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ORIGINAL ARTICLE

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Increased delivery of erucylphosphocholine to C6 gliomas by chemical opening of the blood-brain barrier using intracarotid pentylglycerol in rats

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Abstract *Background:* Erucylphosphocholine (ErPC) has been shown to exert strong antineoplastic effects against various brain tumor cell lines in vitro. Since ErPC only enters the brain after long-term treatment, ineffective drug delivery to the tumor is considered to be the reason for the moderate responses to chemotherapy with ErPC observed in animal brain tumor models. We investigated a recently described method for chemically opening the blood-brain barrier (BBB) using intraarterial administration of alkylglycerols to increase the transfer of ErPC into the brain. *Methods:* ErPC (40 mg/kg) was given to C6 glioma-bearing rats either as a single intracarotid bolus injection in the presence or absence of 1-*O*-pentylglycerol (300 m*M*) or as an intracarotid infusion in conjunction with bradykinin. Brain tissue concentrations were analyzed and compared to values obtained after intravenous ErPC treatment over 14 and 30 days (cumulative ErPC doses of 210 and 350 mg/kg, respectively). *Results:* Pentylglycerol-induced BBB opening resulted in a significant increase in ErPC delivery to the tumor (17-fold) and, to a lesser extent, to the surrounding ipsilateral brain (7-fold) compared to intraarterial ErPC administration without alkylglycerol ($P < 0.05$). Furthermore, the resulting ErPC concentrations in the brain tumor exceeded those obtained in tumor and tumor-free brain after long-term intravenous ErPC administration. In contrast to this, intracarotid bradykinin did not increase the transfer of ErPC to the tumor or tumor-free brain. *Conclusions:* The intracarotid administration of pentylglycerol represents a novel and nontoxic method of overcoming the limited access of ErPC to both brain tumors and brain tissue adjacent to tumors. The present results provide further evidence that chemical opening of the BBB by intraarterial alkylglycerols is a promising new concept for improving delivery of chemotherapeutic agents to brain tumors.

Keywords Alkylglycerol · Erucylphosphocholine · Blood-brain barrier · Brain tumor · Chemotherapy

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Introduction

Erucylphosphocholine (ErPC) is a new derivative of the alkylphosphocholine (APC) family exerting strong antineoplastic effects against a variety of tumor cells in vitro and in vivo [4, 14, 17, 18, 19]. In contrast to hexadecylphosphocholine, the prototypical APC, ErPC can be administered intravenously without causing hemolysis [3, 5]. Biodistribution studies have shown that ErPC does not enter the brain after a single bolus injection. However, intravenous (i.v.) injections repeated every 48 h over periods of 2 and 4 weeks were followed by a significant accumulation of ErPC in the brain parenchyma [5]. Consequently, the effectiveness and mechanisms of action of ErPC in the treatment of brain tumor cell lines have been investigated in more detail [14, 15]. Due to its cell membrane-mediated mechanisms of action, ErPC is thought to be capable of overcoming the chemoresistance against standard anticancer agents frequently observed in high-grade gliomas [16]. Surprisingly, in animal experiments, i.v. ErPC in the treatment of brain tumors has shown only moderate antitumor effects. This has been attributed mainly to the slow increase in brain tissue concentrations after repeated ErPC administration [4].

Recently, we described a new and very effective method of enhancing the delivery of chemotherapeutic drugs to the brain [6]. The intraarterial (i.a.) coinjection of short-chain alkylglycerols and anticancer drugs

results in a well-controllable increase in drug concentrations in both normal brain and brain tumor tissue. Furthermore, no organotoxic side effects have been observed in long-term toxicity studies [8]. An intracarotid bolus administration of pentylglycerol is followed by rapid urinary elimination and there is no accumulation of the substance within the brain. Thus, alkylglycerols are thought to offer an effective strategy for increasing drug transfer to the brain without significant toxicity.

In the present study, the access of ErPC to brain tumor tissue and to normal brain was investigated using i.a. ErPC administered in the absence and in the presence of 1-*O*-pentylglycerol. The results were compared with those obtained using intracarotid bradykinin for chemical opening of the blood-brain barrier (BBB) and with those after long-term i.v. ErPC treatment.

