concentration *versus* time in the elimination phase of drug disposition.

RESULTS

Patients. Between July 1994 and January 1995, 14 patients were enrolled whose details are shown in Table 2.

Of those 14 patients, 1 entered the protocol at 15 years of age (patient 6), and one (patient 7) died within 1 week after the second therapy from causes (sepsis) not related to magnetic drug targeting. There was no second therapy in a patient who experienced a 15-min episode of chills with associated tachycardia and arterial hypertension 2 h after the beginning of the infusion of the ferrofluid and within 10 min after release of the magnetic field from the left shoulder. Because there were some positive subjective and objective responses to the treatment, a third cycle of magnetic therapy was administered in two patients. After analysis of the results up to patient 12, the study committee decided to apply 100 mg epirubicin/m^2 in the form of magnetic drug targeting in two more patients only once, whereas the same dose of regular epirubicin was given 3 weeks later to obtain the first data with regard to better interpretability of intraindividual tolerance of the two treatment forms. The mean age of the patients was 52 ± 5 (SEM); the range was 15–73 years.

Toxicity. All patients tolerated the test dose well. There were no changes in systemic hemodynamic parameters both during and after application of the test dose of the ferrofluid, as well as during and after magnetic drug targeting itself. It was believed important for the best outcome of the treatment that the patient remained without motion in relation to the magnetic field that was built up close to the tumor. In three patients, this was not possible over 1–2 h. Due to pain or other causes, sometimes the movements or discomforts from the disease had been so severe that the magnet had to be relocated or reattached. Overall, the magnetic fluid had been given 43 times either as as test dose (without epirubicin) or as magnetic epirubicin (epirubicin bound to the ferrofluid). The dose escalation stopped at 100 mg/m^2 before the maximum tolerable dose was reached because of nonlinear pharmacokinetics and the decision to progress with intraindividual comparisons of the two treatment forms, as described above.

Hematological Toxicity. Myelosuppression was manifest by either leukopenia or thrombocytopenia or both. The nadir counts and severity grades are depicted in Table 3. Because there were two treatments in patients 1–12 within 1 week, data are presented at various intervals prior to and after the treatments. The leukocyte nadir occurred at 10 days, with recovery being observed before day 21. The platelet nadir occurred at 14 days, with recovery being noted after day 21. There was considerable variability in the 50–75-mg/m^2 dose range, which was probably attributable to patient factors. No patient required antibiotics for neutropenic fever episodes. No growth factors were given within the treatment phase and thereafter. Hematological data of patients 13 and 14, who received magnetic drug targeting once and systemic epirubicin 3 weeks later, are shown in Table 4. There was less hematological toxicity with magnetic drug targeting.

Nonhematological Toxicity. With regard to serum electrolytes, renal function and hepatic parameters, serum proteins, albumin, uric acid, electrophoresis, partial thromboplastin and prothrombin times, lactate dehydrogenase, and C-reactive protein, there were no changes from baseline parameters prior to magnetic drug targeting to those obtained at regular intervals after that therapy. Table 5 depicts WHO data for nonhematological toxicities before and after magnetic drug targeting. Basically, magnetic drug targeting was tolerated well without antiemetic or other supportive therapy.

Because iron was a key ingredient of the ferrofluid, particular attention was focused on the serum iron and ferritin levels over time in the blood. Those values are shown in Tables 6 (patients 1–12) and 7 (patients 13 and 14). Although there were serum iron elevations from the baseline level in almost all patients, those elevations were transient for 24–48 h after therapy and did not cause any symptoms. Ferritin baseline values were very variable, as could be expected from the heterogeneous patient group. Those values were also increased after the therapy but remained at this level for a longer, yet variable, time interval. Urine iron levels (done in the last 7 patients) were always within the normal range.

Proof of Concentration of Magnetites in the Tumor. In 10 of the 14 patients, intact skin covered the tumors. The other four demonstrated exulcerated, superficially open wounds. In four of the former cases, an uptake of the magnetites into the tumor could be visualized easily, in that the magnetic field caused a darker area with the shape of the magnetic blocks that had been attached to the tumor. This discoloration lasted for 24–36 h and then disappeared completely. There were no local toxicities in those regions. It was assured that the discoloration could not be wiped away to rule out the possibility of iron deposits from the magnetic blocks at the superficial skin layer.

