Visualisation of Tumour Regression after Local Chemotherapy with Magnetic Nanoparticles – A Pilot Study

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Abstract. *Magnetic drug targeting (MDT) is a new locoregional chemotherapy method that increases the drug dose in the tumour region, while simultaneously reducing the overall dose through the application of chemotherapeutic-bound superparamagnetic nanoparticles, which are focused by an external magnetic field to the desired body compartment. An important factor in this kind of therapy is the vascularisation of the targeted tumour. In this pilot study, the visualisation of the tumour-vascularisation before and after MDT was investigated. Materials and Methods: In a rabbit VX-2 tumour model, mitoxantrone bound to Fe_3O_4-nanoparticles was applied through the femoral artery close to the tumour. The visualisation of vascularisation and tumour size before and after MDT was performed using a biplane angiographic system (Siemens Axiom Artis dBA) to obtain conventional angiographic series with a standard iodine contrast agent. In addition, cross-sectional images were obtained with the new technique of flat panel detector computed tomography (FD-CT) called DYNA-CT. Results: The tumours and the supplying vessels were clearly displayed by FD-CT before and after MDT. The tumours of the study group showed considerable size reductions and the angiography showed a drastic reduction of the tumour supporting vessels following MDT. Conclusion: MDT leads to significant tumour size reductions within several weeks after a single administration of chemotherapy. In this pilot study, FD-CT offered an excellent possibility to monitor the vascularisation and the size of the tumours before and after MDT.*

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In Europe every year, approximately 1.6 million people are diagnosed with malignancies and approximately one million die from their disease (1, 2). Hence, cancer is not only an illness with high incidence but also with high mortality in many cancer types. The standard treatments for cancer patients are surgery, radiotherapy and systemic chemotherapy. New approaches with monoclonal antibodies (mAB) are currently emerging as standard second-line and even first-line modality (3-5). These new methods have shown significant improvement in combination with standard chemotherapy in tumour free progression and overall survival of patients with cancer types *e.g.* localized in the breast or the lung (6-8). Despite these successes in treatment of patients with malignancies, there are still some reasons to look for improvements. The surgical removal of malignant tissue often requires a massive intervention with difficult surgical reconstructions (9, 10). Often tumours are inoperable, if their position or extent is unfavourable. In these cases, radiotherapy as well as systemic chemotherapy are the means of choice for a palliative treatment. Under these treatments, the patients often suffer from severe side-effects (11-16). Although these causes of discomfort can be effectively managed under clinical as well as ambulant conditions, they affect not only the quality of life of the patients, often facing only a palliative situation, but also raise the costs of cancer treatment. These undesired effects are the result of the compromise of killing tumour cells without killing the patient by radiation therapy or intravenous administration of cytotoxic drugs.

Therefore the aim of local tumour therapy is to increase the dose of the therapeutic agent administered in the tumour and simultaneously reduce the overall dose. The benefit for the patients is a better treatment of the malignancy with a reduction of the often severe side-effects of the therapy. Magnetic drug

targeting (MDT) is one promising attempt to realise this ambitious endeavour (17, 18). With this method it is intended to concentrate the therapeutics by intra-arterial application of chemotherapeutics bound to superparamagnetic nanoparticles close to the tumour. These drug-loaded ferrofluids are focused at the tumour by a strong external magnetic field during the application of the nanoparticles (19, 20). Preliminary results show that by this method it is possible to enrich the therapeutic agent by up to 50 times in the desired body compartment (*i.e.* the tumour region) compared to the commonly used systemic application (21). By this means complete tumour remissions devoid of observable side-effects have been achieved in an *in vivo* VX-2 rabbit tumour model (17).

A critical factor for the effectiveness of MDT is an even distribution of the drug-loaded nanoparticles in the tumour tissue. Hence, a detailed knowledge of the tumour vascularisation is of high importance for an optimal positioning of the application catheter as well as the magnetic field. Flat panel computed tomography (FD-CT) (22) was initially developed to obtain cross-sectional images in order to improve workflow during standard cerebral angiography (23), and in a remarkably short time period it has become widely accepted for neuroangiographic imaging. The aim of this technique is to be able to obtain cross sectional CT-like images to control haemorrhage of any kind during a cerebral angiogram or intervention to overcome transport to the CT suite. In comparison to standard CT, FD-CT provides a higher spatial resolution; however soft-tissue contrast is limited. This new generation of angiographic systems also provide an excellent resolution in conventional angiographic series. Taken together, these new angiographic systems may suit the requirement for the detailed display of vascularisation in the tumour region by using conventional angiographic series and FD-CT to visualise the tumour on cross-sectional CT-like images before MDT and to survey its outcome.