Materials and methods

Chemicals

ErPC was synthesized by H. Eibl as previously described [5]. For i.v. injections, ErPC was dissolved in ethanol and water (4:96, w/v) to a final ErPC concentration of 8 mg/ml. This stock solution was sterilized by filtration (Minisart 0.2 μm; Sartorius, Göttingen, Germany) and stored at 4°C. For i.a. administration, three different ErPC formulas (1–3) were used. The ErPC dose was set at 40 mg/kg body weight (BW) and all solutions were freshly prepared before injection as follows: (1) ErPC was dissolved in ethanol and distilled water as for i.v. administration (4:96 w/v; ethanolic ErPC); (2) to avoid ethanol-induced effects at the BBB, an ethanol-free solution containing ErPC (10.25 mg/ml) and the adjuvants 1.2-myristoyl-*sn*-glycerol-3-phosphoglycerol-sodium (1.5 mg/ml), and propandiol-(1.2) (12 mg/ml) was prepared (aqueous ErPC = control, and bradykinin group); (3) ErPC was dissolved in pentylglycerol and the mixture was diluted with water to a final pentylglycerol concentration of 300 m*M* (pentylglycerol-ErPC). The synthesis of (*rac*)-1-*O*-pentyl-glycerol (3-pentoxy-propylene glycol) has been described elsewhere [5] and the purity was assessed by HPLC. The adjuvants were added to the control solution to counteract the hyperviscosity of ErPC in water that would otherwise have resulted in the formation of gel phases. The osmolalities of the solutions averaged 242, 258 and 276 mOsm/kg, respectively. Bradykinin was purchased from Sigma (Deisenhofen, Germany).

Animals

Male Wistar rats weighing between 230 and 305 g were used. The animals were kept under conventional controlled conditions and had free access to a standard diet (Altromin) and tap-water during the whole experimental period. The experiments were carried out in accordance with the German Law on the Protection of Animals.

Implantation of central venous catheters

Silicone catheters (Silastic, 0.51 mm ID × 0.94 mm OD; Dow Corning Corporation, Midland, Mich.) were prepared as described by Harms and Ojeda [10]. The tubes were filled with heparinized normal saline and the ends were sealed with a 24-gauge pin. Rats were anesthetized using intraperitoneal ketamine/xylazine hydrochloride (90 μg/7.5 μg per gram BW) and the catheters were inserted into the superior vena cava via the external jugular vein as described elsewhere [5].

Tumor implantation

Animals received intraperitoneal ketamine/xylazine hydrochloride (90 μg/7.5 μg per gram BW) and C6 cells were inoculated stereotactically into the right putamen of rats weighing 190 to 230 g as described by Erdlenbruch et al. [4]. A volume of 10 μl of C6 cell suspension (10^7 cells/ml) was injected.

Treatment groups

Intravenous ErPC treatment

ErPC was given repeatedly by i.v. injections via a central venous catheter. Two different treatment regimens were administered. In a first group (constant dose regimen), ErPC was injected repeatedly over 30 days at intervals of 48 h at a dose of 25 mg/kg BW ($n = 6$). Due to the rapid growth of C6 gliomas the inoculation of C6 cells was performed 10 days after the beginning of the ErPC treatment to guarantee a treatment period of 30 days. In a second group, ErPC was given immediately after implantation of C6 cells. Animals in this experiment (saturation regimen) were treated over 14 days and received a rapid i.v. ErPC saturation (day 1, 50 mg/kg; day 2, 40 mg/kg; day 4 to day 8, 20 mg/kg; and day 11, 20 mg/kg; $n = 6$). For i.v. injections, the ErPC solution was heated to 37°C followed by sterile filtration.