In one patient with a malignant schwannoma, the left forearm was amputated 6 weeks after the second therapy. The tissue was stained with the Turnbull histological iron staining technique, and magnetites were clearly seen in some regions of the tumor as an indication of local concentration as a result of magnetic drug targeting.

All but three patients received MRT at least once prior to and after magnetic drug targeting. From those 11 patients, there were 2 in

Table 3 *Hematological data prior to and after magnetic drug targeting*

Patient	Hematological measure[a]	Day[b] −3	0	7	10	21	28	40
1	Hb	12.5	12	11.6	12.8			
	Ery	4.2	4	3.9	4.2			
	L	6.5	5.6	4.5	6.4			
	Thr	316	304	289	329			
2	Hb	6	9.3	10	9.9			7
	Ery	2.1	3.2	3.4	3.3			2.2
	L	6.6	9.6	13	7.5			8.9
	Thr	461	585	550	631			724
3[c]	Hb	9.8	9.7	9.9	8.9	13.4	14.1	
	Ery	3.4	3.3	3.4	3.1			
	L	2.1	42.4	16.4	10.5	31	25	
	Thr	177	230	129	163	267	273	
4	Hb	13.4	13.3	12	9	11	12	
	Ery	4.4	4.4	4	3	3.4		
	L	9.2	8.5	5.4	2.6	5.8	10.6	
	Thr	259	251	205	108	253	288	
5	Hb	10.6	10.8	10.3	9	11.3	12	
	Ery	3.9	4	3.7	3.2	4	4.2	
	L	5.6	5.9	4	3	4.1	5.2	
	Thr	398	426	346	274	564	414	
6	Hb	11	11.1	10.7	9.4		10.2	11.7
	Ery	4.2	4.2	3.9	3.6		3.9	
	L	4.7	4	4.1	3.1		16	9.5
	Thr	267	285	224	217		132	472
7	Hb	10.7	9.8	9.6	9.1			
	Ery	3.6	3.2	3.3	3.2			
	L	11.5	12.6	6.2	1.8			
	Thr	532	528	539	337			
8	Hb	12.9	11.7	11.7	11.6	13	15.1	15.1
	Ery	4.2		3.9	3.9	4.2	4.9	5
	L	7.1	7.6	5.3	5.8	4.3	6.8	5.8
	Thr	203	177	225	229	140	178	158
9	Hb	9.5		9.2	8.6	7.9	8.2	10.8
	Ery	3.1		3.1	2.7	2.4	3.4	
	L	3.1		2.4	1.4	1.3	7.8	4.2
	Thr	126		132	118	50	24	154
10	Hb	11.4	10.4	9.5				
	Ery	3.4	3.1	2.8				
	L	7.3	5.7	3.9				
	Thr	228	295	407				
11	Hb	12.9	12.9	11.1	10	11.9	11.9	13.6
	Ery	3.9	3.9	3.4	3.2	3.7		4.2
	L	6	5.3	4	1.5	2.9	9.7	7.5
	Thr	353	355	278	237	392	337	346
12	Hb	10.6	10.1	9.7	8.8	9.7		11.1
	Ery	3.6	3.3	3.1	2.9	3.1		3.5
	L	3.9	4.4	4.6	4.9	1.5		6.6
	Thr	290	281	193	163	143		265

[a] Hb, hemoglobin (g/dl); Ery, number of RBC ($10^6/\mu l$); L, number of WBC ($10^3/\mu l$); Thr, number of platelets ($10^3/\mu l$).
[b] Day −3, before test dose; day 0, day of first magnetic drug targeting; day 7, day of second drug targeting.
[c] Patient 3 received growth factors.

whom there was a complete or at least a significant loss of signal intensity in the T2-weighted sequences after the magnetic therapy. In four patients there was some signal loss, and in another five patients no signal loss could be detected. In three patients the liver, as the key organ of elimination of magnetic particles, was also analyzed by MRT techniques. Here, the complete loss of signal intensity within 2 days of the second treatment of magnetic drug targeting proved to be reversible in the course of 60 days.