The aim of this pilot study was to explore the possibilities of visualising the tumour behaviour after MDT with a new imaging technique using flat panel detectors.

Materials and Methods

Tumour model. An experimental VX-2 tumour was implanted subcutaneously into the left hind limb of rabbits (female New Zealand white rabbits, age at tumour implantation: 18-22 weeks, body weight range 3.1-3.9 kg). After four to six weeks, the tumours had grown to a size that made intervention necessary to avoid unnecessary pain caused by the tumour. MDT was performed using a single cycle of treatment. Mitoxantrone was bound to superparamagnetic Fe_3O_4-nanoparticles (hydrodynamic diameter: ~100 nm) in an aqueous solution (ferrofluid). The applied doses were approximately 10% compared to the regular systemic dose (10 mg/m^2) of mitoxantrone.

Application procedure. For the application of the nanoparticles, access through the femoral artery close to the tumour was chosen. Therefore the left thigh of the rabbits was carefully opened under sterile conditions in an animal operating room. The drug-loaded nanoparticles were then applied by injection through an *i.v.* catheter with wings (BD Insite-W, 24GA 0.75IN). The magnetic nanoparticles were attracted to the tumours by a focused external magnetic field during the application. The water-cooled electromagnet (Siemens Healthcare, Erlangen, Germany) had a magnetic field gradient of 72 T/m directly under the tip of the pole shoe during the whole procedure (19).

Table I. *Summary of the animal data of the pilot study.*

Weight before MDT (kg)	No. of weeks between implantation and MDT	No. of weeks between MDT and control angiography	Tumour size before MDT (cm^3)
3.1-3.9	4-6	5-11	2.0-11.2

Dose of MTO: ≤10.5%, compared to regular systemic dose of Mitoxantrone (=10 mg/m^2), intra-arterial application.

Visualisation. Visualisation of vascularisation and tumour size before and after MDT was performed using a biplane angiographic system with FD-CT (22). The standard contrast agent used had an iodine concentration of 300 mg/ml. Conventional angiographic series were obtained in order to visualise the vascularisation in the tumour region. Additionally FD-CT was performed to reconstruct cross-sectional CT images.

Results

The initial tumour sizes before MDT were between 2.0 cm^3 and 11.2 cm^3 at 4 to 6 weeks post implantation (Table I). Angiography was performed 1-3 days before MDT with angiographic equipment used in routine human angiography (Siemens Axiom Artis dBA). A standard contrast agent was used with a concentration of 300 mg/ml iodine. The contrast agent was tolerated very well without visible side-effects. The reconstruction of CT-like images revealed the tumour mass with a contrast and resolution prodigious for such small structures (Figure 1). The two-dimensional (2D) angiography displayed the small vessels of the rabbit leg very clearly, with excellent resolution. The angiography showed that the pathological vascularisation of the tumour was branching off the *arteria tibialis posterior* with large and irregular vessels (Figure 2A). The three-dimensional reconstruction (3D) of the vascularisation, bones and soft tissue of the tumour was performed with a volume rendering technique (VRT) using a dedicated Leonardo Workstation (Siemens Healthcare, Forchheim, Germany) (Figure 2 B-C). The large pathological vessels branching off the *arteria tibialis posterior* were displayed with an outstanding resolution and contrast.

One to three days after angiography each animal was treated once with MDT by applying the mitoxantrone-loaded

ferrofluid through the femoral artery, near to the tumour. During the application (10-12 min) and up to 15 minutes afterwards, the tumour-bearing region was exposed to a magnetic field gradient with its maximum of 72 T/m at the tip of the poleshoe. The administered doses for each individual animal corresponded to reduced doses up to 10.5% compared to the regular systemic dose of 10 mg/m^2, respectively (Table I). The animals showed no visible side-effects and behaved normally after the treatment.

A second angiography was performed to assess the treatment 7-11 weeks after MDT. These angiographies revealed that even at this early time point, when a small but still measurable tumour remained in the legs of the animals, the pathological vascularisation had disappeared and the tumour mass was barely visible (Figures 3A-C). This image-supported assessment showed that the single treatment with MDT had a profound effect not only on tumour vascularisation but also on the tumour tissue itself. Taken together, it was demonstrated, that a single treatment of MDT with a highly reduced dose of mitoxantrone compared to the normal systemic administration had significant effects on tumour size and vascularisation without visible side-effects. The tumour size and vascularisation of the implanted VX-2 tumours were displayed with excellent resolution by conventional angiography and FD-CT. In the same manner, reduction in tumour size as well as the disappearance of the pathological vessels supplying the tumours was demonstrated with this new angiography platform.