Intraarterial ErPC treatment

C6 gliomas were inoculated as described above and tumors were allowed to grow until clinically manifest (12 to 23 days after tumor implantation). ErPC was then given into the right internal carotid artery in the presence of 1-*O*-pentylglycerol (300 m*M*, $n = 6$) or in the absence of 1-*O*-pentylglycerol either as an ethanolic ($n = 5$) or ethanol-free aqueous solution ($n = 5$). The i.a. injection procedure has been described elsewhere in detail [5]. Briefly, the right external carotid artery was cannulated in a retrograde manner. Blood pressure and heart rate were recorded via the left femoral artery with a Statham transducer (Gould, Oxnard, Calif.). ErPC solutions were slowly injected at a flow rate of 6 ml/min, for a total of 800 to 1175 μl of drug solution (depending on the ErPC formulation and individual dose) followed by rinsing with isotonic saline (325–400 μl). Antegrade blood flow was interrupted during the injection by clamping the common carotid artery. A total volume of 1.2 to 1.5 ml ErPC and physiological saline were injected over 12 to 15 s using a Hamilton dispenser (Microlab, Hamilton, Bonaduz, Switzerland). The pentylglycerol-induced ErPC transfer to the brain was compared with the effects of bradykinin-mediated BBB opening ($n = 6$). Bradykinin was infused into the right internal carotid artery at a rate of 10 μg/kg per min for 15 min as described by Nomura et al. [27]. The intracarotid administration of ErPC (40 mg/kg; formula 2) was started 5 min after the start of bradykinin, and ErPC was infused within 15 min. In all experiments, 5 min after completion of the i.a. ErPC administration, blood samples were withdrawn and brains were removed.

Analysis of ErPC concentrations

At the end of the i.v. ErPC treatment (day 14 or 30), rats were anesthetized using ketamine (60 mg/kg i.v.) and heparinized with an i.v. bolus of 300 U/kg. The left ventricle was cannulated and blood samples were withdrawn. The animals were then perfused with 100 ml Ringer's solution. In the 30-day treatment group (constant dose regimen), brain, fat tissue, liver and kidneys were removed for quantitative ErPC analysis and histological evaluation of the organs. In the 14-day treatment group (saturation regimen), only the brains were removed and stored at –20°C until ErPC analysis.

In the intracarotid ErPC groups, blood samples were withdrawn 5 min after ErPC administration. Again, the animals were

perfused with Ringer's solution before removal of the brains and these were frozen until further analyses could be carried out.

ErPC concentrations were analyzed by high-performance thin-layer chromatography (HPTLC) as previously described [5, 22]. Tissue concentrations were determined separately in the tumor and surrounding tumor-free brain ipsilateral to the injection site, as well as in the contralateral hemisphere and the cerebellum. The lower detection limit of this method was 10 to 12 nmol/ml. The results are presented as picomoles per milligram wet weight.

Hematological and clinical chemical parameters

Hematological and serum parameters were determined in order to evaluate the toxicity of the alkylglycerols. Parameters were determined twice before and once after i.v. ErPC treatment as well as after intracarotid ErPC injection with and without BBB modification. Blood cells were counted using a hematocytometer (Minos STE; ABX, Göppingen, Germany). The serum parameters Na, K, Ca, glucose, protein, aminotransferases (GOT, GPT), lactate dehydrogenase, bilirubin and creatinine were analyzed using a Beckman autoanalyzer (Synchron CX5D; Beckman, Munich, Germany).

Statistical evaluation

For statistical analysis of the data, Student's *t*-test was used to compare means across treatment groups. Mean values ± SD are reported.

Results

Using increasing doses from 10 to 40 mg/kg, both single i.v. and single intracarotid bolus injection of ErPC resulted in brain concentrations under the detection limit of 12 nmol/mg (data not shown). However, in the first group receiving repeated i.v. ErPC injections (constant dose regimen; 25 mg/kg every 48 h over 30 days; cumulative dose 350 mg/kg), an ErPC accumulation was found in the brain with tissue concentrations averaging 152 ± 53 pmol/mg, which exceeded serum levels by about 1.7-fold (Fig. 1A). Tissue levels of ErPC in liver and kidney were higher and those of fat tissue lower than the CNS levels. In view of earlier biodistribution studies using lower ErPC doses (20 mg/kg) [5], we expected the concentrations of ErPC in the tumor-free brain parenchyma to be higher than the levels found in the present study. Unfortunately, brain tumor concentrations could not be determined in this group of animals since large parts of the tumor had been used for histopathological evaluation (data not shown). ErPC treatment was well tolerated by the animals. The clinical and histological examinations showed no signs of ErPC-induced toxicity. Furthermore, assessment of laboratory parameters revealed no changes during ErPC treatment.

