

## Intraperitoneal Use of Doxorubicin Drug-eluting Beads in Sheep: A Pilot Safety Study

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**Abstract.** *Background: Intraperitoneal carcinomatosis and intra-abdominal tumour cell dissemination may occur with several tumour entities. At this final stage of the tumour disease, systemic chemotherapy remains only palliative and may be accompanied by severe side-effects. Alternatively, compartment-based therapy by intraperitoneal (i.p.) instillation of doxorubicin drug-eluting beads (dox-DEB) has been proposed. Here we investigated the safety, pharmacokinetics and side-effects of i.p. dox-DEB in a large animal trial. Materials and Methods: Ten black-headed meat-sheep received i.p. polyvinyl-alcohol-encapsulated doxorubicin. The plasma concentration of doxorubicin, blood count and laboratory findings were recorded and histological examination of the organs was performed. Results: After i.p. instillation, elevated serum levels of doxorubicin were obtained. After this initial phase, serum levels remained constant. Upon autopsy, no signs of systemic toxicity were detected. Beads remained within the peritoneal cavity and were not systemically distributed. However, we found significant local toxicity in five sheep, three of which died due to severe peritonitis. Conclusion: Doxorubicin can be successfully targeted to the peritoneal cavity by bead encapsulation. Local toxic effects must be controlled in order to facilitate clinical utility.*

Peritoneal carcinomatosis (PC) may occur in patients with ovarian, pancreatic, gastric and colorectal cancer (47). In spite of novel multimodal treatment options, which include systemic

chemotherapy, radiation therapy, hormonal therapy, targeted-therapy, surgical resection and hyperthermic intraperitoneal chemotherapy (HIPEC), only a few selected patients may benefit from these treatments with a full tumour remission (11, 12). The prognosis of patients suffering from PC is usually poor. The median survival rates are 5.2 months for advanced colorectal cancer and 3.1 months for advanced gastric cancer, respectively (35). PC is often the end-stage of the tumour disease; palliative systemic chemotherapy remains the only therapeutic option. This therapy may lead to longer survival (21, 30), however, systemic chemotherapy may be accompanied by severe systemic side-effects (16). One promising approach to circumvent systemic side-effects of a palliative therapy for PC is compartment-restricted therapy, which, avoids the systemic administration of chemotherapeutic agents (12). Encapsulation devices, which are loaded with chemotherapeutic agents, may help to limit the chemotherapeutic drug to the peritoneal compartment.

Drug-eluting beads (DEBs) are already in clinical use for trans-arterial chemoembolisation (TACE) of hypervascularized tumours (4). These DC-Beads® consist of a sulphonate-modified polyvinyl-alcohol-based polymer, synthesized by a suspension polymerisation reaction and formulated into beads (23). They can be easily loaded with doxorubicin (DOX). As dox-DEB they provide an accurate dosage of drug per unit volume of beads *in vitro* (23), which they are able to liberate consistently over weeks (13, 45). *In vitro*, DC-Beads are mechanically robust and are consistent of size and shape after drug loading (18). This is important, since any bead damage may rapidly liberate the encapsulated active agent and may lead to significant systemic distribution of the drug. The dox-DEB utilized in this study are composed of the same polymer material as DC-Beads which are pre-loaded with doxorubicin and lyophilized. When administered selectively into the liver vasculature proximal of the neoplasm, they embolize the

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tumour-feeding vessels and distribute high levels of the chemotherapeutic compound into the neoplasm (40).

In the treatment of peritoneal carcinomatosis, these encapsulation systems have shown a benefit in experimental animal models for PC, using murine colorectal carcinoma cells (19, 37). Milder systemic side-effects at equal tumour toxicity suggested that a sustained, controlled release with lower plasma levels could be achieved. The rationale of this study was to investigate in a large animal experiment how dox-DEB interact with the organism, how the drug distributes and whether toxic concentrations are reached. Furthermore we searched for damage in end-organs due to dox-DEB or high doxorubicin levels.

## Materials and Methods

**Drug-eluting beads.** Doxorubicin-loaded drug-eluting beads (dox-DEB; 75 mg, sizes 75-275 µm) were provided by Biocompatibles UK Ltd. (Farnham, UK).

**Animal model.** Animal experiments were performed after obtaining permission from the Regierungspräsidium Thüringen in accordance with German legislation on the protection of animals and the Guide for the Care and Use of Laboratory Animals. Ten German black-headed meat-sheep were purchased and caged at the Institute for Innovative Medicine in Beichlingen, Germany. Throughout the experiments, animals were kept with a natural night-day schedule with food *ad libitum*. Vascular access was obtained *via* the external jugular vein for the sequential blood tests. Animals were sedated by 0.7 ml 2% Xylazine (Rompun®, Bayer AG, Vienna, Austria) *i.m.* for application of the DEBs. After determination of their body weight animals were shaved and marked. Lidocaine (2%) (Belapharm, Vechta, Germany) was injected subcutaneously. For intraperitoneal instillation of the beads, a stab incision was used and a Verres-Needle (150 mm Disposable Insufflation Needle; Richard-Allan Medical Industries, Richland, MI, USA) was introduced. Intraperitoneal positioning of the needle was tested by free injectability of Ringers solution and by the 'hanging drop test'. DC-DOX beads were suspended in 40 ml water and 5 mg/kg were injected into the peritoneal cavity of each animal. Animals were visited twice daily and clinically examined for side effects. At day 28, the animals were sedated using 20 mg 2% xylazine (Rompun®, Bayer AG) *i.m.* and 200 mg ketamine (Ketavet; Parke-Davis, Berlin, Germany) and then sacrificed using 10 ml T61® (2000 mg embutramide, 500 mg mebezonium and 50 mg tetracaine; Intervet Deutschland GmbH, Merck, Whitehouse Station, NJ, USA).

