Evaluation of Efficient Chemoembolization Mixtures by Magnetic Resonance Imaging Therapy Monitoring: An Experimental Study on the VX2 Tumor in the Rabbit Liver

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ABSTRACT

To find effective chemoembolization mixtures, we tested combinations of carboplatin with the embolizates Spherex and Gelfoam in comparison to a therapy with NaCl-solution, a treatment with the cytostatic drug only, and a therapy with each of the embolizates alone. The experiments were carried out using as a model the VX2 tumor in the liver of male chinchilla rabbits (five for each group). Carboplatin was revealed by the 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide test to be a potent cytostatic drug for VX2 rabbit tumor cells. We used magnetic resonance imaging to examine the tumor volume and signal intensity enhancement up to 15 min after Gd-DTPA administration within the tumor and liver before and after the different therapies. These parameters allowed us to evaluate tumor growth and vitality as well as liver injury for the different therapy types. The results found by magnetic resonance imaging corresponded very well to those obtained by histological analysis of the tumors. The chemoembolization therapies were significantly more efficient than the other therapies, as indicated by the reduction of signal intensity enhancement after contrast agent administration within the tumor and by the histologically determined necrotic fraction after therapy. In addition, we found a significant decrease of the tumor volume and no significant liver injury for a therapy with Carboplatin and Gelfoam.

INTRODUCTION

The liver is the most common site of long distance metastasis after potentially curative resection of gastrointestinal carcinomas (1–4). Surgical resection of liver metastases of colorectal carcinomas is possible in only 5–20% of the cases (5–7); therefore, it is very important to make their chemotherapy as effective as possible. Three main approaches are currently under investigation to improve chemotherapy: transport by 'tumor-specific' drug carriers, locoregional injection of the cytostatic drug, and locoregional application of an embolizate together with a cytostatic drug. Chemoembolization in combination with cytostatic drugs is an established palliative therapy for liver tumors (8–10), but the search for the best embolizate-cytostatic mixture is still in progress. A favorable mixture should simultaneously achieve the following: a) short-term ischemia of the tumor; b) long contact between the tumor tissue and the cytostatic drug; and c) low injury to the liver parenchyma. The VX2 carcinoma implanted in the rabbit liver is an established model for investigation of chemoembolization therapies of hepatocellular carcinomas (11). In this study, we used Gd-DTPA-enhanced MRI3 to examine rabbits with the VX2 carcinoma for the therapeutic efficacy of each of the embolizates Spherex and Gelfoam combined with the chemotherapeutic agent carboplatin in comparison with Gelfoam, Spherex, and carboplatin alone.

1 The abbreviations used are: MRI, magnetic resonance imaging; SI, signal intensity; MTT, 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide.

The experimental design was as follows: a) checking the efficacy of carboplatin as a potent cytostatic drug against VX2-tumor cells by the MTT test; b) comparing the efficacy of the two chemoembolization mixtures in reducing tumor volume and vitality by dynamic Gd-DTPA-enhanced MRI therapy monitoring; and c) evaluating the last- ing of signal intensity enhancement within the tumor after locoregional injection of Gd-DTPA and the respective embolizate.

MATERIALS AND METHODS

Drugs. Carboplatin (Carboplat) was purchased from Bristol Arzneimittel GmbH (Munich, Germany). Spherex (DSM) degradable starch microspheres with a diameter of 30–50 μm were provided by Pharmacia (Uppsala, Sweden), and Gelfoam (diameter of the particles, 90 μm) was obtained from Upjohn (Kalamazoo, MI). Gd-DTPA/dimeglumine (Magnevist) was obtained from Schering (Berlin, Germany).

Assay of Cell Proliferation. Rabbit VX2 tumor (12–14) suspension was obtained from the German Cancer Center (Heidelberg) and passaged as described earlier (15). The carcinoma cells were derived from a solid tumor [squamous cell carcinoma (16, 17)] and grown in MEM (Life Technologies, Inc., Egggenstein, Germany) supplemented with 10% fetal calf serum (Biochrom), 2 mm l-glutamine, 1% nonessential amino acids, and 1% Antibiotic-Antimycotic 1008 (Life Technologies, Inc.). Cells (5 × 105) were seeded in 96-well plates and used for the MTT test after 24 hours.

MTT Assay. The colorimetric MTT assay (Ref. 18, Sigma Chemical Co., Deisenhofen, Germany) was used to determine tumor cell growth inhibition by different amounts of carboplatin (0.1–50 μg/ml). After incubation periods of 24, 48, and 72 h, 10 μl of a 5 mg/ml stock solution of MTT were added to each well plate and incubated for 3 hours at 37°C. The supernatant was aspirated from the wells, and formazan crystals were dissolved in 100 μl DMSO (Sigma). The absorbance was directly recorded on an ELISA reader/96-multiscaner (SLT, Zepernick, Germany) at a wavelength of 540 nm. The reduction in cell number was calculated from the absorbance given in percent. Carboplatin did not show any direct MTT reduction.