Pharmacokinetics. The first 12 patients received a total of 25 treatments. In the one patient in whom no second therapy was administered, as well as in the third courses of those two patients who responded to the first two cycles with magnetic drug targeting, no

Table 4 *Hematological data of patients 13 and 14*[a]

Patient	Hematological measure	Day[b] −3	0	7	10	21	28	40
13	Hb	11.5	11.6	11.4	11.4	12.5	12	11.7
	Ery	4.1	4.3	4.1	4.2	4.5	4.2	4.1
	L	10.1	8	7.7	7.7	10	4.9	2.2
	Thr	231	232	252	251	231	167	270
14	Hb	10.7	9.7	10	9.9	10.6	7	6.5
	Ery	3.3	3		2.9	3.3	2.1	2
	L	10.6	9.3	10.4	3.7	19.1	5.5	3.5
	Thr	298	279	351	169	483	210	195

[a] See Table 3 for details.
[b] Day 40: after 100 mg/m^2 systemic epirubicin.

Table 5 *WHO toxicity data before (first data point) and after (second data point) two magnetic drug targetings with epirubicin*

	Patient													
Toxicity	1	2	3	4	5	6	7	8	9	10	11	12	13	14
General Behavior	2/2	1/1	2/2	3/3	1/1	2/2	4	0/0	3/3	2/2	2/2	2/2	3/3	3/3
Hemorrhage	0/0	0/0	0/0	0/0	0/0	0/0	0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Nausea/emesis	0/0	0/0	0/0	0/0	0/0	0/0	3	0/0	0/0	0/1	0/0	0/0	0/0	0/0
Diarrhea	0/0	0/0	0/0	0/0	0/0	0/0	0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Renal toxicity	0/0	0/0	0/0	0/0	0/0	0/0	0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Pulmonary toxicity	0/0	0/0	0/0	2/2	0/0	0/0	3	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Allergic reactions	0/0	0/0	0/0	0/0	0/0	0/0	0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Skin affliction	0/0	0/0	0/0	0/0	0/0	0/0	0	0/0	0/0	0/0	0/2	0/0	0/2	0/0
Alopecia	1/1	0/0	3/3	3/3	1/2	4/4	3	0/1	2/3	2/2	2/2	2/2	1/2	2/2
Neurological toxicity	0/0	0/0	0/0	0/0	1/1	0/0	1	0/0	0/0	0/0	0/0	0/0	0/0	0/0
State of consciousness	0/0	0/0	0/0	0/0	0/0	0/0	2	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Pain	1/1	0/0	1/1	3/3	1/1	1/1	3	0/0	2/2	2/2	3/3	3/3	3/3	3/3
Ototoxicity	0/0	1/1	0/0	2/2	0/0	0/0	1	0/0	1/1	0/0	1/1	0/0	0/0	2/2
Fever	0/0	0/0	0/0	0/0	0/0	0/0	0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Local infection	0/0	0/0	0/0	2/2	0/0	0/0	3	0/0	2/2	0/0	2/2	2/2	0/0	0/0
Cardiac toxicity	0/0	0/0	0/0	2/2	0/0	1/1	2	0/0	1/1	1/1	2/2	1/1	1/1	0/0

pharmacokinetic analysis had been performed. Data from 19 treatments were completely analyzed (methodological problems occurred in 6 cases). Patients 13 and 14 were analyzed after magnetic drug targeting and the systemic administration of epirubicin. Table 8 shows the three half-lives of epirubicin and epirubicinol (the primary metabolite), as well as the AUC at the respective dose level in comparison with selected values from the literature. Independent of the variable drug targeting (and, thus, a different form of administration of this drug), the resulting values lie within what is typical for this substance. Relevant data for patients 13 and 14 are depicted in Table 9. The AUC was considerably smaller when drug targeting had been conducted. Particularly in the early phase of administration (during the first 60 min), peak concentrations are much less than those after systemic application (Figs. 1 and 2).

Antitumor Activity. Table 10 shows the results after magnetic drug targeting. There were four slight tumor reductions at day 10 and some little responses at day 40.

