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ORIGINAL ARTICLE

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# Microencapsulated, CYP2B1-transfected cells activating ifosfamide at the site of the tumor: the magic bullets of the 21st century

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**Abstract** *Background:* Conventional chemotherapy of pancreatic carcinoma is only marginally effective. This is in part due to the severity of side effects following systemic administration of the cytostatic drug. The aim was to create a therapeutic tool allowing the targeting of the conversion site of a cytotoxic prodrug to the site of the tumor. This was realized by transfection of the CYP2B1 gene, the major ifosfamide-converting P450 enzyme, in cells with subsequent microencapsulation and administration of these microcapsules to or into the tumor. The enzyme activity (resorufin assay) remained stable for weeks in vitro and in vivo within the microencapsulated CYP2B1-expressing cells. We demonstrated a significant antitumor effect of the intratumorally injected capsules against xenotransplanted human pancreatic carcinomas in the nude mouse. Angiographic experiments in the pig confirmed the feasibility of an intraarterial placement of the capsules into the pancreas. A clinical protocol was established and approved. *Patients, material and methods:* L293 cells were transfected with the CYP2B1 gene, microencapsulated (diameter 0.7 mm) under GCP conditions and packed sterile. Patients with confirmed inoperable adenocarcinoma of the pancreas underwent angiography, and capsules were injected into a vessel leading into the tumor. The patients were monitored for 48 h to exclude allergic reactions or pancreatitis. A day later, ifosfamide was administered for three consecutive days to be repeated on days 21–23. The patients were followed up for 5 months. *Results:* A total of 17 patients were enrolled. The patients tolerated the procedure without any complications. No allergic reactions or pancreatitis were encountered. Chemotherapy was uneventful. All patients had stable disease, and two patients a partial remission. The median survival was 44 weeks which compared favorably with that of a historical control group (22 weeks). *Conclusions:* The intraarterial administration of microcapsules for targeted chemotherapy was well tolerated. Control of local tumor growth was achieved.

**Keywords** Ifosfamide · CYP2B1-transfected cells

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## Introduction

Ifosfamide has been used for the treatment of many solid tumors including pancreatic carcinoma. The response rates are in the range of about 20% with median survival times between 8 and 10 months [1, 2, 3, 4, 5]. This is well within the range of current quasi-standard single-agent chemotherapy [6]. However, the side effects at ifosfamide dosages utilized in these studies are severe and eventually intolerable older patients suffering from pancreatic adenocarcinoma.

The rationale for this project was to improve the therapeutic index of a cytotoxic drug that has been shown to work in pancreatic carcinoma, is registered for this disease and is a prodrug that needs activation by defined enzymes. The aim was then to develop an approach that allows local conversion of the prodrug at the site of the tumor by genetically engineering cells to

express the respective enzyme and allow treatment with a low-dose of the cytotoxic prodrug. Ifosfamide fulfils all these criteria. In order to protect genetically engineered cells from the host immune system and to protect the host from the allogenic, genetically modified cells, another feature was added to this concept. The cells were microencapsulated, a rather old idea [7] that took decades to put into clinical practice [8].

We established a system to encapsulate genetically engineered cells expressing the 2B1 isomer of the rat cytochrome p450 gene (CYP2B1), which is capable of converting ifosfamide to its active compounds, phosphoramide mustard and acrolein [9]. In contrast to the widely used alginate/L-polylysine system [10], we utilized sodium cellulose sulfate (NaCS) as polyanion and poly(dialyldimethylammonium chloride (PDADMAC) as polycation [11]. We have used this system for cancer gene therapy [12, 13].

## Methodology and results

### Vector construct

The cDNA coding for the CYP2B1 enzyme [14] was cloned into the plasmid pcDNA3 [15]. Plasmid pC3/2B1, which carries a neomycin (G418) resistance gene, was transferred into qualified 293 cells (human embryonic kidney cell line; ATCC). The expression of biologically active CYP2B1 in the transfectants was determined using a biochemical assay, which is specific for the cytochrome P450 isoforms 1A1 and 2B1 [16]. The cell clone 22P1G [17] showed the highest enzymatic activity and was chosen for further experimentation.

### Encapsulation

For encapsulation, CYP2B1-transfected 22P1G or 293 cells were suspended in NaCS or phosphate-buffered saline (pH 7) containing 2–5% NaCS depending on the degree of sulfation (polyanionic solution). The suspension was passed through an adjustable droplet generation system (Inotech, Switzerland) with droplets eventually falling into a precipitation bath containing 3–4% PDADMAC in NaCl depending on the concentration of the NaCS (polycationic solution). Upon contact of the polyanion with the polycationic solution, a polyelectrolyte complex starts forming producing a capsule membrane, which forms from the outside towards the centre. The capsules were then washed twice with saline and stored at 4°C [15]. The extent of cell survival after encapsulation was measured with the two-color a Life&-Dead viability/cytotoxicity kit (Molecular Probes).