In the second group receiving i.v. ErPC treatment (saturation regimen), a rapid and more pronounced drug accumulation was achieved in both tumor tissue and surrounding tumor-free brain (Fig. 1B). ErPC concentrations averaged 264 pmol/mg in the tumor and 244 pmol/mg in the remaining tumor-free brain. Thus, even though the cumulative ErPC dose was significantly

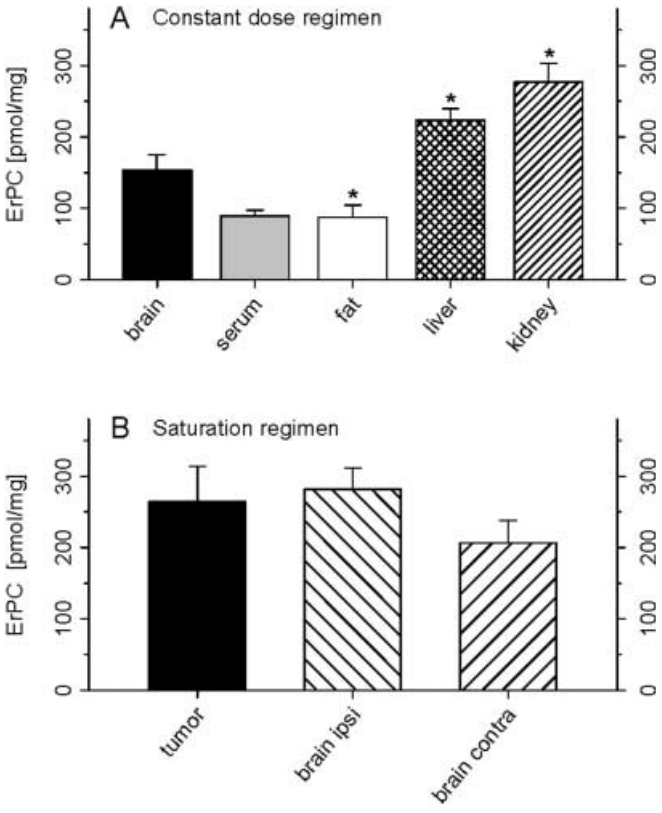

Fig. 1A, B. ErPC concentrations in brain tumors, surrounding brain and peripheral organs after long-term i.v. ErPC treatment. **A** Constant dose regimen: treatment over 30 days, ErPC (25 mg/kg) given repeatedly at 48-h intervals (cumulative dose 350 mg/kg). ErPC concentrations were only determined in tumor-free brain (*$P < 0.05$ compared to brain). **B** Saturation regimen: ErPC treatment over 14 days, ErPC injected on day 1 at 50 mg/kg, day 2 at 40 mg/kg, day 4 to day 8 at 20 mg/kg and day 11 at 20 mg/kg (cumulative dose 210 mg/kg). Concentrations are given as picomoles per milligram wet weight, means ± SD

lower using the saturation regimen (210 mg/kg compared to 350 mg/kg given in the constant i.v. ErPC group), a significantly higher ErPC transfer into the CNS was achieved ($P < 0.05$). On the other hand, the higher initial ErPC doses were associated with attenuated weight gain or stagnant body weight, a clinical sign of ErPC-induced toxicity (Fig. 2). However, laboratory and morphological examinations revealed no other signs of toxicity.

The single i.a. ErPC injection in the presence of 1-*O*-pentylglycerol resulted in very high ErPC concentrations in brain tumor tissue (Fig. 3D). Compared to control animals receiving i.a. ErPC without pentylglycerol (aqueous and ethanolic ErPC groups) and with those receiving intracarotid bradykinin, a marked increase in drug delivery to the brain tumor and to the ipsilateral and contralateral tumor-free brain was achieved ($P < 0.05$). The increased access of ErPC to the brain after coinjection with pentylglycerol was also associated with significantly lower plasma levels of ErPC ($P < 0.05$). Of particular interest is the fact that the pentylglycerol-mediated BBB opening appeared to be more