**Blood samples.** Blood samples were sequentially obtained immediately before application, immediately after application and at 5, 10, 30, 60, 120 (2 h), 240 (4 h), 360 (6 h), 1440 (1 d), 2880 (2 d), 10080 (7 d), 20160 (14 d) and 40320 (28 d) min after application. The investigations were performed at the Institute for Clinical Chemistry at the University Hospital Mannheim. EDTA samples were used for a full blood count. These samples were kept at 3°C for transportation and measurements were taken within 48 h. The measurements included white blood cells (WBC), red blood cells (RBC), concentration of hemoglobin (Hb), haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets (Plt), red blood cell distribution width (RDW), basophilic

granulocytes (Baso), percentage of basophilic granulocytes in white blood cells (Baso%) using a Sysmex XE-2100 instrument (Sysmex Corporation, Kobe, Japan). For plasma parameters and doxorubicin concentration measurements, the samples were centrifuged at 2500 rcf (Hettich Rotanta, DJB Labcare Ltd., Buckinghamshire, UK). The supernatant was stored in Eppendorf vials (Eppendorf AG, Hamburg, Germany) at -20°C for transportation. Plasma parameters included sodium, potassium, bilirubin, triacylglyceride, cholesterol, alkaline phosphatase, alanine transaminase (ALT), aspartate transaminase (AST), uric acid, glucose, γ-glutamyl-transpeptidase (γGT), lactate dehydrogenase, creatinine, cholinesterase (CHE), albumin, urea, calcium and amylase. They were measured within 48 h using a Siemens Dimension Vista 1500 instrument (Siemens Healthcare Diagnostics GmbH, Eschbronn, Germany).

To measure for doxorubicin levels, DOX had to be separated from the plasma proteins and lipids *via* liquid-liquid-extraction. A precipitation with zinc and acetone had to be used since the doxorubicin showed a strong protein binding. The doxorubicin plasma concentrations were determined *via* high performance liquid chromatography-mass spectrometry (HPLC-MS). Five hundred microlitres of plasma were added to the internal standard (100 ng 25 µl of a 4 ng/µl-daunorubicin-solution, daunorubicin hydrochloride; Sigma Aldrich, St. Louis, USA). This was added to 100 µl of a 70% (0.3 M) zinc sulfate dilution. After mixing using a Vibrofix VF1 (Janke & Kunkel IKA-Werk, Staufen, Germany), 500 µl of acetone at -20°C were added. After another round of mixing and incubating for 10 min at ambient temperature, 100 µl of acetonitrile at -20°C were added. After an incubation time of 10 min, samples were centrifuged at 18500 rcf for 15 min (Abbott Laboratories, North Chicago, IL, USA). The clear supernatant was placed in Eppendorf Safe-Lock Tubes (1.5 ml; Eppendorf AG) and processed in a SpeedVac (Concentrator 5301; Eppendorf AG) where the analyte was concentrated and isolated by vaporizing under vacuum at 60°C. The processed samples were dissolved in 50 µl water and applied to the HPLC system. The direct infusion contained 0.5 mg/ml daunorubicin as an internal standard. For liquid chromatography a C8 column was used (3 µm, 2.1 mm × 100 mm, Thermo Fisher Scientific Inc., Waltham, MA, USA). The mobile phase consisted of 4 mM ammonium formate and 90% methanol (in 4 mM ammonium formate). The flow rate was set to 0.3 ml/min. The gauging in the ion trap used the multiple reaction monitoring (MRM) mode so that not only the main mass but also the fragments would be identified using tandem mass-spectrometry. The scan range of the mass spectrometer was set to m/z 250 to m/z 750, the temperature at the sprayshield was 300°C, the pressure at the nebulizer was set to 30 psi (=206,84 kPa), the dry gas flow was set to 8 l/min. The voltage used at the skimmer was 40 V, and 12 V respectively and 1.70V at the octopoles, whereas the capillary voltage was -4200 V. The evaluation of the measured samples was carried out with QuantAnalysis software Version 1.9 (Bruker Daltonics, Bremen, Germany). The external calibration with daunorubicin generated an analyte range between 2 ng/ml and 120 ng/ml. The concentrations were calculated by comparing the area under the curve of the peaks with those of the internal standard peaks. For data analysis and figure design, JMP the 9.0.0 Software (SAS Institute Inc., Cary, NC, USA) was used. The area under the curve, half-lives, maximum concentration ( $c_{max}$ ), time after the maximum concentration was reached ( $t_{max}$ ) were calculated using the Microsoft Excel 2007 (Microsoft Corporation, Redmond, Washington, USA) plug-in PK Engine (provided by the Department of Pharmacokinetics and Drug Metabolism, Allergan, Irvine, CA, USA).

**Tissue samples.** Organ samples were taken at autopsy and stored in Falcon Blue Max™ 50 ml Polypropylene Conical Tubes (BD Biosciences, San Jose, CA, USA) in 4% formaldehyde solution in a dry and cool environment. After a fixation period, haematoxylin and eosin (H&E) staining was performed according to standard protocols at the Institute for Pathology, Mannheim. Slides were examined using a ZEISS Axioplan 2 imaging with Axiophot2-attachment (Carl ZEISS AG, Oberkochen, Germany). Photo documentation was performed using a ZEISS AxioCam MRc5, supported by AxioVision40 V 4.5.0.0 software (Carl ZEISS Imaging Solutions GmbH, Freising, Germany).

## Results

At present, to our knowledge there is no published work regarding the median lethal dose (LD<sub>50</sub>) and maximum lifetime dose of tolerated doxorubicin in sheep. We had to align to doses according to experiments which had been performed in other species. The LD<sub>50</sub> of an *i.v.* bolus-injection for dogs has been described to be as low as 2.5 mg/kg, for mice 22 mg/kg, and for rats between 8 and 14 mg/kg (32). Since after *i.p.* injection of dox-DEB, a slow release of doxorubicin was expected and furthermore an unveiling of as yet unknown side-effects was intended, we chose 5 mg/kg for the sheep. This dose was applied as single *i.p.* injection. All animals survived the initial application of the dox-DEB. Seven out of ten German black-headed meat-sheep reached the end-point of 28 days survival after application (Figure 1). Six days after application, one animal developed a septic condition whereupon it had to be sacrificed early. Fifteen days after application, a second animal exhibited the same symptoms whereupon sacrifice was also carried out ahead of schedule. A third animal was found dead at day 28. Autopsy was performed on all animals either on the day 28 or earlier, at the point of death. No signs of adverse drug reaction were clinically detected in four animals. In these animals normal autopsy without pathological findings was performed (group I). Six animals (group II) had developed severe fibrinous peritonitis, with up to 3 l of ascites (Figure 2a). Fibrin was found in all four quadrants (Figure 2b).