### Animal models

Initial studies were aimed at detection of immediate or delayed toxic effects in rodents. In all these experiments, no toxicity could be detected. To ascertain the tolerance of the capsule material at the planned site of clinical application, empty capsules were injected orthotopically into the pancreas of both nude and immunocompetent mice [18]. A mild foreign body reaction was seen surrounding the capsules, but no systemic reaction or granuloma formation was observed [19].

The antitumor effect of the administration of microencapsulated CYP2B1-producing cells, and subsequent systemic ifosfamide administration were investigated using encapsulated CYP2B1-expressing Crandell feline kidney (CrFK) cells [15]. Briefly, a suspension of $1\times10^6$ PANC-1 cells was injected subcutaneously into athymic nude mice to establish preformed tumors [20]. Once the tumors had reached a size of 1 $cm^3$, 20 to 40 capsules were injected. Animals were then treated with low-dose ifosfamide and mesna (both at 100 mg/kg) intraperitoneally every 3 days for 2 weeks [15]. Most dramatically this resulted in a complete remission of the established tumors in almost 20% of the animals and partial remission in a further 50% [15].

The added value of the local conversion of ifosfamide was demonstrated in a second experiment. Preformed tumors were established but this time 40 capsules were injected only into one side of the tumor, which was marked. The mice were then treated with a single dose of ifosfamide (100 $mg/m^2$). After 30 min, when the plateau phase of ifosfamide in blood plasma had been reached, the animals were anaesthetized and tumor tissue was removed and divided into three parts: site of the capsule implantation, the middle portion, and the side furthest away from the capsule implantation. Ifosfamide and its active metabolites were measured in the snap-frozen tissue as well as in blood plasma. While the levels of ifosfamide were similar at all three sites, at the site where the capsules had been implanted the concentrations of activated 4-OH-ifosfamide were at least double [21].

### Angiographic route of delivery

In order to take full advantage of the proposed system in humans, a vascular route was considered necessary. To test the feasibility of such an approach, large-animal studies were mandatory. Adolescent pigs underwent angiography by the femoral artery route. A 4F introducer system (Terumo) was inserted by the Seldinger technique [22]. Under fluoroscopy, the celiac trunk was catheterized. Supraselective cannulation was achieved by further advancement of the guide-wire and a coaxial 2.3F microcatheter system (Cordis). In general, the splenic lobe was cannulated. After successful placement of the catheter in the main vessel leading into the splenic lobe of the pancreas, 100 capsules were delivered slowly. The animals were monitored clinically and by laboratory tests for a week. In all animals, it was possible to selectively cannulate both the splenic and the duodenal lobe arteries. Further, the instillation of capsules was successful in all of the pigs. Importantly, none of the 18

animals receiving the capsules developed obvious pancreatic symptoms. The level of markers, such as amylase, remained within normal limits in all of the pigs. Finally, the animals were killed and the pancreas as well as other inner organs were removed. Careful examination of the pancreas revealed no visible macroscopic or microscopic damage. The capsules were found in capillaries, partly in conjunction with thrombotic material, but they were never found to occlude the entire vessel (Löhr et al., submitted for publication).

### Clinical studies

A non-randomized, phase I/II study was designed in which patients with advanced-stage pancreatic carcinoma that was not suitable for curative surgery were to be treated [23]. The study was approved by the relevant bodies including the state Ethics Committee, the Somatic Gene Therapy Commission of the German Federal Medical Association (KSG-BAK), the Working Party for Oncology (AGO) of the German Society for Gastroenterology (DGVS), and the German Working Party for Gene Therapy (DAG-GT). For the clinical study, the entire process of culturing the cells carrying the therapeutic gene and the encapsulation process was transferred to a contract research organization to ensure compliance with GCP-GCH guidelines. The cells were cultured under constant GMP conditions. After passing the necessary quality assurance tests, including sterility and functional tests, the encapsulated cells were released for use in the clinical trial.

A total of 14 patients were treated according to the protocol. After giving informed consent, the selected patients underwent angiography. Supraselective catheterization of the transversal artery was performed with a coaxial 2.3F microcatheter system (Cordis) [22]. After catheterization, 300 microcapsules were slowly instilled through the catheter one by one. After capsule delivery, which typically took 15–30 min, the patients rested for 48 h. During this period, the patients were monitored clinically and by follow-up laboratory tests as well as by abdominal ultrasound in order to detect any abnormality that might occur in the upper abdomen, such as pancreatitis or ischemia.