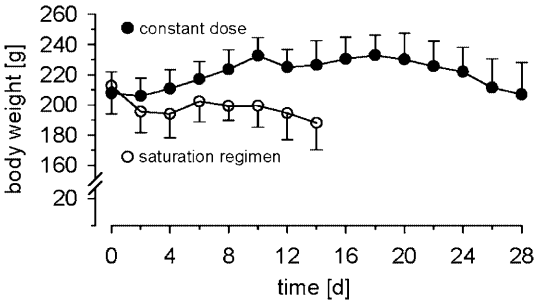

Fig. 2. Change in body weight of animals treated with i.v. ErPC (*closed circles* constant dose regimen over 30 days, *open circles* saturation regimen over 14 days; see Fig. 1)

pronounced in the tumor tissue, as evidenced by the concentration difference between tumor (289 pmol/mg) and surrounding brain tissue (ipsilateral, 163 pmol/mg; $P < 0.05$, Fig. 3). In contrast to this, i.a. bradykinin was not followed by an increase in ErPC concentrations in either tumor tissue or surrounding tumor-free brain.

Using ethanolic ErPC, there were higher ErPC concentrations in all brain regions than after the use of aqueous ErPC ($P < 0.05$). Thus, ethanol itself induced an increase in BBB permeability and the ethanolic ErPC solution was therefore not suitable as a control to assess the pentylglycerol-associated effects at the BBB.

Discussion

Serial i.v. ErPC administrations have been shown to result in an accumulation of this new APC derivative in the brain and considerable amounts of the drug have been found within the CNS after 4 weeks of ErPC treatment [5]. However, in contrast to the potent cytotoxic effects of ErPC against numerous brain tumor cell lines in vitro, only a moderate therapeutic effect has been achieved when using ErPC for the treatment of rat gliomas [4]. This has been attributed at least in part to ineffective ErPC concentrations within the brain at the beginning of the treatment. Since the induction of brain tumors in these experiments was followed by rapid tumor progression and death, the treatment periods were too short to allow significant ErPC-induced tumor reduction. The need for adequate drug delivery to the tumor or for longer treatment periods had already been inferred from previous studies in MNU-induced mammary carcinomas using oral chemotherapy with hexadecylphosphocholine or ErPC [19, 24]. Thus, to prove the putative excellent antitumor effects of ErPC against highly malignant brain tumors, new treatment concepts for opening the BBB and increasing drug delivery to the tumor target have to be developed.