Microscopically, these findings were confirmed. In HE histology, the *omentum majus* exhibited a surface layer of necrotic cells, with lymphocytes and foreign body cells (Figure 3). Within the peritoneal cavity, oedematous lymphatic nodes were found. The inner structure of the nodes was intact. Lymph follicles were detectable. No beads were detected outside of the peritoneal cavity. Intraperitoneally, the beads remained in contact with foreign body cells and white blood cells throughout the *omentum majus* and mesentery (Figure 3). No beads were found in any histological slides of heart, liver, spleen, kidney, jejunum, colon or mesenteric lymphatic nodes. There was no sign of systemic distribution of beads into the systemic circulation *via* blood or lymphatic vessels. In close proximity to dox-DEB, necrotic cells were detected (Figure 3). No other organ displayed signs of toxicity, macroscopically.

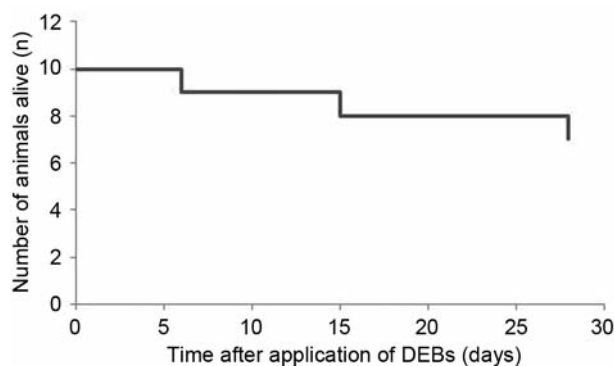


Figure 1. Kaplan-Meier survival analysis of sheep after *i.p.* instillation of doxorubicin-eluting beads (DEBs).

Specifically, no pathological findings were found in the gastrointestinal tract or in the heart. In the liver, areas of piecemeal necrosis were detectable. These were found in the proximity of dox-DEB but also unrelated in the middle of the parenchyma (Figure 4).

To search for changes in liver function ALT, AST,  $\gamma$ GT levels and CHE were determined. Concentration levels of ALT,  $\gamma$ GT and CHE did not rise. After an initial increase AST dropped to normal levels on day 14. All other serum parameters remained unchanged. All sheep exhibited a macrocytic anaemia (Figure 5), which had been compensated for on day 28. The red blood cell distribution width increased over time. In most animals, thrombocyte levels remained normal. Elevated levels of leucocytes were found with the highest levels recorded on day seven and a continuous decline until day 28. In the sheep lost early, persistent anaemia, thrombocytopenia and a persistent elevation of leucocytes were found.

Determination of doxorubicin was performed using mass spectroscopy. Doxorubicin exhibited a major peak of  $m/z$  544.1 which was fragmented into smaller masses, with the biggest peak at  $m/z$  397.1 (Figure 6). Daunorubicin was used as an internal standard. Its main mass was detected at  $m/z$  528.1, which equates molecularly to its  $H^+$ -ion. Daunorubicin itself has a molecular weight of 527.5 g/mol. The fragmental peaks were 527.1→362.9+320.9. The main peak was found at  $m/z$  362.9. The calculated area under the curve for doxorubicin for the averaged data was 1.81 mg h/ml (Figure 7a). For the specific groups, the area under the curve was calculated as: 1.65 mg h/ml for group I (Figure 7b) and 2.19 mg h/ml for group II (Figure 7b), respectively. The maximal doxorubicin concentration was reached after 60 min ( $t_{max}$ ) in group I and 30 min in group II respectively. Mean peak concentrations ( $c_{max}$ ) reached were 30.99 ng/ml in group I and 50.23 ng/ml in group II (43.65 ng/ml in the animals which survived and 73.55 ng/ml in the animals which died). To understand the liberation, distribution and excretion characteristics, three compartments were considered: the beads, the systemic blood circulation and



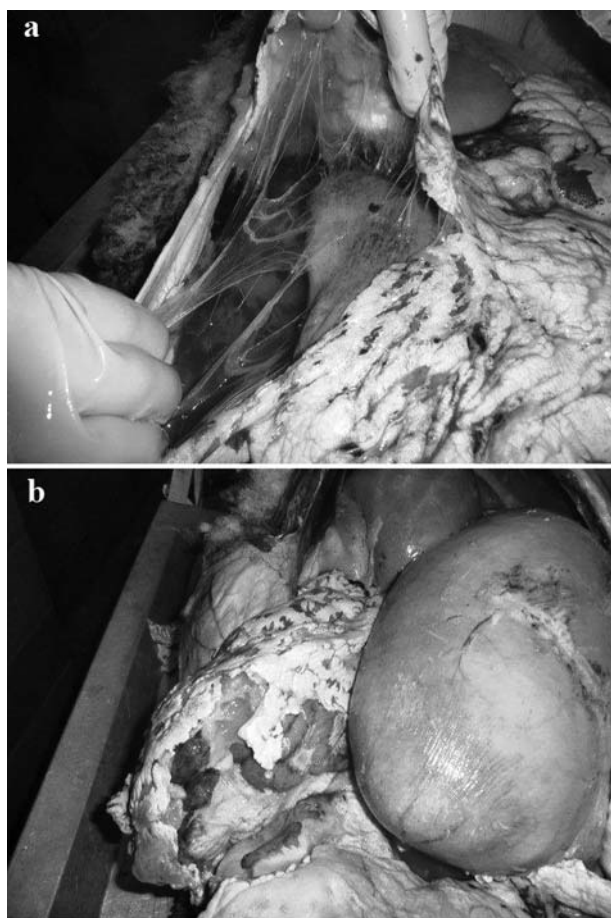


Figure 2. Autopsy 28 days after instillation of i.p. dox-DEBs a: Situs after laparotomy with the omentum majus adherent to the small bowel; 3 l of ascites were found. b: Macroscopic situs with the greater omentum and intestinal loops with fibrinous adhesions.