The patients then received 1 g/m$^2$ body surface ifosfamide for three consecutive days (days 2–4). A second cycle of ifosfamide was given according to this regimen starting on day 22. Routine laboratory monitoring was performed during the entire period according to oncological standards. Each of the patients was followed up for at least 5 months. The whole procedure was well tolerated with none of the patients developing signs of an allergic reaction or pancreatitis. There was not a single adverse event or toxicity related to the low-dose chemotherapy. Two of the patients showed partial remissions and the other patients exhibited stable disease. The survival time of the patients was extended from 22 weeks (control group at study centre) to 44 weeks [21].

Based on these results, a phase II trial is currently ongoing in which the effects of three concentrations of ifosfamide with optimized capsules are being investigated in a multicentre study.

## Discussion

Our initial experiments demonstrated proof of principle for the use of genetically engineered, microencapsulated cells in cancer therapy with ifosfamide as the prodrug and pancreatic carcinoma as the model system. We suggest that the combination of the local delivery of the microcapsules and low-dose systemic administration of the chemotherapy represents a modern interpretation of the 'magic bullet' concept envisaged by Paul Ehrlich a century ago [24].

Several gene therapy approaches have been described in experimental pancreatic cancer [25, 26, 27, 28, 29]. In animal models, biodegradable polylactic acid microspheres releasing recombinant interleukin-12 have been used for in situ tumor immunotherapy and these have resulted in tumor reductions [30]. The cellulose sulfate-based microencapsulation technology described in this report has a number of advantages over other encapsulation systems [11]. In addition to the use outlined above for pancreatic cancer, it has been shown to allow secretion of larger molecules such as monoclonal antibodies produced by encapsulated hybridoma cells [31] even resulting in protection from a virally induced disease [32], and the technology could be used to deliver other biomolecules. Furthermore, administration of capsules containing CYP2B1-expressing cells in conjunction with ifosfamide, as used in our experiments, may prove effective for other cancers, e.g. peritoneal metastasis/malignant ascites, soft tissue sarcoma, and hepatic masses (primary and metastatic). In the meantime, such capsules have been successfully utilized to treat breast cancer in mouse models of mammary cancer [13] as well as more recently in an ongoing clinical trial in dogs (Hain et al., submitted for publication).

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## References

1. Cerny T, Martinelli G, Goldhirsch A, Terrier F, Joss R, Fey MF, et al (1991) Continuous 5-day infusion of ifosfamide with mesna in inoperable pancreatic cancer patients: a phase II study. J Cancer Res Clin Oncol 117 [Suppl IV]:S135–S138