DISCUSSION

Magnetic drug targeting appears to be a safe procedure that could be administered easily to patients. Only the arrangement of the magnets around an individual tumor sometimes took more than 30 min to cover the largest possible tumor region. Due to the relatively small particle size of the magnetites (100 nm), good capillary organ perfusion was assured. Because of the low iron load with each treatment (Table 1), no iron toxicities were detected. Thus, we interpret the repeated elevations of the iron concentration after the application of

Table 6 *Iron (μM) and ferritin (ng/ml) concentrations prior to and after magnetic drug targeting*

	Day −3[a]	Day −2[a]	Before first therapy	12 h after first therapy	Days 1, 2, 3	Before second therapy	12 h after second therapy	Days 8, 9, 10	Days 14, 21, 40
Patient 1									
Iron	13		13	20	16, 16, 15	16		20, 14	
Ferritin	21	35	60	86	121, 136, 136	101	117	171, 217	
Patient 2									
Iron	6	3	10	6	5, 4, 5	3	4	4	2
Ferritin	1201				1039, 1208	725	969	794	1793
Patient 3									
Iron	7	16	19	41	43, 21	15	14	20, 9	
Ferritin		71	237	316	367, 429, 417	303	310	486, 363	
Patient 4									
Iron	11	7	7	19	29, 31, 36	14		38, 37	0, 14, 16
Ferritin	315	384	393	436	254, 252	1947		1236, 1260	0, 740, 320
Patient 5									
Iron	5	7	6	40	42, 35, 35	7	22	29	0, 7
Ferritin	274		291	389	489, 566, 621	1130	1014	1505	0, 679
Patient 6									
Iron	6		10	36	27, 47, 49			20, 46, 47	
Ferritin	74		149		180, 291, 291	366	388	478, 569	
Patient 7									
Iron	3	4	3	9	10, 0, 13	6		8, 11	
Ferritin	1497	1409	1460	1578	2840, 1845			2139, 2073	
Patient 8									
Iron	10	11	9	33	35, 24, 22	12	52	51, 39	12, 14, 13
Ferritin	173		193	156	258, 281, 400	273	309	339, 397	257, 173, 196
Patient 9									
Iron	22	3	21	44	41, 46	37	45	46, 46	0, 44, 22
Ferritin	760	845	944	939	879, 840	106	1073	1005, 1076	0, 1775, 2000
Patient 10									
Iron	4	5	5	9	23, 40, 40				
Ferritin	187	402	449	638	723, 825, 752				
Patient 11									
Iron	15		13		49, 49, 47	15	45	45, 46	
Ferritin			127		297, 198, 509	768	769	769, 1037	
Patient 12									
Iron	15		13	49	49, 49, 47	15	45	45, 46, 50	12, 20, 0
Ferritin	41		127		297, 438, 509	761	747	769, 1037, 1032	1330, 597, 0

[a] Day −3, prior to; and day −2, after application of test dose of the ferrofluid (0.2% of the estimated blood volume).

Table 7 *Iron and ferritin concentrations in patients 13 and 14*[a]

	Day −3	Day −2	Before first therapy	12 h after first therapy	Days 1, 2, 3	Before second therapy	12 h after second therapy	Days 8, 9, 10	Days 14, 21, 40
Patient 13									
Iron	10		10	33	14, 8, 13	10	8	10	10, 7
Ferritin	66		74	133	278, 276, 235	235	207	147	211, 152
Patient 14									
Iron	10		28	19	12, 12			11	16
Ferritin	282		470	1300	1350, 1000			992	778

[a] See Table 6 for details.

Table 8 *Pharmacokinetic parameters of epirubicin in comparison to the literature*

Data are from studies cited by Scheulen (16). Our own data are in the last two rows. In the last column, data are shown from patients who had been treated with conventional epirubicin (among those, patients 13 and 14).

Study	Half-life 1 (min)	Half-life 2 (h)	Half-life 3 (h)	AUC/dose (nM/h/m^2/mg)
Speth (1986)	9.6	20		83
Eksborg (1986)	3.4	0.87	14	28
Scheulen (1985)	4.5	1.9	42	85
Vrignaud (1985)	2.3	1.3	27	48
Vrignaud (1985)	2.8	0.72	32	48
Mross (1985)	1.8	0.49	19	38
Robert (1985)	3.4	1.1	18	66
Camaggi (1988)	2.9	1.1	31	46
Weenen (1983)	4.8	2.6	38	17
Scheulen (1986)	3.7	2.3	33	74
Weenen (1983)	4.8	2.6	38	20
With ferrofluid (n = 19)	3.8	0.98	32	46
Without ferrofluid (n = 3)	4.8	2.9	16	34

the ferrofluid as most likely representing analytical errors, rather than true elevated iron values.

