Biodegradable microspheres for local drug delivery in the treatment of rat glioblastoma

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Introduction: Glioblastoma multiforme is the most common type of primary brain tumors, which has a very poor prognosis despite therapy. Due to the fact that local monotherapy was not really successful until now, the idea for a "drug cocktail" came up to combat the tumor in multiple ways simultaneously. Therefore, biodegradable Poly(D,L-lactide-co-glycolide) microspheres loaded with Celecoxib, Etoposide or Elacridar were tested for the local treatment of glioblastoma in a flank model of male Fischer 344 rats.

Materials and Methods:

Materials

PLGA was a kind gift of Boehringer Ingelheim (Ingelheim, Germany) and Celecoxib was a kind gift of Grünenthal (Aachen, Germany). Etoposide was purchased from Sigma-Aldrich (Steinheim, Germany) and Elacridar was synthesized by M. Christlieb (Pharmaceutical Chemistry, University of Bonn, Germany). All other chemicals were of analytical grade. F11 cells were obtained from A. Lamprecht (Pharmaceutical Engineering, University of Franche-Comté, Besançon, France). Cells were cultivated in DMEM-AQua media, Sigma-Aldrich, (Steinheim, Germany) and supplemented with 10% fetal bovine serum, 100 U/ml Penicillin and 1.0 mg/ml Streptomycin (Sigma-Aldrich, Steinheim, Germany) under standard conditions at 37 °C, 5% CO2 and 95% humidity.

Methods

Microsphere preparation

Microspheres were prepared by a conventional solvent evaporation method. 200 mg PLGA RG 502 H + 50 mg Etoposide or 125 mg PLGA RG 502 H + 25 mg Elacridar was dissolved in 3 ml methylene chloride, which built the internal phase. The internal phase was emulsified under stirring with an UltraTurrax, IKA, (Staufen, Germany) at 6000 rpm for 3 minutes with a 5% F11 solution, which built the external phase. An O/W emulsion was obtained and evaporation of methylene chloride was performed under magnetic stirring at 650 rpm for 1 overnight. In the end microspheres were washed extensively with distilled water and dried under vacuum at a desiccator.

In-vitro characterization

Morphology of microspheres were analyzed by a scanning electron microscope, Hitachi S-4400 N, (Tokyo, Japan) at 15 kV. Particle size and particle size distribution were analyzed by laser diffraction, Sympatec, (Cassault-Zellenberg, Germany). Due to the toxicity of Etoposide and small available amounts of Elacridar only Celecoxib microspheres were analyzed for their morphology and particle size. Encapsulation efficiency and in-vitro release behaviour were determined by high performance liquid chromatography, Kontron instrument, Siegburg, (France).

Animals

Male Fischer 344 rats (50 to 55 days) were purchased from Charles River Laboratories (Sulzfeld, Germany). Animals were housed with three animals per cage and received autoclaved food and water ad libitum. Rats were treated in accordance to the specifications of the Landesamt für Natur, Umwelt und Verbraucherschutz (LMUVR) (Recklinghausen, Germany) and the University and the hospital in the city of Bonn.