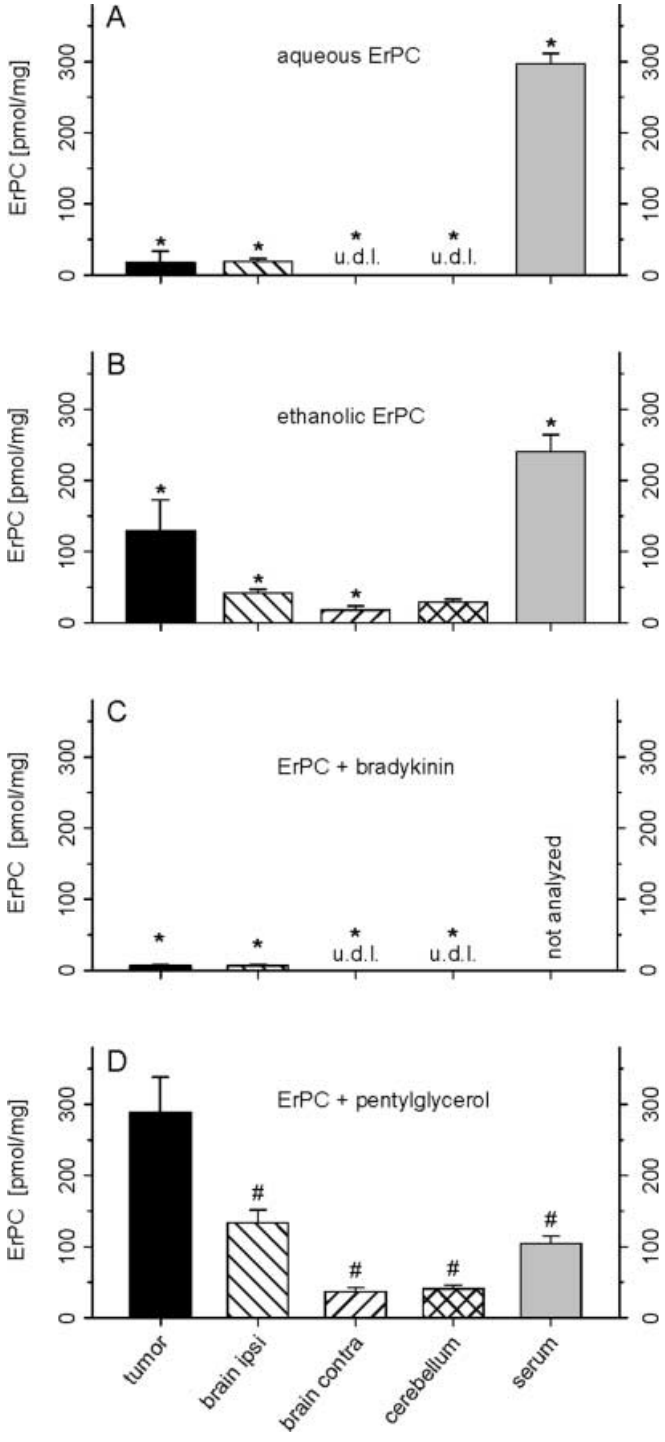

Fig. 3A–D. ErPC concentration in brain tumors, tumor-free brain and serum after a single i.a. administration of ErPC in the absence or presence of intracarotid 1-*O*-pentylglycerol or bradykinin. **A** Intracarotid injection of aqueous ErPC 40 mg/kg without alkylglycerol. **B** Intracarotid administration of ethanolic ErPC (water/ethanol 96/4 w/v; 40 mg/kg) without alkylglycerol. **C** Intracarotid infusion of aqueous ErPC 40 mg/kg in conjunction with intracarotid bradykinin infusion (10 µg/kg per min). **D** Intracarotid coinjection of 40 mg/kg ErPC and 300 m*M* pentylglycerol (*brain ipsi* brain tissue ipsilateral to the ErPC injection, *brain contra* brain tissue contralateral to the ErPC injection, *u.d.l.* under detection limit of 10 nmol/ml). Values are given as picomoles per milligram wet weight, means ± SD ($^{\#}P < 0.05$ normal brain vs tumor tissue, $^{*}P < 0.05$ vs respective specimen in **D**)

The BBB constitutes a major impediment to chemotherapy of malignant tumors in the CNS, since most anticancer drugs are unable to reach the tumor target at therapeutic concentrations [20, 26]. The osmotic disruption of the BBB by i.a. infusion of hypertonic mannitol significantly increases drug delivery to normal brain tissue and to brain tumors, and it has been reported to be effective in the chemotherapy of patients with primary lymphoma of the CNS [2] and malignant glioma [9, 20]. However, mannitol increases drug transfer predominantly to the normal brain rather than to the tumor itself [11, 13, 25] and this effect lasts for several hours [28], thereby increasing the potential for toxicity. In contrast, pharmacological modification of the BBB by bradykinin or its analog lobradimil (RMP-7) allows a highly specific increase in drug delivery to brain tumors [12, 27]. In experimental tumor studies as well as in clinical trials, the increase in drug transport to the tumor has been described as weak [1, 21, 23]. Thus, there is a need for new concepts to overcome the limited access of chemotherapeutic drugs to the brain and to brain tumors.

Chemical opening of the BBB by i.a. alkylglycerols has already been described in earlier animal experiments and a significant accumulation of several drugs was achieved in the brain after coinjection with different alkylglycerol derivatives, as compared to controls without pentylglycerol [6]. In the present study, we demonstrated an impressive pentylglycerol-mediated increase in ErPC concentrations in both brain tumor (17-fold) and surrounding brain tissue (7-fold), whereas intracarotid bradykinin failed to enhance the concentrations of ErPC in the CNS. In order to verify the surprisingly low ErPC levels found in the tumors of bradykinin-treated animals, tissue samples were reevaluated by HPLC analysis. ErPC concentrations found using HPLC were as low as those found using HPTLC and tissue levels in the tumor and ipsilateral brain were less than 5 pmol/mg.