2. Keizer HJ, Ouwerkerk J, Welvaart K, van der Velde CJH, Cleton FJ (1995) Ifosfamide treatment as a 10-day continuous intravenous infusion. J Cancer Res Clin Oncol 121:297–302
3. Einhorn LH, Loehrer PJ (1986) Ifosfamide chemotherapy for pancreatic carcinoma. Cancer Chemother Pharmacol 18 [Suppl 2]:51–54
4. Gad-el-Mawla N (1986) Ifosfamide in advanced pancreatic cancer. Cancer Chemother Pharmacol 18 [Suppl 2]:55–56
5. Loehrer PJ, Williams SD, Einhorn LH, Ansari R (1985) Ifosfamide: an active drug in the treatment of adenocarcinoma of the pancreas. J Clin Oncol 3:367–372
6. Storniolo AM, Enas NH, Brown CA, Voi M, Rothenberg ML, Schilsky R (1999) An investigational new drug treatment program for patients with gemcitabine. Cancer 85:1261–1268
7. Chang TMS (1957) Hemoglobin corpuscles. Research report for Honours Physiology, Medical Library, McGill University. McGill University Press, Montreal
8. Lim F, Sun AM (1980) Microencapsulated islets as bioartificial endocrine pancreas. Science 210:908–909
9. Dirven HAAM, van Ommen B, van Blaederen PJ (1996) Glutathione conjugation of alkylating cytostatic drugs with a nitrogen mustard group and the role of glutathione S-transferases. Chem Res Toxicol 9:351–360
10. Okada N, Miyamoto H, Yoshioka T, Sakamoto K, Katsume A, Saito H, et al (1997) Immunological studies of SK2 hybridoma cells microencapsulated with alginate-poly(L)lysine-alginate (APA) membrane following allogenic transplantation. Biochem Biophys Res Commun 230:524–527
11. Dautzenberg H, Schuldt U, Grasnik G, Karle PMP, Löhr M, Pelegrin M, et al (1999) Development of cellulose sulphate-based polyelectrolyte complex microcapsules for medical applications. Ann N Y Acad Sci 875:46–63
12. Löhr M, Hoffmeyer A, Kröger JC, Freund M, Hain J, Holle A, et al (2001) Microencapsulated, cell mediated treatment of inoperable pancreatic carcinoma. Lancet 357:1591–1592
13. Kammertöns T, Gelbmann W, Karle P, Salmons B, Günzburg WH, Uckert W (2000) Combined chemotherapy of murine mammary tumors by local activation of prodrugs ifosfamide and 5-fluorocytosine. Cancer Gene Ther 7:629–636
14. Kedzie KM, Balfour CA, Escobar GY, Grimm SW, He YA, Pepperl DJ, et al (1991) Molecular basis for a functionally unique cytochrome P450IIB1 variant. J Biol Chem 266:22515–22521
15. Löhr M, Müller P, Karle P, Stange J, Mitzner S, Nizze H, et al (1998) Targeted chemotherapy by encapsulating cells engineered to deliver CYP2B1, an ifosfamide activating cytochrome P450 gene. Gene Ther 5:1070–1078
16. Kurowski V, Wagner T (1993) Comparative pharmacokinetics of ifosfamide, 4-hydroxyifosfamide, chloracetaldehyde, and 2- and 3-dechloroethylifosfamide in patients on fractionated intravenous ifosfamide therapy. Cancer Chemother Pharmacol 33:36–42
17. Günzburg WH, Karle P, Renz R, Salmons B, Renner M (1999) Characterization of a human cell clone expressing cytochrome P450 for safe use in human somatic cell therapy. Ann N Y Acad Sci 880:326–337
18. Löhr M, Schmidt C, Ringel J, Kluth M, Müller P, Nizze H, et al (2001) Transforming growth factor-beta 1 induces desmoplasia in an experimental model of human pancreatic carcinoma. Cancer Res 61:550–555
19. Müller P, Jesnowski R, Karle P, Renz R, Saller R, Stein H, et al (1999) Injection of encapsulated cells producing an ifosfamide-activating cytochrome P450 for targeted chemotherapy to pancreatic tumors. Ann N Y Acad Sci 880:337–351
20. Löhr M, Trautmann B, Göttler M, Peters S, Zauner I, Maillet B, et al (1994) Human ductal adenocarcinomas of the pancreas express extracellular matrix proteins. Br J Cancer 69:144–151
21. Löhr M, Saller R, Salmons B, Günzburg WH (2002) Microencapsulation of genetically engineered cells for cancer therapy. Methods Enzymol 346:603–618
22. Kröger JC, Bergmeister H, Hoffmeyer A, Ceijna M, Karle P, Saller R, et al (1999) Intraarterial instillation of microencapsulated cells in the pancreatic arteries in pig. Ann N Y Acad Sci 880:374–378
23. Löhr M, Bago ZT, Bergmeister H, Ceijka M, Freund M, Gelbmann W, et al (1999) Cell therapy using microencapsulated 293 cells transfected a gene construct expressing CYP2B1, an ifosfamide converting enzyme, instilled intra-arterially in patients with advanced-stage pancreatic carcinoma. A phase I-study. J Mol Med 77:393–398
24. Witkop B (1999) Paul Ehrlich and his magic bullets – revisited. Proc Am Philos Soc 143:540–557
25. Makinen K, Loimas S, Wahlfors J, Alhava E, Janne J (2000) Evaluation of herpes simplex thymidine kinase mediated gene therapy in experimental pancreatic cancer. J Gene Med 2:361–367
26. Makinen K, Loimas S, Kosma VM, Wahlfors J, Yla-Herttuala S, Alhava E, et al (2000) Adenovirus-mediated gene transfer into an experimental pancreatic tumour. Ann Chir Gynaecol 89:99–103
27. Kijima H, Scanlon KJ (2000) Ribozyme as an approach for growth suppression of human pancreatic cancer. Mol Biotechnol 14:59–72
28. Evoy D, Hirschowitz EA, Naama HA, Li XK, Crystal RG, Daly JM, et al (1997) In vivo adenoviral-mediated gene transfer in the treatment of pancreatic cancer. J Surg Res 69:226–231
29. DiMaio JM, Clary BM, Via DF, Coveney E, Pappas TN, Lyerly HK (1994) Directed enzyme pro-drug gene therapy for pancreatic cancer in vivo. Surgery 116:205–213
30. Egilmez NK, Jong YS, Sabel MS, Jacob JS, Mathiowitz E, Bankert RB (2000) In situ tumor vaccination with interleukin-12-encapsulated biodegradable microspheres: induction of tumor regression and potent antitumor immunity. Cancer Res 60:3832–3837
31. Pelegrin M, Marin M, Noel D, Del Rio M, Saller R, Stange S, et al (1998) Systemic long-term delivery of antibodies in immunocompetent animals using cellulose sulphate capsules containing antibody-producing cells. Gene Ther 5:828–834
32. Pelegrin M, Marin M, Oates A, Noel D, Saller R, Salmons B, et al (2000) Immunotherapy of a viral disease by in vivo production of therapeutic monoclonal antibodies. Hum Gene Ther 11:1407–1415