tissue/ascites. These three compartments were both taking up doxorubicin and liberating it into other compartments depending on concentration gradients. Considering these three phases of drug kinetics (Figure 7), three different half-lives were calculated. The first phase was dominated by liberation of doxorubicin from the beads and its distribution in the systemic blood circulation, which led to a fast rise of its plasma levels until  $c_{max}$  was reached. This phase from application until  $t_{max}$ , lasted 60 min in group I and 30 min in group II. The calculated half-life for phase I was 20 min in group I and 7 min in group II. The second phase was dominated by an accumulation of doxorubicin in tissue and ascites, which lead to a slow drop in plasma concentration; this phase, from  $t_{max}$  until reaching the steady state, lasted from 60-360 min after application in group I and 30-360 min after application in group II. The calculated half-life for phase II was 4.7 h in group I and 3.7 h in group II. In the third phase, liberation, excretion, distribution of

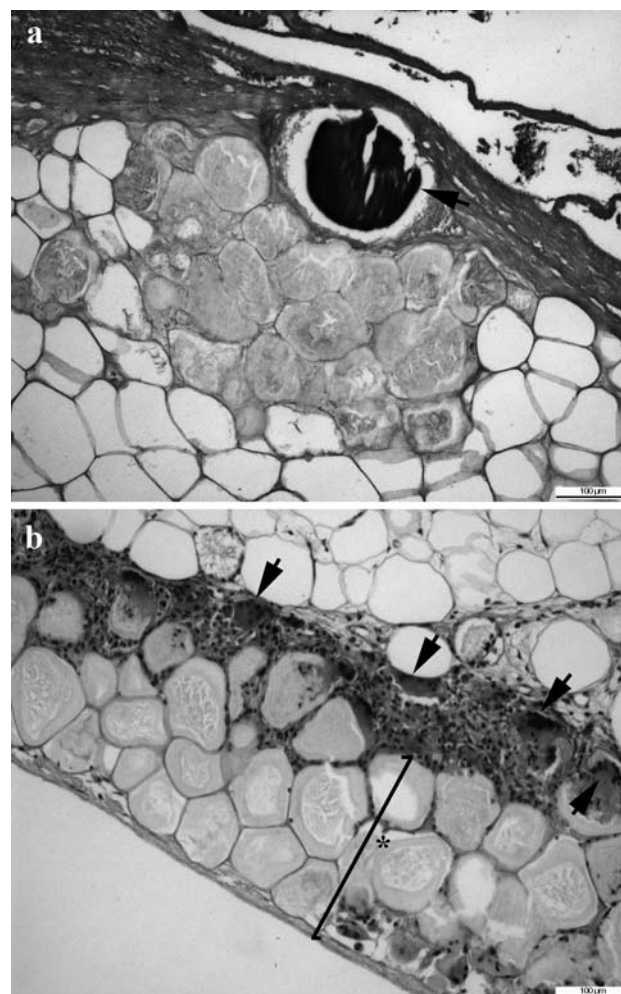


Figure 3. Adipose tissue, omentum majus, H&E stained. a: Necrotic adipocytes beneath a drug-eluting bead (arrow); b: Layer of necrotic adipocytes (\*), foreign body cells (arrows) and lymphocytes within the omentum majus. Original magnification,  $\times 75$ .

doxorubicin in tissue and re-distribution from tissue into systemic circulation balanced-out, which lead to a steady state; this phase lasted from obtaining steady state after 360 min after application until the end of the experiment. The calculated half-life was 792 h for group I and 596 h for group II.

## Discussion

Changes in the management of patients with PC have shown favourable results (8). In these new treatment regimes, the chemotherapeutic drugs are administered directly into the peritoneal cavity at the time of tumour resection (42). Only patients with medium-sized intraperitoneal tumour nodules with limited distribution within the abdomen and pelvis are

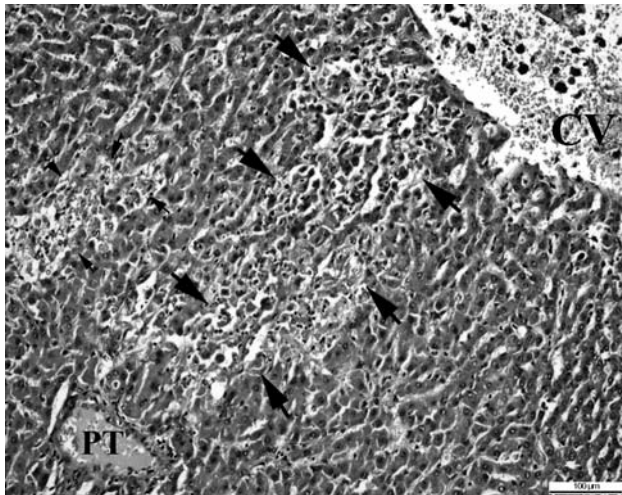


Figure 4. Hepatic tissue, H&E-stained: Piecemeal necrosis (area in arrows) in between portal triads (PT) and central vein (CV). Original magnification,  $\times 75$ .

likely to exhibit prolonged benefits (36). If PC is diagnosed at an advanced stage of disease, systemic therapy is indicated which is often accompanied by severe systemic side-effects (10, 26). One of the drugs used for the treatment of PC is doxorubicin (9, 36, 43). Doxorubicin was discovered in 1969 by Arcamone *et al.* (6). Chemically, it is a hydroxyl-derivative of daunorubicin. It intercalates with DNA like all anthracyclines (25). Inhibition of DNA-polymerase (44) and topoisomerase II (46) and production of reactive oxygen species (ROS) (14), are known modes of tumour cell toxicity. Furthermore, direct interaction and destruction of cell membranes has also been implicated (48).

At present, there are several tumour entities treated by systemic administration of doxorubicin including breast, lung and ovarian cancer (32). In superficial urothelial carcinoma of the bladder a locoregional application of doxorubicin is in clinical use (32). Approximately 20% of patients treated with doxorubicin develop side-effects, including increased urinary frequency, dysuria, hematuria, urinary infection and bladder spasms (17). The most dreaded complication however, is bladder perforation (29). This indicates a potential local toxicity of doxorubicin.