Discussion

Biodistribution studies with rabbits bearing VX-2 tumours at the left hind limb have shown that of a 100% systemic dose of mitoxantrone, less than 1% reaches the tumour (21). Even an intra-arterial administration of the drug without attempting any means to concentrate it in the tumour region results in an only marginally higher deposition. Under these conditions and even with lower doses (75% of the standard dose), the side-effects are extremely severe (24). In contrary, the deposition of mitoxantrone-bound to magnetic nanoparticles can be significantly increased by the application of a strong external magnetic field gradient during administration (21). The aim of the present study was to gain insight into the potential for an image-controlled therapy using a new angiographic technique with electronic signal acquisition. A crucial basis of tumour growth and development is the vascularisation of the malignant tissue. For the treatment of this tissue by MDT, the access through the supporting vessels is of high importance for being able to deposit much higher amounts of magnetic nanoparticles – and chemotherapeutic agent – in the tumour compared to intravenous application. Hence, we thought of using an imaging technique which enables the display of vessels as well as tumour tissue. A new angiographic platform with flat detectors that is able to reconstruct CT-like images by using the software DYNA-CT was shown to meet these requirements. This platform has been used for routine interventions in humans with excellent results. For imaging the rabbit tumours, standard contrast agent was used without visible side-effects. The results showed that it was possible for the small vessels of the rabbits, compared with human vessels, to be displayed with high resolution in 2D and 3D. The CT-like reconstruction revealed sufficient contrast of the soft tissue. The 3D-reconstruction overlaying the angiography, bone and soft tissue of the tumour (VRT) produced unique images of the tumour and its vascularisation in the present rabbit model. The tumour-supplying vessels were displayed with high resolution before treatment of the tumours with MDT.

The application procedure requires a minimally invasive surgery of the left hind limb and there is a possibility of side-effects occurring through this procedure that might affect the animals as well as the outcome of the treatment. At the medial thigh, the femoral artery is in close contact to the *nervus ischiadicus* and near the femoral vein. Injury of either of these can cause severe complications during and after MDT. Nevertheless, neither during surgery and particle administration nor afterwards did the rabbits in this pilot study show any visible side-effects of the treatment. The wound healing appeared normal and the left hind limb remained fully functional.

Five to eleven weeks after the treatment of the tumours by MDT with a single and reduced dose of mitoxantrone applied through the *arteria femoralis*, the angiographies showed the loss of the pathological vessels branching off of the *arteria tibialis posterior*. At this time, the tumour sizes had been reduced significantly such that it was difficult to display the tumour mass by FD-CT.

Taken together, this study shows that FD-CT offers an excellent opportunity for an image-controlled therapy in tumour-bearing rabbits. The small vessels of the rabbit leg were displayed with high resolution. The VRT technique was able to display the vascularisation in detail and the tumour mass in 3D before the treatment with MDT and the loss of the pathological vascularisation several weeks after MDT.

Future studies are needed to investigate the effects of different vascularisation types *e.g.* the diameter and or the angle of the branching vessels, by using rotational flat detector angiography. Finally, FD-CT should be compared to other standard imaging techniques, *e.g.* MRT to demonstrate the opportunities offered by this new technique for displaying therapy control.

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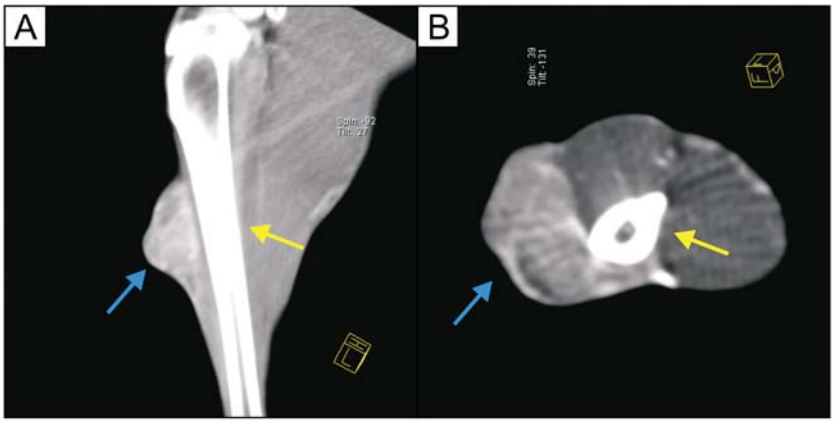

Figure 1. *CT-like visualization of the left hind limb of animal Pi-01 reconstructed by the program DYNA-CT (Siemens Healthcare, Forchheim, Germany). A: The longitudinal section shows the tumour (blue arrows) left of the shank bones (yellow arrow). B: The axial section displays the tumour with substructures in front of the tibia.*