With i.a. administration of 1-*O*-pentylglycerol and ErPC, a slight tumor-to-brain selectivity was demonstrated resulting in ErPC concentrations in the tumor exceeding those of the surrounding brain by 2.2-fold (see Fig. 2). In contrast, we have recently found no tumor-to-brain selectivity in C6 glioma-bearing rats after intracarotid coinjection of 1-*O*-pentylglycerol and methotrexate [6]. The delivery of methotrexate was increased 18-fold in the tumor and 28-fold in surrounding brain, as compared with controls without pentylglycerol. Thus, the extent of drug penetration and the spatial distribution in the brain appear to be influenced not only by structural modifications of the alkylglycerols [7], but also by the physicochemical properties of the coadministered cytotoxic drug. Regarding the tumor tissue, however, the pentylglycerol-induced increase in drug concentrations was equal for both methotrexate and ErPC.

The lack of acute toxicity of the pentylglycerol-associated BBB opening, as assessed by circulatory parameters, and hematological and clinical chemistry values, is consistent with results recently obtained after intracarotid administration of several short-chain alkylglycerols (pentyl and hexyl analogs). Moreover, no clinical, laboratory or histopathological signs of long-term pentylglycerol-induced toxicity have been observed [8].

In conclusion, pentylglycerol-induced BBB opening dramatically increased the tissue concentrations of ErPC in the tumor and surrounding brain after i.a. injection, whereas intracarotid bradykinin was ineffective. The ErPC levels measured in the CNS also exceeded those obtained after long-term i.v. treatment. Thus, alkylglycerols represent a novel instrument to increase delivery of chemotherapeutic agents to brain tumors but also to more distant regions of the brain containing infiltrating and migrating cells that might be responsible for tumor recurrence. The selectiveness of the effect in favor of the tumor tissue reduces the potential for toxicity in the normal brain. I.a. administration of ErPC in the presence of alkylglycerols is a promising new concept for brain tumor chemotherapy.

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References

1. Black KL, Clougheshi T, Huang SC, Gobin YP, Zhou Y, Grous J, Nelson G, Faharani K, Hoh CK, Phelps M (1997) Intracarotid infusion of RMP-7, a bradykinin analog, and transport of gallium-68 EDTA into human gliomas. J Neurosurg 86:603
2. Dahlborg SA, Petrillo A, Crossen JR, Roman-Goldstein S, Doolitle ND, Fuller KH, Neuwelt EA (1998) The potential for complete and durable response in nonglial primary brain tumors in children and young adults with enhanced chemotherapy delivery. Cancer J Sci Am 4:110
3. Eibl H, Kaufmann-Kolle P (1995) Medical application of synthetic phospholipids as liposomes and drugs. J Liposome Res 5:131
4. Erdlenbruch B, Jendrossek V, Marx M, Hunold A, Eibl H, Lakomek M (1998) Antitumor effects of erucylphosphocholine on brain tumor cells in vitro and in vivo. Anticancer Res 18:2551
5. Erdlenbruch B, Jendrossek V, Gerriets A, Vetterlein F, Eibl H, Lakomek M (1999) Erucylphosphocholine: pharmacokinetics, biodistribution and CNS accumulation in the rat after intravenous administration. Cancer Chemother Pharmacol 44:484
6. Erdlenbruch B, Jendrossek V, Eibl H, Lakomek M (2000) Transient and controllable opening of the blood-brain barrier to cytostatic and antibiotic agents by alkylglycerols in rats. Exp Brain Res 135:417
7. Erdlenbruch B, Schinkhof C, Kugler W, Eibl H, Lakomek M (2000) Enhanced drug delivery to the brain and brain tumors in rats by alkylglycerols and regulation of the effect by structural modifications. Int J Mol Med 6 [Suppl 1]:S36
8. Erdlenbruch B, Schinkhof C, Kugler W, Alipour M, Eibl H, Lakomek M (2001) Opening of the blood-brain barrier by alkylglycerols in rats: pharmacokinetics, biodistribution and toxicity. Neuro-oncology 3:289
9. Gumerlock MK, Belshe BD, Madsen R, Watts C (1992) Osmotic blood-brain barrier disruption and chemotherapy in the