In this pilot experiment, the aim was to detect side-effects which occur with *i.p.* instillation of the dox-DEB. Therefore a dose close to the  $LD_{50}$  had to be administered. The  $LD_{50}$  is usually given as dose/body surface (BS). Since we used dose/body weight, the calculation may be facilitated by the formula of Benedict (7). Here BS of a sheep is estimated as:  $BS (m^2) = 0.124 \times \text{Weight (kg)}^{0.56}$ . The smallest sheep (43 kg) had a BS of  $1.02 m^2$  and the largest had a BS of  $1.54 m^2$  with a body weight of 89 kg. Therefore, the applied doses calculated by BS were  $210 mg/m^2$  and  $288 mg/m^2$ , which were between

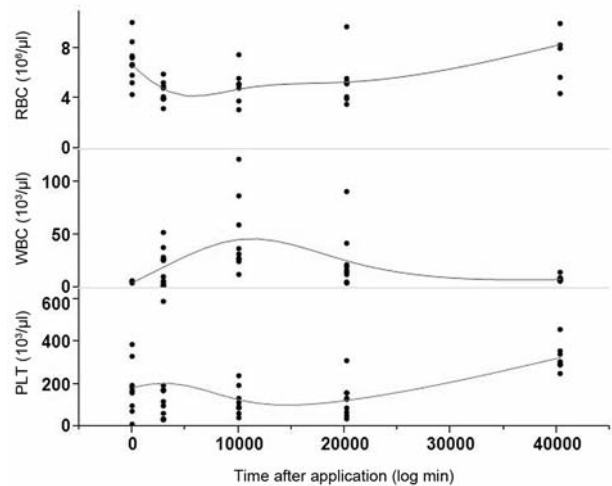


Figure 5. Red blood cell, white blood cell and platelet count over time, after *i.p.* administration of drug-eluting beads.

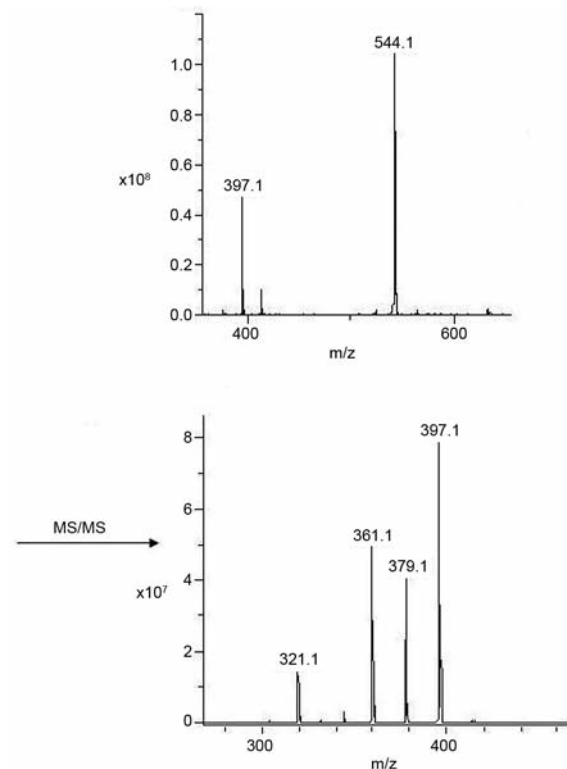


Figure 6. Fragment pattern of doxorubicin by mass spectrometry.

38 and 46% of the maximal cumulative doses suggested for humans [ $550 mg/m^2$  BS (32)]. Three out of 10 animals were lost. This showed that the dose administered was indeed close to the  $LD_{50}$  of doxorubicin for sheep.

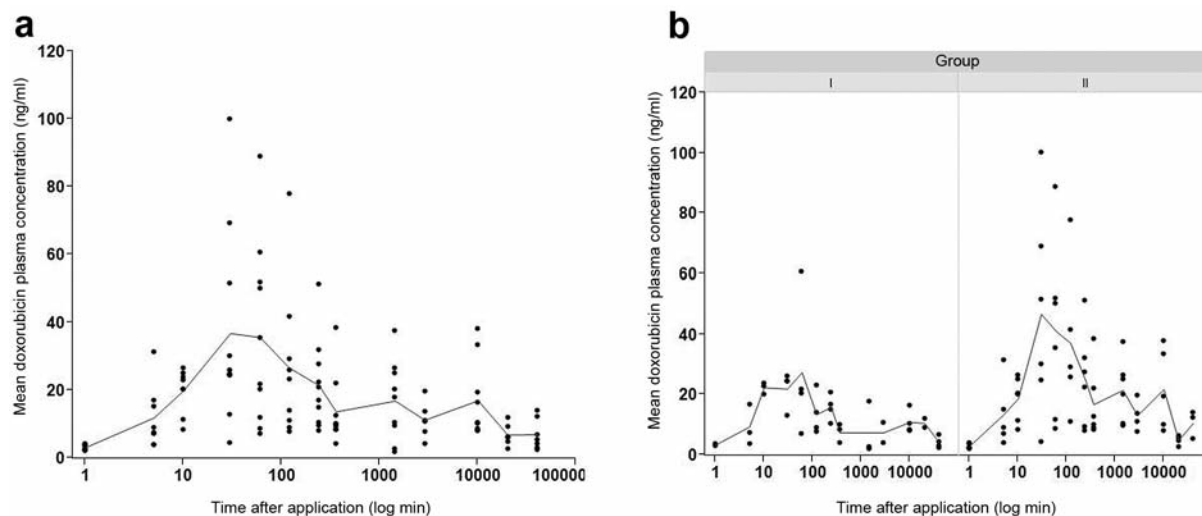


Figure 7. Concentration–time curve of doxorubicin in plasma after i.p. administration of doxorubicin-eluting DC beads. a: Mean values of all animals; b: Mean values for group I and II.