The patients tolerated the procedure well and did not complain of any inconveniences. Also, cardiovascular parameters and body temperatures did not change during and after the treatment. In one patient, due to her dyspnea and pain from a thoracic wall recurrence, the magnetic field had to be taken away briefly but repeatedly; therefore, the magnetic field could not be attached consistently. In two other patients the magnetic field was also not consistently applied. As a result, in those three cases we could not verify the accumulation of magnetites. The chill episode in another patient 10 min after release of the magnetic field most probably was due to a tumor lysis syndrome (there was a 50% tumor regression in the following week).

Because it was important to observe the overall toxicity, no antiemetic drug had been given throughout the protocol. With regard to nausea and emesis, we did not notice significant occurrences even in the higher dose ranges of epirubicin. Other typical side effects of epirubicin (17, 18) did occur under magnetic drug targeting (Tables 3–5). Because of the known interindividual variability with regard to those parameters (19, 20), we began a treatment protocol with an intraindividual comparison between magnetic drug targeting with epirubicin and systemic epirubicin in patients 13 and 14. Thus, at this point, no conclusion can be drawn with regard to the possible reduction of hematological or nonhematological side effects with magnetic drug targeting. In patients 13 and 14 there appears to be a tendency toward an intraindividual benefit of magnetic drug targeting. In particular, leukocyte nadir appears to be smaller with systemic epirubicin compared with magnetic drug targeting in patient 13 (Table 4). If this were true for more patients, then the one could establish the hypothesis that magnetic drug targeting with epirubicin results in the concentration of the drug in the tumor and consequently reduces systemic toxicity. But more patients must be treated in this comparative form to arrive at valid conclusions. Taken together, our results indicate that treatment with magnetic drug targeting seems not to harm the patient and appears to be safe.

With regard to the local accumulation of the magnetites and, thus, an index of the feasibility of using the ferrofluid as a vehicle to carry drugs within the organism, there were five different ways to test for this. First, systemic side effects were reduced with magnetic drug targeting. As said above, interindividual differences in the tolerance of the drug are too large, and larger sample sizes and/or a different study design must be used to test for those. Second, there was macroscopic evidence of discoloration at the skin surface in some cases in which the skin above the tumor was intact. Third, it was possible to detect iron particles in the amputated forearm tissue of a malignant schwannoma as evidence of the deposition of magnetites in the tumor. Fourth, the MRT data showed that it was possible to accumulate magnetites in six patients. Because ferrite-containing contrast agents have been clinically evaluated for MRT diagnostics over the last 15 years, MRT is well suited for identifying magnetic particles within the organism (20–23). Fifth, the AUC pharmacokinetic data from the first 12 patients confirm the patient variability and the fact that AUC data after magnetic drug targeting lie within the range of what has previously been shown for systemic epirubicin (16–20). Data from patients 13 and 14 show a difference in the curves that had been obtained from systemic application and drug targeting. There was a lower overall epirubicin concentration with magnetic drug targeting compared with systemic application, and there was a pronounced reduction in the peak concentration of epirubicin within the first 60 min after drug targeting to suggest early concentration of that substance in the tumor.

Ferrite-containing fluids for MRT diagnostics share certain characteristics with the ferrofluid that was used in this study. Yet, there were important differences. Although both types of ferrofluids contain magnetic particles, particle size and concentration between the two

Table 9 *Intraindividual comparison of the AUC and the AUC/dose in two patients who received 100 mg/m^2 epirubicin with magnetic drug targeting and conventional epirubin administration by a 15-min infusion*

Patient	AUC (h/μg/liter)	AUC/dose [h/nM/(m^2/mg)]
13	2.642 (drug targeting)	45.57 (drug targeting)
	19.640 (without drug targeting)	338.6 (without drug targeting)
14	656.3 (drug targeting)	11.31 (drug targeting)
	1.642 (without drug targeting)	28.31 (without drug targeting)