% reduction in cell number =

\[
\frac{\text{absorbance of cells + carboplatin}}{- (\text{absorbance of cells without carboplatin})} \times 100
\]

Animal Model. Tumors were induced by injection of 1 × 107 to 5 × 107 viable VX2 carcinoma cells into the left lobe of the liver of male chinchilla rabbits weighing 2800–3200 g. Anesthesia for tumor implantation and MRI was induced by injection of a mixture of 5 mg xylazine (Bayer AG, Leverkusen, Germany) and 50 mg ketamine hydrochloride (Parke-Davis, Berlin, Germany) per kg body weight. Five male chinchilla rabbits were examined for each therapy group (Table 1).

For locoregional administration, the therapy mixtures were injected through the proper hepatic artery by a catheter and implanted into the gastroduodenal artery. The animals were investigated by MRI 14 days after tumor implantation, as well as before and 7 days after therapy. After completion of the MRI experiments, the animals were sacrificed under deep anesthesia by intravenous injection of 2 ml T61. The tumor-bearing liver was dissected, and after careful removal from the liver tissue, the tumors were fixed in 5% formaldehyde solution for histological preparation. Experiments for evaluating the dwell time of a contrast agent within the
Table 1 Drug administration in the different therapy groups

<table>
<thead>
<tr>
<th>Therapy group</th>
<th>Drug administration</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Control group, injection of 5 ml 0.9% NaCl solution</td>
</tr>
<tr>
<td>2</td>
<td>Injection of 60 mg Spherex in 5 ml 0.9% NaCl solution</td>
</tr>
<tr>
<td>3</td>
<td>Injection of 10 mg Gelfoam in 5 ml 0.9% NaCl solution</td>
</tr>
<tr>
<td>4</td>
<td>Injection of 50 mg carboplatin in 5 ml 0.9% NaCl solution</td>
</tr>
<tr>
<td>5</td>
<td>Injection of 50 mg carboplatin and 60 mg Spherex in 5 ml 0.9% NaCl solution</td>
</tr>
<tr>
<td>6</td>
<td>Injection of 50 mg carboplatin and 10 mg Gelfoam in 5 ml NaCl solution</td>
</tr>
</tbody>
</table>

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therapy were measured by regions of interest within the whole area of the tumor, the necrotic center, the vital tumor edge, and the surrounding liver tissue. From these values, relative signal intensities ($SI_{rel}$) were calculated according to the following equation:

$$SI_{rel} = \frac{SI_{tot}}{SI_{pre}/SI_{necrotic}}$$

For volumetric analysis, the tumor area of every slice was measured and multiplied by the slice thickness. The whole tumor volume was estimated by addition of these slice volumes.

Statistical Analysis. Randomization of the tumor volumes before therapy was checked by the Kruskal-Wallis test (19). The significance of tumor growth differences after the investigation therapies in comparison to the therapy with 0.9% NaCl solution was determined by the Mann-Whitney test (19). The significance of differences in the necrosis fraction between the different therapy groups was ascertained by the Mann-Whitney test. We calculated the significance of distinction between the therapy groups, we used a one-way analysis of variance with the Student-Newman-Keuls test (19).

RESULTS

Assay of Cell Proliferation. The MTT colorimetric assay (20) uses the general principle involved in the detection of cell growth or cell death based on the selective ability of living cells to reduce the yellow water-soluble salt MTT to a purple-blue insoluble formazan precipitate that can be detected by spectrophotometry. The formation of formazan takes place via intact mitochondria, although other extracellular locations with dehydrogenase activity may contribute to the total formazan production (20).

Our experiments (repeated three times) demonstrated that, at a concentration of 10 $\mu$g/ml over a period of 72 hours, carboplatin caused a significantly inhibited growth (92%) of VX2-tumor cells (see Fig. 1).

MRI Experiments to Evaluate the Efficacy of Chemoembolization. Differentiation of internal tumor structure and liver perfusion was only possible after injection of the contrast medium as shown in Fig. 2. Fig. 3 shows the time dependence of signal enhancement of the whole tumor area and over a region of interest within the liver of selected slices after injection of Gd-DTPA for the different therapy types. Calculation of the SI differences within the whole area of the tumors at the investigated time points before and 7 days after therapy.
yielded a significant distinction between the chemoembolization therapies ($P = 0.014$) and all other groups. The reductions of SI enhancement after administration of the contrast agent after the different types of therapy are given in Table 2 and Fig. 3a. It is obvious that the mixture of carboplatin and Gelfoam is the most effective with respect to SI enhancement reduction within the tumor after therapy. There is no significant difference in the time course of SI enhancement after contrast agent injection within the liver for a therapy of carboplatin and Gelfoam in comparison with the control group and the monotherapies as shown in Fig. 3b. In contrast, the therapy with Spherex and carboplatin caused a significant difference in SI enhancement, comparing the contrast agent dynamics before and after therapy.