To characterize liberation of doxorubicin from beads, serum levels were determined by mass spectroscopy. One difficulty which hampered the reliability of the measurements was the plasma protein binding of doxorubicin. For nearly complete extraction, a setup with zinc sulphate and acetone was established. For the mobile phase, we chose methanol over acetone since a better linearity has been described (41). By this, the linear range remained highly reliably from 2 ng/ml to 120 ng/ml. Using a three-compartment model, the half-life of doxorubicin could be deduced. In this model, drug liberation from the beads, drug redistribution and drug excretion/metabolism were considered. As expected, after a short initial rise in serum, the doxorubicin levels remained constant. This indicated a continuous release of the doxorubicin from the drug-eluting beads.

Doxorubicin has been described to produce ROS (14) and to interact with cell membranes (20, 27, 28, 49). Therefore, it not only reduces cell proliferation, but also initiates apoptosis in non-dividing cells (50). As cardiac myocytes exhibit little expression of catalase and dismutase (which metabolize ROS), they are prone to ROS-mediated damage. Here, former findings of cardiotoxicity (1, 2, 5, 22, 34, 39) could not be confirmed. Neither macroscopically nor microscopically did the heart show any sign of toxic damage. This is surprising since the cumulative dose and the peak concentration are considered as the best predictive values of cardiotoxicity (32, 39). Clinically, there have been multiple reports on myelosuppression when doxorubicin was administered (3, 15, 32, 33). In the sheep, no clear signs of myelosuppression were found. The initial drop in red blood cell and platelet counts were compensated. Other side-effects of doxorubicin such as alopecia and reduction of general health (32), were only observed in group II, which

include the animals that died as a consequence of the peritonitis. Peritonitis has been described with the intraperitoneal use of doxorubicin (31, 38). Chemical peritonitis is the dose-limiting factor in the intraperitoneal use of anthracyclines (24, 31). Since in our experiment no bowel perforation and stool contamination were detected, we assume that peritonitis was induced chemically by the local toxicity of doxorubicin. Peritonitis was found in six animals (Figure 2a and b) and led to the death of three animals. The reason is potentially explained by the drug liberation characteristics within the animals which died. Regarding the differences in half-lives of almost 300% in the first phase and only 27% in the second phase, the main difference is to be found in liberation characteristics. Therefore liberation and not the distribution, metabolism or excretion of the drug is the important parameter. Furthermore it was remarkable that although the maximal concentration in group II was about two-fold higher than that in group I (47 ng/ml vs. 27 ng/ml), it took less time to decline. The calculated half-lives of the third phase were 791 h and 595 h respectively, and can be described as steady-state. Again concentrations in group II showed a faster decline by 33%. As the main liberation stimulators are ion exchange mechanisms (45), we assume that the intra abdominal milieu may have differed between the groups.

In summary, we conclude that dox-DEB administration into the abdominal cavity shows a good safety profile. There is no danger of bead distribution *via* blood or lymphatic vessels. We found no evidence of organ-related damage or systemic toxicity. Local toxicity of doxorubicin may, however, occur as a consequence of individual reaction. Therefore, care is indicated if dox-DEB are to be used in patients with PC. More information on specificity of



placement within the peritoneal cavity, subsequent dissemination and dose-finding studies are required. Due to tumour-related adhesions, the free distribution of the beads in the diseased peritoneal cavity may be even more limited, leading to higher local concentrations as in individuals without pathological findings in the abdominal cavity. An additional approach could also include the evaluation of other chemotherapeutic agents, such as irinotecan.