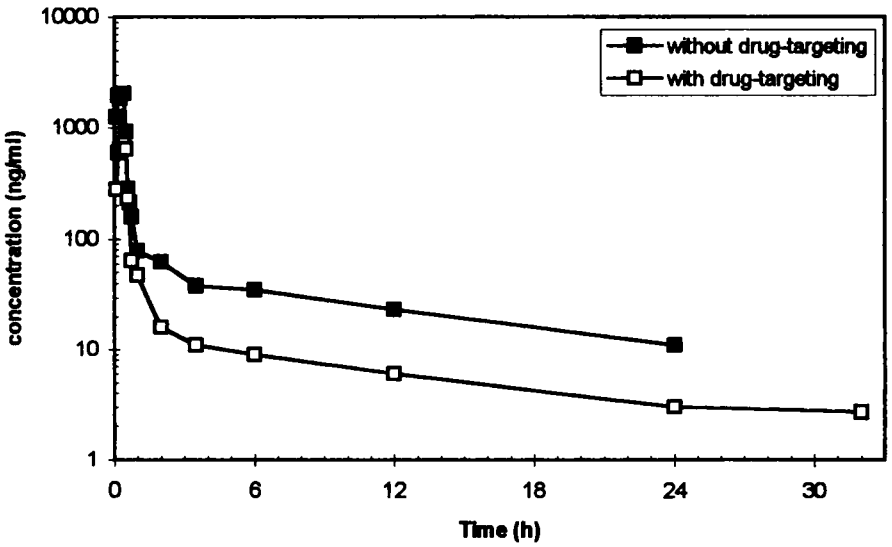

Fig. 1. Epirubicin concentration profile of a patient who received drug targeting with epirubicin (□) and conventionally applied epirubicin 3 weeks later (■).

fluid types vary, both parameters being higher in the therapeutic fluid. A certain particle size of at least 100 nm was found to be crucial to ensure a good influence by the inhomogeneous magnetic field that was applied to the tumor. Also, the magnetites had to be configured such that epirubicin could be bound reversibly to it. Only with the particular arrangement of the multidomain particles and a special carbohydrate cover to ensure sedimentation stability and the adsorptive binding of the drug could the kinetic behavior of the drug be reached.

Because certain physiological parameters, such as circulation time, blood volume, tumor volume, and tumor blood vessel content, as well as tumor blood flow were considerably different between small-animal models and human patients, practical experiences with the minimum of the magnetic fluid necessary to obtain good clinical results in small animals and theoretical assumptions, *i.e.*, extrapolations to man, formed the basis for the volume of the ferrofluid (0.5% of the estimated blood volume) and the duration of the magnetic field application (at least 60 and ideally 120 min). Assuming a total tumor volume of 1000 ml and a tumor blood vessel content of between 1 and 10%, in addition to the fact that between 1 and 10% of those vessels are constantly perfused, then the fluid volume to reach those vessel ranges between 1 and 100 ml. Assuming a strong magnetic field and data from animal experiments, 0.5% of the estimated blood volume (28 ml in a 70-kg male) was considered an appropriate volume. Given different circulation times and tumor volumes in relation to body weight and body surface among small animals and human beings, a factor of 10 seemed necessary to increase the minimum duration of magnetic field application from small animals (10 min) to patients (100 min) to ensure maximum retention of the magnetites in the tumor.

It was a requirement to set the half-life of desorption of epirubicin from the ferrofluid to 60 min. Obviously, this is a critical variable in relation to the magnetic field strength and the characteristics of the magnetites. The intravasal availability of the magnetites had been determined to be 30 min (German patent 19624426.9).

There was a clear clinical correlation between the quality and quantity of the signal loss and the particular tumor and the quality of the treatment performed. In other words, in those cases in which the magnetic field could not be applied consistently, there was little or no accumulation of magnetites in the tumor, as seen with MRT techniques. We think that unless the magnetic field is applied for certain minimum times, magnetites will not arrest within the tumor microcirculation and will be washed away by physical forces from the flowing blood stream. We have data to confirm this by using various microcirculation models to test for minimum times necessary to reach irreversible magnetite accumulation.[4] Also, in cases in which the tumor was very large and little perfused, such as in the patient with a chondrosarcoma, no signal loss could be detected, probably because either the magnetic field was not strong enough or the particle size of the magnetites was not large enough and, consequently, the magnetic influence on the particles by the magnetic field was too small. On the other hand, in a patient with a small and well-perfused tumor to which the magnetic field had been applied for 2 h firmly, there was complete signal loss and, thus, excellent accumulation of magnetites within the tumor.

Because one can only speculate how much of the magnetic fluid can be concentrated in the tumor, it is unclear how many particles follow their normal distribution pattern, *i.e.*, vanish in the liver and the spleen of the patient. In those cases in which signal intensities of the liver had been observed within 48 h after the second therapy and 5 weeks later, a complete loss and partial regain of the signal intensity of the liver could be detected. This could mean that a significant amount of magnetic particles will not be permanently attached in the tumor but will end up in the liver within the first 48 h after magnetic drug targeting and that a significant amount of those particles will be eliminated from that tissue within several weeks. Those data confirm the literature on iron-containing MRT contrast agents and our data in animal experiments (15, 21–23).