treatment of high grade malignant glioma: patient series and literature review. J Neurooncol 12:33
10. Harms PG, Ojeda SR (1974) A rapid and simple method for chronic cannulation of the jugular vein. J Appl Physiol 36:391
11. Hiesinger EM, Voorhies RM, Basler GA, Lipschutz LE, Posner JB, Shapiro WR (1986) Opening the blood-brain and blood-tumor barriers in experimental rat brain tumors: the effect of intracarotid hyperosmolar mannitol on capillary permeability and blood flow. Ann Neurol 19:50
12. Inamura T, Nomura T, Bartus RT, Black KL (1994) Intracarotid infusion of RMP-7, a bradykinin analog: a method for selective drug delivery to brain tumors. J Neurosurg 81:752
13. Inoue T, Fukui M, Nishio S, Kitamura K, Nagara H (1987) Hyperosmotic blood-brain barrier disruption in brains of rats with an intracerebrally transplanted RG-C6 tumor. J Neurosurg 66:257
14. Jendrossek V, Erdlenbruch B, Hunold A, Kugler W, Eibl H, Lakomek M (1999) Erucylphosphocholine, a novel antineoplastic ether lipid, blocks growth and induces apoptosis in brain tumor cell lines in vitro. Int J Oncol 14:15
15. Jendrossek V, Kugler W, Erdlenbruch B, Eibl H, Lakomek M (2001) Induction of differentiation and tetraploidy by long-term treatment of C6 rat glioma cells with erucylphosphocholine. Int J Oncol 19:673
16. Jendrossek V, Kugler W, Erdlenbruch B, Eibl H, Lang F, Lakomek M (2001) Erucylphosphocholine-induced apoptosis in chemoresistant glioblastoma cell lines: involvement of caspase activation and mitochondrial alterations. Anticancer Res 21:3389
17. Konstantinov SM, Berger MR (1999) Human urinary bladder carcinoma cell lines respond to treatment with alkylphosphocholines. Cancer Lett 144:153
18. Konstantinov SM, Eibl H, Berger MR (1998) Alkylphosphocholines induce apoptosis in HL-60 and U-973 leukemic cells. Cancer Chemother Pharmacol 41:210
19. Kötting J, Berger MR, Unger C, Eibl H (1992) Alkylphosphocholines: influence of structural variation on biodistribution at antineoplastically active concentrations. Cancer Chemother Pharmacol 30:105
20. Kroll RA, Neuwelt EA (1998) Outwitting the blood-brain barrier for therapeutic purposes: osmotic opening and other means. Neurosurgery 42:1083
21. Kroll RA, Pagel MA, Muldoon LL, Roman-Goldstein S, Fiamengo SA, Neuwelt EA (1998) Improving drug delivery to intracerebral tumor and surrounding brain in a rodent model: a comparison of osmotic versus bradykinin modification of the blood-brain and/or blood-tumor barriers. Neurosurgery 43:879
22. Marschner N, Kötting J, Eibl H, Unger C (1992) Distribution of hexadecylphosphocholine and octadecyl-methyl-glycero-3-phosphocholine in rat tissues during steady-state treatment. Cancer Chemother Pharmacol 31:18
23. Matsukado K, Inamura T, Nakano S, Fukui M, Bartus RT, Black KL (1996) Enhanced tumor uptake of carboplatin and survival in glioma-bearing rats by intracarotid infusion of bradykinin analog, RMP-7. Neurosurgery 39:125
24. Muschiol C, Berger MR, Schuler B, Scherf HR, Garzon FT, Zeller WJ, Unger C, Eibl H (1987) Alkylphosphocholines: toxicity and anticancer properties. Lipids 22:930
25. Nakagawa H, Groothuis D, Blasberg RG (1984) The effect of graded infusions on drug delivery to experimental RG-2 gliomas. Neurology 34:1571
26. Neuwelt EA, Abbott NJ, Drewes L, Smith QR, Couraud PO, Chiocca EA, Audus KL, Greig NH, Doolittle ND (1999) Cerebrovascular biology and the various neural barriers: challenges and future directions. Neurosurgery 44:604
27. Nomura T, Inamura T, Black KL (1994) Intracarotid infusion of bradykinin selectively increases blood-tumor permeability in 9L and C6 brain tumors. Brain Res 659:62
28. Siegal T, Rubinstein R, Bokstein F, Schwartz A, Lossos A, Shalom E, Chisin R, Gomori JM (2000) In vivo assessment of the window of barrier opening after osmotic blood-brain barrier disruption in humans. J Neurosurg 92:599