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## References

- 1 Abraham R, Bassar RL and Green MD: A risk-benefit assessment of anthracycline antibiotics in antineoplastic therapy. *Drug Saf* 15: 406-429, 1996.
- 2 Abu-Khalaf MM and Harris L: Anthracycline-induced cardiotoxicity: risk assessment and management. *Oncology* 23: 239, 244, 252, 2009.
- 3 Ackland SP, Ratain MJ, Vogelzang NJ, Choi KE, Ruane M and Sinkule JA: Pharmacokinetics and pharmacodynamics of long-term continuous-infusion doxorubicin. *Clin Pharmacol Ther* 45: 340-347, 1989.
- 4 Aliberti C, Benea G, Tilli M, and Fiorentini G: Chemoembolization (TACE) of unresectable intrahepatic cholangiocarcinoma with slow-release doxorubicin-eluting beads: preliminary results. *Cardiovasc Intervent Radiol* 31: 883-888, 2008.
- 5 Appel JM, Nielsen D, Zerahn B, Jensen BV and Skagen K: Anthracycline-induced chronic cardiotoxicity and heart failure. *Acta Oncol* 46: 576-580, 2007.
- 6 Arcamone F, Cassinelli G, Fantini G, Grein A, Orezzi P, Pol C and Spalla C: Adriamycin, 14-hydroxydaunomycin, a new antitumor antibiotic from *S. peucetius* var. *caesius*. *Biotechnol Bioeng* 11: 1101-1110, 1969.
- 7 Benedict FG: Ueber die den Energieumsatz bei Schafen beeinflussenden Faktoren. *Arch, für Tierern und Tierzucht*: 5, 1931.
- 8 Cao C, Yan TD, Black D and Morris DL: A systematic review and meta-analysis of cytoreductive surgery with perioperative intraperitoneal chemotherapy for peritoneal carcinomatosis of colorectal origin. *Ann Surg Oncol* 16: 2152-2165, 2009.
- 9 Deraco M, Virzi S, Iusco DR, Puccio F, Macri A, Famulari C, Solazzo M, Bonomi S, Grassi A, Baratti D and Kusamura S: Secondary cytoreductive surgery and hyperthermic intraperitoneal chemotherapy for recurrent epithelial ovarian cancer: a multi-institutional study. *Bjog* 119: 800-809, 2012.
- 10 Esquivel J, Sticca R, Sugarbaker P, Levine E, Yan TD, Alexander R, Baratti D, Bartlett D, Barone R, Barrios P, Bieligg S, Bretcha-Boix P, Chang CK, Chu F, Chu Q, Daniel S, de Bree E, Deraco M, Dominguez-Parra L, Elias D, Flynn R, Foster J, Garofalo A, Gilly FN, Glehen O, Gomez-Portilla A, Gonzalez-Bayon L, Gonzalez-Moreno S, Goodman M, Gushchin V, Hanna N, Hartmann J, Harrison L, Hoefer R, Kane J, Kecmanovic D, Kelley S, Kuhn J, Lamont J, Lange J, Li B, Loggie B, Mahteme H, Mann G, Martin R, Misihi RA, Moran B, Morris D, Onate-Ocana L, Petrelli N, Philippe G, Pingpank J, Pitroff A, Piso P, Quinones M, Riley L, Rutstein L, Saha S, Alrawi S, Sardi A, Schneebaum S, Shen P, Shibata D, Spellman J, Stojadinovic A, Stewart J, Torres-Melero J, Tuttle T, Verwaal V, Villar J, Wilkinson N, Younan R, Zeh H, Zoetmulder F and Sebbag G: Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy in the management of peritoneal surface malignancies of colonic origin: a consensus statement. *Society of Surgical Oncology. Ann Surg Oncol* 14: 128-133, 2007.
- 11 Glockzin G, Ghali N, Lang SA, Agha A, Schlitt HJ and Piso P: Peritoneal carcinomatosis. Surgical treatment, including hyperthermal intraperitoneal chemotherapy. *Chirurg* 78: 1100, 1102-1106, 1108-1110, 2007, original article in German.
- 12 Glockzin G, Schlitt HJ and Piso P: Peritoneal carcinomatosis: patients selection, perioperative complications and quality of life related to cytoreductive surgery and hyperthermic intraperitoneal chemotherapy. *World J Surg Oncol* 7: 5, 2009.
- 13 Gonzalez MV, Tang Y, Phillips GJ, Lloyd AW, Hall B, Stratford PW and Lewis AL: Doxorubicin eluting beads-2: methods for evaluating drug elution and *in vitro*:*in vivo* correlation. *J Mater Sci Mater Med* 19: 767-775, 2008.
- 14 Goodman J and Hochstein P: Generation of free radicals and lipid peroxidation by redox cycling of adriamycin and daunomycin. *Biochem Biophys Res Commun* 77: 797-803, 1977.
- 15 Gottlieb JA, Gutterman JU, McCredie KB, Rodriguez V and Frei E, 3rd: Chemotherapy of malignant lymphoma with adriamycin. *Cancer Res* 33: 3024-3028, 1973.
- 16 Griffin AM, Butow PN, Coates AS, Childs AM, Ellis PM, Dunn SM and Tattersall MH: On the receiving end. V: Patient perceptions of the side effects of cancer chemotherapy in 1993. *Ann Oncol* 7: 189-195, 1996.
- 17 Huang JS, Chen WH, Lin CC, Liaw CC, Wang CH, Lan YJ, Lai CH and Liu JP: A randomized trial comparing intravesical instillations of mitoxantrone and doxorubicin in patients with superficial bladder cancer. *Chang Gung Med J* 26: 91-97, 2003.
- 18 Jordan O, Denys A, De Baere T, Boulens N and Doelker E: Comparative study of chemoembolization loadable beads: *in vitro* drug release and physical properties of DC bead and hepasphere loaded with doxorubicin and irinotecan. *J Vasc Interv Radiol* 21: 1084-1090, 2010.
- 19 Keese M, Gasimova L, Schwenke K, Yagublu V, Shang E, Faissner R, Lewis A, Samel S and Lohr M: Doxorubicin and mitoxantrone drug eluting beads for the treatment of experimental peritoneal carcinomatosis in colorectal cancer. *Int J Cancer* 124: 2701-2708, 2009.
- 20 Kessel D: Enhanced glycosylation induced by adriamycin. *Mol Pharmacol* 16: 306-312, 1979.
- 21 Klaver YL, Simkens LH, Lemmens VE, Koopman M, Teerenstra S, Bleichrodt RP, de Hingh IH and Punt CJ: Outcomes of colorectal cancer patients with peritoneal carcinomatosis treated with chemotherapy with and without targeted therapy. *Eur J Surg Oncol* 38: 617-623, 2012.
- 22 Klein HO: Anthracycline-induced cardiotoxicity. *Ann Intern Med* 126: 827; author reply 827-828, 1997.