The future potential of magnetic drug targeting is linked to the proposed ferrofluid, equipment (magnetic field), and way of application. According to this study, the procedure seems safe and somewhat effective. However, because of limited reduction of hematological side effects and variable degrees of magnetite accumulation in the tumor, magnetic drug targeting must be improved. The size of the magnetic particles could be the key determinant to increase the accumulation of the magnetites in tumors and, consequently, the concentration of the drug. Then, patient-dependent factors, such as tumor blood flow and histology, would be less important variables in the success rate of magnetite/drug concentration. The larger the magnetic particles are, the better they are attracted over a given distance by a constant magnetic

[4] A. S. Lübbe and C. Bergemann, unpublished data.

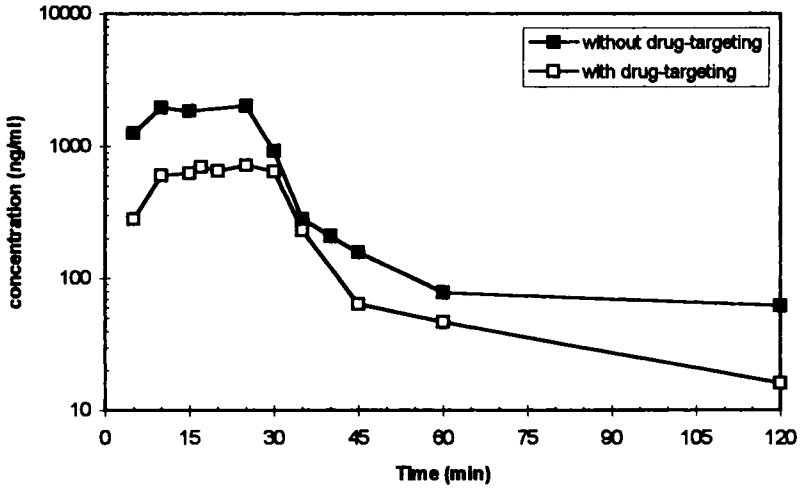

Fig. 2. Epirubicin plasma concentration with and without drug targeting with epirubicin depicted over 120 min. *Lower peak*, concentrations of epirubicin with drug targeting.

Table 10 *Description of tumor responses after magnetic drug targeting*

Patient	Physical examination	Nuclear magnetic resonance and other studies, days 40 (Patients 1–12) and 20 (Patients 13 and 14)
1	Constant	Amputation
2	Constant	Slight reduction of tumor size
3	Constant	Exitus letalis
4	Constant	Slight reduction of tumor size
5	Slight reduction of tumor size	Surgery
6	Slight reduction of tumor size	Constant
7	Constant	Exitus letalis
8	Slight reduction of tumor size	Constant
9	Constant	Constant
10	Slight reduction of tumor size	
11	Constant	Progress
12	Constant	Progress
13	Constant	Progress
14	Constant	Constant

field and the higher the number of particles that are attracted to and retained, even in less well-perfused and large tumors. In the current study, particle sizes of 100 nm were used. Particle sizes up to 1 μm, however, show significant advantages in experimental tumors without harming the organism (the smallest blood vessels are 5 μm thick, inner diameter). Here, much better accumulation of magnetic particles with no apparent side effects has been shown possible in small animal models.[4] The other important variable that offers possible ways of improvement is the time of desorption of epirubicin from the magnetites. Those manipulations, however, seem appropriate only after the optimal particle size of the magnetites has been determined. The increase of the magnetic field strength as well as the extension of the duration of the magnetic field application are not realistic options (too costly and too inconvenient for the patient). Until such crucial variables are satisfactorily clarified, magnetic drug targeting cannot find extended entrance into the hands of medical doctors.

We have shown the first data on magnetic drug targeting in human patients. Although there are encouraging results with regard to the tolerance and applicability of the systems, more improvements must be made with respect to future study designs and the system being used. Specifically, the next step could be to use ferrofluids with increasing particles sizes and to test this new form of treatment intraindividually in more patients against the systemic application of the same drug.

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