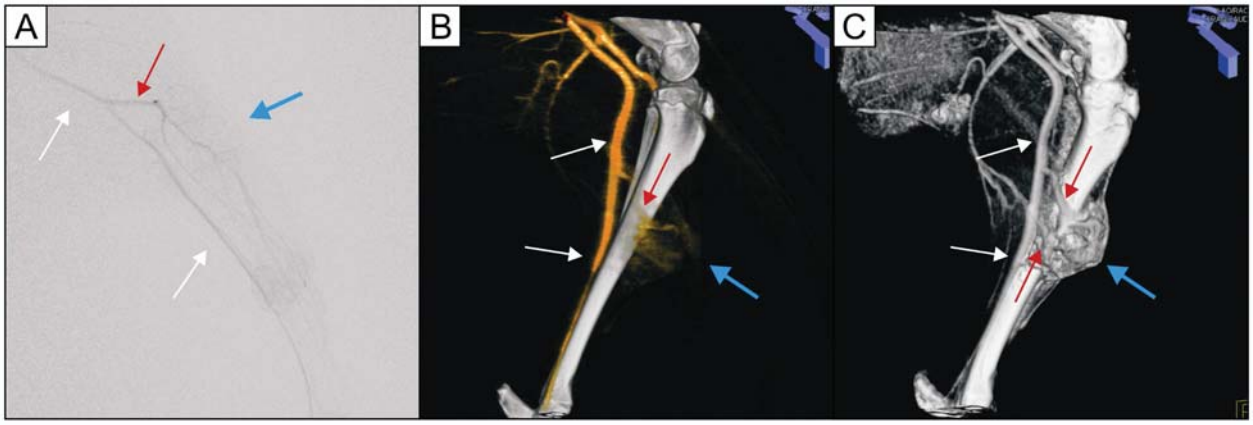

Figure 2. *Angiography of animal Pi-01 one day before MDT. White arrows: arteria tibialis posterior; red arrows: large pathological arterial branches supporting the tumour (blue arrow). A: 2D-angiography. B: 3D-angiography combined with DYNA-CT reconstruction of the left knee and the tibia. The vessels are displayed in orange; the bone is displayed in grey. C: 3D-combination of angiography and CT of the bone and CT of the tumour tissue.*

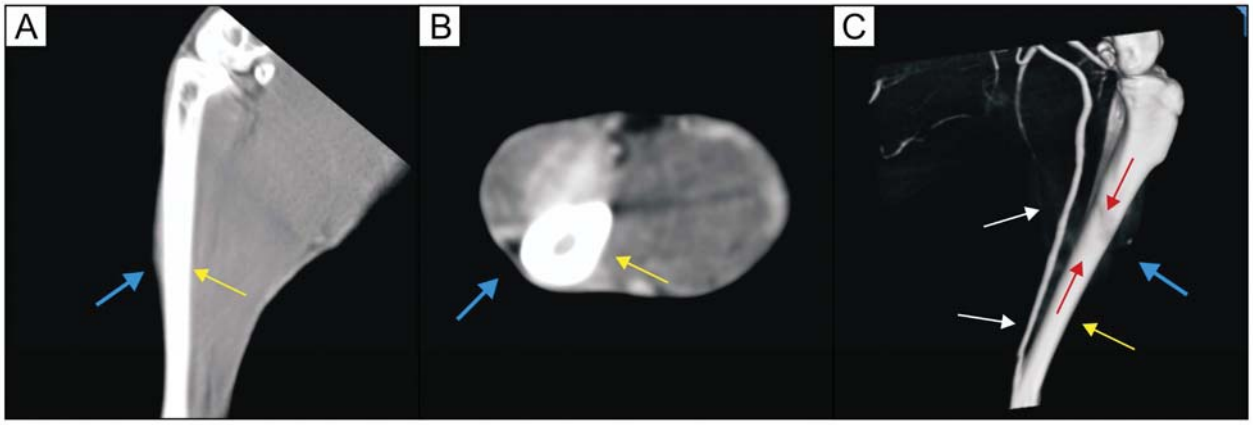

Figure 3. *FD-CT-reconstructions of the left tibia of rabbit Pi-01 eleven weeks after MDT. A: The CT-like imaging in a sagital section shows only marginal remaining tumour mass (blue arrows) left of the tibia (yellow arrows). B: The axial sections shows no distinguishable remaining tumour mass. C: VRT shows the complete reduction of the pathological vascularisation (red arrows) compared to Figure 2C. The arteria tibialis posterior (white arrows) has no larger branches at the passage from the knee to the distal tibia. The remaining tumour mass is fairly visible (blue arrows).*

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