- 23 Lewis AL, Gonzalez MV, Lloyd AW, Hall B, Tang Y, Willis SL, Leppard SW, Wolfenden LC, Palmer RR and Stratford PW: DC bead: *in vitro* characterization of a drug-delivery device for transarterial chemoembolization. *J Vasc Interv Radiol* 17: 335-342, 2006.
- 24 Litterst CL, Collins JM, Lowe MC, Arnold ST, Powell DM and Guarino AM: Local and systemic toxicity resulting from large-volume ip administration of doxorubicin in the rat. *Cancer Treat Rep* 66: 157-161, 1982.
- 25 Manfait M, Alix AJ, Jeannesson P, Jardillier JC and Theophanides T: Interaction of adriamycin with DNA as studied by resonance Raman spectroscopy. *Nucleic Acids Res* 10: 3803-3816, 1982.
- 26 Moehler M, Al-Batran SE, Andus T, Anthuber M, Arends J, Arnold D, Aust D, Baier P, Baretton G, Bernhardt J, Boeing H, Bohle E, Bokemeyer C, Bornschein J, Budach W, Burmester E, Caca K, Diemer WA, Dietrich CF, Ebert M, Eickhoff A, Ell C, Fahlke J, Feussner H, Fietkau R, Fischbach W, Fleig W, Flentje M, Gabbert HE, Galle PR, Geissler M, Gockel I, Graeven U, Grenacher L, Gross S, Hartmann JT, Heike M, Heinemann V, Herbst B, Herrmann T, Hocht S, Hofheinz RD, Hofer H, Hohler T, Holscher AH, Horneber M, Hubner J, Izbicki JR, Jakobs R, Jenssen C, Kanzler S, Keller M, Kiesslich R, Klautke G, Korber J, Krause BJ, Kuhn C, Kullmann F, Lang H, Link H, Lordick F, Ludwig K, Lutz M, Mahlberg R, Malfertheiner P, Merkel S, Messmann H, Meyer HJ, Monig S, Piso P, Pistorius S, Porschen R, Rabenstein T, Reichardt P, Ridwelski K, Rocken C, Roetzer I, Rohr P, Schepp W, Schlag PM, Schmid RM, Schmidberger H, Schmiegel WH, Schmoll HJ, Schuch G, Schuhmacher C, Schutte K, Schwenk W, Selgrad M, Sendler A, Seraphin J, Seufferlein T, Stahl M, Stein H, Stoll C, Stuschke M, Tannapfel A, Tholen R, Thuss-Patience P, Tremel K, Vanhoefer U, Vieth M, Vogelsang H, Wagner D, Wedding U, Weimann A, Wilke H and Wittekind C: German S3-guideline "Diagnosis and treatment of esophagogastric cancer". *Z Gastroenterol* 49: 461-531, 2011, original article in German.
- 27 Murphree SA, Cunningham LS, Hwang KM and Sartorelli AC: Effects of adriamycin on surface properties of sarcoma 180 ascites cells. *Biochem Pharmacol* 25: 1227-1231, 1976.
- 28 Murphree SA, Tritton TR, Smith PL and Sartorelli AC: Adriamycin-induced changes in the surface membrane of sarcoma 180 ascites cells. *Biochim Biophys Acta* 649: 317-324, 1981.
- 29 Oddsens JR, van der Meijden AP and Sylvester R: One immediate postoperative instillation of chemotherapy in low risk Ta, T1 bladder cancer patients. Is it always safe? *Eur Urol* 46: 336-338, 2004.
- 30 Oneyama M, Sekikawa K, Goto M, Kitamura M, Kawahara Y, Ohta R, Takahashi Y, Hirata Y, Nakayama M and Shimoda Y: A long-surviving patient with unresectable advanced gastric cancer treated with S-1 and biweekly paclitaxel combination chemotherapy as second-line treatment. *Gan To Kagaku Ryoho* 39: 1131-1133, 2012, original article in Japanese.
- 31 Ozols RF, Young RC, Speyer JL, Sugarbaker PH, Greene R, Jenkins J and Myers CE: Phase I and pharmacological studies of adriamycin administered intraperitoneally to patients with ovarian cancer. *Cancer Res* 42: 4265-4269, 1982.
- 32 Pfizer: Adriblastin - Fachinformation. 2009. Rote Liste Service GmbH, Berlin, original statement in German.
- 33 Piscitelli SC, Rodvold KA, Rushing DA and Tewksbury DA: Pharmacokinetics and pharmacodynamics of doxorubicin in patients with small cell lung cancer. *Clin Pharmacol Ther* 53: 555-561, 1993.
- 34 Rodrigues M, Ferreira R and Marques MS: Anthracycline cardiotoxicity. Review of the literature and presentation of a study protocol. *Rev Port Cardiol* 8: 871-876, 1989, original article in Portuguese.
- 35 Sadeghi B, Arvieux C, Glehen O, Beaujard AC, Rivoire M, Baulieux J, Fontaumard E, Brachet A, Caillot JL, Faure JL, Porcheron J, Peix JL, Francois Y, Vignal J and Gilly FN: Peritoneal carcinomatosis from non-gynecologic malignancies: results of the EVOCAPE 1 multicentric prospective study. *Cancer* 88: 358-363, 2000.
- 36 Salti GI, Ailabouni L and Undevia S: Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy for the treatment of peritoneal sarcomatosis. *Ann Surg Oncol* 19: 1410-1415, 2012.
- 37 Samel S, Keese M, Lux A, Jesnowski R, Proszt R, Saller R, Hafner M, Sturm J, Post S, and Lohr M: Peritoneal cancer treatment with CYP2B1 transfected, microencapsulated cells and ifosfamide. *Cancer Gene Ther* 13: 65-73, 2006.
- 38 Schmoll H, Possinger: Kompendium Internistische Onkologie: Standards in Diagnostik und Therapie. Therapiekonzepte maligner Tumoren. Heidelberg: Springer Medizin Verlag, 2006.
- 39 Shan K, Lincoff AM, and Young JB: Anthracycline-induced cardiotoxicity. *Ann Intern Med* 125: 47-58, 1996.
- 40 Skowasch M, Schneider J, Otto G, Weinmann A, Woerns MA, Dueber C and Pitton MB: Midterm follow-up after DC-BEAD-TACE of Hepatocellular Carcinoma (HCC). *Eur J Radiol* 2012.
- 41 Sproll C: Quantitative Analyse von Sudanfarbstoffen mittels HPLC-DAD und HPLC-MS/MS in unterschiedlichen Lebensmittelmatrizen. (Veterinäruntersuchungsamt C ed.). Karlsruhe, 2009.
- 42 Sugarbaker PH: Management of peritoneal-surface malignancy: the surgeon's role. *Langenbecks Arch Surg* 384: 576-587, 1999.
- 43 Sugarbaker PH, Van der Speeten K, Anthony Stuart O and Chang D: Impact of surgical and clinical factors on the pharmacology of intraperitoneal doxorubicin in 145 patients with peritoneal carcinomatosis. *Eur J Surg Oncol* 37: 719-726, 2011.
- 44 Tanaka M and Yoshida S: Mechanism of the inhibition of calf thymus DNA polymerases alpha and beta by daunomycin and adriamycin. *J Biochem* 87: 911-918, 1980.
- 45 Taylor RR, Tang Y, Gonzalez MV, Stratford PW and Lewis AL: Irinotecan drug eluting beads for use in chemoembolization: *in vitro* and *in vivo* evaluation of drug release properties. *Eur J Pharm Sci* 30: 7-14, 2007.
- 46 Tewey KM, Rowe TC, Yang L, Halligan BD and Liu LF: Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II. *Science* 226: 466-468, 1984.
- 47 Tischoff I and Tannapfel A: Pathologic and anatomic evidence of peritoneal metastases. *Chirurg* 78: 1085-1086, 1088-1090, 2007.
- 48 Tritton TR and Yee G: The anticancer agent adriamycin can be actively cytotoxic without entering cells. *Science* 217: 248-250, 1982.
- 49 Tritton TR, Murphree SA and Sartorelli AC: Adriamycin: a proposal on the specificity of drug action. *Biochem Biophys Res Commun* 84: 802-808, 1978.
- 50 Tritton TR, Yee G and Wingard LB Jr.: Immobilized adriamycin: a tool for separating cell surface from intracellular mechanisms. *Fed Proc* 42: 284-287, 1983.

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