7 Summary

Investigation of accumulation of liposomes containing both contrast agent and anti-cancer drug in the liver of WAG/Rij rats

A crucial problem for treatment of liver metastases is the question, whether the therapeutical agent reaches the tumor bearing organ, or ideally the tumor itself. Therefore the aim of this study was the use of magnetic resonance imaging (MRI) methods for the monitoring of the accumulation of the anti-cancer drug .
In a new approach the cytostatic agent 5-fluorouracil (5-FU) was encapsulated into stealth® liposomes (SUV-PEG) together with Gd-DTPA as an MRI contrast agent (5-FU-Gd-SUV-PEG).

To simulate human liver metastases of colorectal carcinomas, CC531 adenocarcinoma cells were implanted into the liver of male WAG/Rij rats. Before and after the treatment with 5-FU-Gd-SUV-PEG i.v., i.a. and i.a. together with degradable starch microspheres (DSM) the animals were examined with proton density and t1-weighted MRI techniques at 2.35 T (BRUKER biospec 24/40). Relative signal intensities of liver and tumor tissue were determined. After sacrifying animals concentrations of 5-FU and its active intracellular metabolite M3 were measured for tumor, liver, spleen, stomach, pancreas, kidney, lung, heart and serum by high performance liquid chromatography (HPLC) after 15, 30, 30, 45, 90, 120 min, 4, 8, 12, 24 and 48 h. The data of liver and tumor were correlated with the relative MR signal intensities.
To assess influences of 5-FU and liposomes on the relative signal intensities, control measurements were performed: a) Gd-DTPA-encapsulated liposomes not containing 5-FU (Gd-SUV-PEG) were applied i.v., i.a., and i.a. with embolisation; b) Gd-DTPA was applied i.v. without liposomes. To asses the influence of Gd-DTPA on the pharmacocinetics of 5-FU, data of any time point of 5-FU-Gd-SUV-PEG groups were compared with data of historical 5-FU-SUV-PEG collectives.
For statistical testing a correlation matrix and analyses of variance were used. P levels $< 0,05$ were considered significant.

In the group with embolisation (group 7) the highest correlation between the relative signal intensity and 5-FU concentration in tumor tissue was found between 90 min and 4 h ($c = 0.82$). In the i.a. group without embolisation (group 5) a high correlation was found at 60 min ($c = 0.84$) and at 4 h ($c = 0.92$). No correlation was found for the i.v. group (group 2).
The highest concentrations of 5-FU between 15 min and 48 h were measured in the tumor tissue after regional chemoembolisation (group 7) while concentrations were much lower in the liver and other organs. In this group some animals exhibited high levels of M3 in

tumor tissue. The influence of bound contrast agent may be one reason that lower levels of M3 were found in other animals of the same group.
In the i.a. group with embolisation without 5-FU (group 6) the signal intensity in tumor tissue was 166 % that of liver tissue. In the same group with 5-FU (group 7) a delayed signal intensity increase was observed probably because of the step by step release of 5-FU by DSM. Between 15 and 120 min both i.a. groups with embolisation (with and without 5-FU; groups 6 and 7) showed an strongly increased tumor signal compared to all other groups ($p < 0.05$). In contrast, after i.v. (groups 1 and 2) and i.a application of Gd-DTPA liposomes without embolisation (groups 4 and 5) a slight signal intensity increase was observed in the liver tissue only. After i.v. application of Gd-DTPA without liposomes (groups 3 and 8) animals showed similar signal intensities in liver and tumor independent of the contrast agent concentration (33 and 250 µmol/kg).

In conclusion, a high correlation between 5-FU tumor tissue concentration determined by HPLC and MRI signal intensities of 5-FU-Gd-SUV-PEG in tumor tissue was found in the i.a. group with embolisation between 90 and 240 min after application. Therefore, the accumulation of 5-FU in tumor tissue may be estimated by non-invasive MRI. However, an adverse effect of Gd-DTPA bearing liposomes on their efficacy to transfer 5-FU to the target tumor and/or on the metabolization from 5-FU to M3 may be concluded from lacking correlation between MRI signal intensities and M3 in the same groups of animals. Future investigations have to show whether completely encapsulated Gd-DTPA will lead to an increased accumulation of the cytostatic agent in the cells.
Furthermore, the strong contrast between liver and tumor tissue after hepatoarterial application with regional embolisation increased the diagnostic sensitivity, since it is often difficult to differentiate between liver tissue and metastases. Whether this is also true after i.v. application of an higher dose encapsulated Gd-DTPA has to be investigated.
The described techniques may improve clinical diagnoses of animals as well as to help to reduce the number of animals to be sacrified in pharmacological trials.