Fig. 4 shows examples of typically analyzed MR images acquired before and 7 days after therapy with carboplatin (Fig. 4b), with Spherex and carboplatin (Fig. 4c), with Gelfoam and carboplatin (Fig. 4d), and without therapy (Fig. 4e). The images shown are those obtained immediately after i.v. injection of Magnevist. Nearly 100% necrosis is evident 7 days after the chemoembolization therapies. We found expansive tumor growth for the control group and tumor growth for all therapy groups except that with carboplatin and Gelfoam, in which we could measure a tumor reduction (Table 2). The tumor volumes of the animals from the different therapy groups were not significantly different before therapy; this was statistically proven by the Kruskal-Wallis test.

**Histology.** The histologically determined necrosis fractions of the tumors (mean values for the different therapy types are given in Table 2) corresponded well with the extended necrosis observed in MR images after a chemoembolization therapy and thus with the calculated SI differences before and after therapy. For therapy with carboplatin and Spherex, we likewise observed areas of liver cell necrosis, which corresponded well with the higher difference between SI enhancement before and after therapy detected by MRI.

**MRI Experiments on the Duration of SI Enhancement after Administration of Embolizate and Contrast Agent.** Fig. 5A shows the time course of signal enhancement within the liver and the VX2 tumor after locoregional injection of 0.17 mmol/kg body weight Magnevist compared to that after locoregional injection of the same dosage Magnevist and 60 mg Spherex (Fig. 5B) and after injection of Magnevist and 10 mg Gelfoam (Fig. 5C). A solution volume of 2 ml was used for all injections. After administration of Magnevist with an embolizate, we found a significantly longer duration of signal enhancement than with Magnevist alone, indicating that even low-molecular substances were retained within the tumor, if they are injected locoregionally together with an embolizate. We were surprised to observe a slightly longer duration of signal enhancement of the liver for Gelfoam than for Spherex because the therapy experiments yielded a stronger liver injury for Spherex.

**DISCUSSION**

Because the temporal change of signal intensity in magnetic resonance images after administration of a contrast agent is related to the local capillary blood supply and the extravasation of the contrast agent into the surrounding tissue, MRI can be used for the assessment of tissue microcirculation (21–23). The diffusion behavior of the contrast agent gives an indication of the necrotic fraction of the tumor. The higher the enhancement immediately after contrast agent injection and the higher the subsequent decrease of the enhanced signal intensity, the more vital the tumor. The time course of SI enhancement of the control group, which received locoregional administration of NaCl solution, characterized the normal tumor development with an increasing necrotic fraction. For the therapy with Gelfoam and carboplatin, we have found the highest difference in SI enhancement after contrast agent injection between the measurement before and after therapy, and we determined 100% necrosis histologically (Table 2).

In conclusion, dynamic Gd-DTPA-enhanced MRI therapy monitoring is well suited to noninvasively evaluate the efficacy of different chemoembolization mixtures. Furthermore, MRI is a valuable tool for determining the dwell time of a substance with the tumor caused by injection of an embolizate and a contrast agent simultaneously. Our findings indicated that chemoembolization by carboplatin with
Table 2. Comparison of results from MRI (reduction of SI enhancement after therapy and tumor growth) and histological analysis

The P values comparing data measured in the different therapy groups and the control group (treatment with NaCl) were calculated by the Mann-Whitney test.

<table>
<thead>
<tr>
<th>Therapy group</th>
<th>Tumor volume after/ mean ± SD</th>
<th>% reduction of SI enhancement within the tumor after therapy, mean value at all time points ± SD</th>
<th>Necrotic fractions in histological sections, mean ± SD</th>
<th>P value vs. control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.64 ± 2.34</td>
<td>11.32 ± 1.64</td>
<td>0.57 ± 0.11</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>2</td>
<td>2.52 ± 1.52</td>
<td>12.60 ± 4.55</td>
<td>0.67 ± 0.07</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>3</td>
<td>2.90 ± 1.16</td>
<td>13.60 ± 5.68</td>
<td>0.55 ± 0.28</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>4</td>
<td>2.81 ± 1.07</td>
<td>26.00 ± 5.70</td>
<td>0.68 ± 0.06</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>5</td>
<td>2.37 ± 1.68</td>
<td>28.04 ± 3.18</td>
<td>0.94 ± 0.07</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>6</td>
<td>0.91 ± 0.54</td>
<td>36.29 ± 4.84</td>
<td>0.99 ± 0.02</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

"NS, not significant.

Fig. 4. MRI images acquired immediately after i.v. injection of 0.17 mmol/kg body weight Magnevist before (left) and 7 days after (right) the following types of therapy: no therapy (a); carboplatin (b); Spherex and carboplatin (c); and Gelfoam and carboplatin (d).
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Fig. 5. Time course of the signal intensity enhancement in the tumor and liver after locoregional injection of an embolizate and Magnevist: A. after Magnevist only; B. with the embolizate Spherex; and C. with the embolizate Gelfoam.

Spherex or Gelfoam considerably increased the efficacy of a chemo-therapy of the VX2 tumor; the combination of Gelfoam and carboplatin was significantly more effective (on the P < 0.01 level) in reducing tumor growth and decreasing tumor vitality.

ACKNOWLEDGMENTS

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REFERENCES


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