In vivo trial

Thirty-4 F11 rats anesthetized by isoflurane were injected 500 µl of 3% F11 Ficoll into the left flank subcutaneously similar as described somewhere else [1]. Tumors were measured by a caliper. When tumors reached a size of 2 cm, microspheres were injected directly into the tumor. Once a tumor reached a size of 12.25 mm, a diameter of 5 cm or 2000 mg of tumor was transplanted into the flanks of the rats. Tumors were analyzed histologically and for histologic peculiarities by a UV-lamp, Germany). After explantation tumors were analysed histologically and for histologic peculiarities by a UV-lamp, Germany). After explantation tumors were analysed histologically and for histologic peculiarities by a UV-lamp, Germany). After explantation tumors were analysed histologically and for histologic peculiarities by a UV-lamp, Germany). After explantation tumors were analysed histologically and for histologic peculiarities by a UV-lamp, Germany). After explantation tumors were analysed histologically and for histologic peculiarities by a UV-lamp, Germany). After explantation tumors were analysed histologically and for histologic peculiarities by a UV-lamp, Germany). After explantation tumors were analysed histologically and for histologic peculiarities by a UV-lamp, Germany). After explantation tumors were analysed histologically and for histologic peculiarities by a UV-lamp, Germany). After explantation tumors were analysed histologically and for histologic peculiarities by a UV-lamp, Germany). After explantation tumors were analysed histologically and for histologic peculiarities by a UV-lamp, Germany). After explantation tumors were analysed histologically and for histologic peculiarities by a UV-lamp, Germany). After explantation tumors were analysed histologically and for histologic peculiarities by a UV-lamp, Germany). After explantation tumors were analysed histologically and for histologic peculiarities by a UV-lamp, Germany). After explantation tumors were analysed histologically and for histologic peculiarities by a UV-lamp, Germany). After explantation tumors were analysed histologically and for histologic peculiarities by a UV-lamp, Germany). After explantation tumors were analysed histologically and for histologic peculiarities by a UV-lamp, Germany). After explantation tumors were analysed histologically and for histologic peculiarities by a UV-lamp, Germany). After explantation tumors were analysed histologically and for histologic peculiarities by a UV-lamp, Germany).

Encapsulation rates of Celecoxib at 97±0.4%, and Elacridar at 98±1.0%, were found to be as expected, whereas the encapsulation rate of Etoposide was significantly lower with 38±7.3% (Table 1). This is most probably due to Etoposide (log P 0.6) being much less lipophilic than Celecoxib (log P 3.4) and Elacridar (log P 5.6). Hence Celecoxib and Elacridar show a lower tendency to diffuse into the external phase, which leads to higher encapsulation rates. Microsphere formulations with Celecoxib and Elacridar were also tested for their in vitro drug release behaviour (Fig. 3), in which various surfactants and concentrations had to be used to maintain sink conditions. The release of Celecoxib and Elacridar was dominated by an initial burst effect of about 85% in the first 24 hours followed by a sustained release. Complete release of Celecoxib and Elacridar were achieved after 7 days. In contrast, Etoposide showed only an initial burst effect of 35% during the first 24 hours followed by a sustained release. After 7 days only 85% of Etoposide were released. However, it was assumed that remaining Etoposide was also released sustained.

Table 1 Characteristics of microspheres for the animal trial

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<th>Encapsulation [%]</th>
<th>Release [%]</th>
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<td>Celecoxib</td>
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<td>Etoposide</td>
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<td>Elacridar</td>
<td>12.5±0.6</td>
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Glioblastomas showed destructive, infiltrative growth and oedema (Fig. 4). Strongly at 365 nm Excimer Laser Elacridar diffused from the particle reserve only few millimeters into the surrounding tumor tissue (Fig. 5).

Results and Discussion:

Celecoxib microspheres appeared spherical with a smooth surface (Fig. 1) and showed monomodal particle size distributions and particles in a size range of 15–25 µm (Fig. 2).

Histological sections demonstrated typical lamellar necrosis, palisade-like arranged cells, a high cell density and multi epidermal nuclei (Fig. 6).

Table 2 Characteristics of microspheres for the animal trial

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Mean survival time of animals treated with Etoposide- or Celecoxib microspheres was prolonged significantly compared to animals treated with blank microspheres (log-rank test) (Fig. 6). Group 2 (blank microspheres) showed a shorter survival time and smallest probability of survival followed by group 1 (no treatment) and 3 (Celecoxib-microspheres) (Fig. 6). Comparing the survival functions of groups 4–5 without the survival probability of the group 1 (Fig. 5). Thus Etoposide microspheres were most effective in prolonging the mean survival time. The minor effect of Celecoxib- and Etoposide-microspheres on mean survival time and survival probability is attributed to their slow diffusion through their tumor tissue (Fig. 5).

Conclusion:

Local delivery of Celecoxib, Etoposide and Elacridar by biodegradable microspheres was found to be suitable for treating glioblastoma in vivo. Prolonged survival was achieved, however the administration of drug combinations did not alter the therapeutic outcome due to their slow diffusion through the tumor tissue. For the future, research in glioma therapy using microparticulate systems should therefore focus on the diffusion of drugs through tumor tissue in solid tumors.

Acknowledgements:

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References: