RESEARCH NOTE

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Transient and controllable opening of the blood-brain barrier to cytostatic and antibiotic agents by alkylglycerols in rats

Abstract The blood-brain barrier hinders progress in the chemotherapy of brain tumors due to insufficient penetration of anticancer drugs into the brain tissue. Short-chain alkylglycerols affect the physicochemical properties of biological membranes. The enhancement of the blood-brain barrier permeability by intra-arterial administration of alkylglycerols was investigated in tumor-free and C6 astroglioma bearing rats. The antineoplastic agents cisplatin and methotrexate and the antibiotics vancomycin and gentamicin were selectively injected into the right internal carotid artery in the absence and presence of various alkylmono-, alkyldi-, and alkyltriglycerols. In normal rats the intra-arterial administration of the drugs without alkylglycerols resulted in low drug concentrations in brain tissue. In the presence of alkylglycerols (0.01–0.3 M) a reversible (within minutes) and concentration-dependent enrichment of the coinjected agents was found, preferentially in the ipsilateral hemisphere. The extent of drug accumulation in the brain was modified by changes in the chemical structure of the alkylglycerols. The effect increased with the chain length of the alkyl group, decreased with the number of glycerols, and varied from 2- to more than 230-fold compared to controls. In rats with C6 tumors 1-*O*-pentylglycerol increased the delivery of methotrexate 18-fold in the tumor, 28-fold in the surrounding brain, 18-fold in the contralateral brain, and 19-fold in the cerebellum compared to controls with methotrexate in the absence of pentylglycerol. In conclusion, the intra-arterial administration of alkylglycerols represents a novel and well controllable method for enhanced drug delivery to the brain and to brain tumors.

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Introduction

The blood-brain barrier presents considerable pharmaco-therapeutic difficulties in the treatment of a variety of disorders of the central nervous system (CNS). The sealing effect of the brain capillary endothelium prevents sufficient penetration of potent therapeutic drugs into the brain (Cornford and Hyman 1999; Jolliet-Riant and Tillement 1999; Neuwelt et al. 1999). Since primary brain tumors and brain metastases of extracranial malignancies are characterized by high frequency and poor prognosis, drug delivery to the brain plays an important role in clinical oncology. In particular, the relative lack of progress in chemotherapy of childhood brain tumors differs from the favorable outcomes of patients with pediatric leukemia or extracranial solid tumors (Packer 1997) and illustrates the necessity for new therapeutic approaches to overcome the blood-brain barrier. Apart from CNS tumors, the treatment of inflammatory diseases of the brain is also impeded by ineffective drug delivery to the brain. Several experimental preclinical studies have been conducted to increase the blood-brain barrier permeability, but few techniques have advanced to the stage of clinical trials (Neuwelt and Rapoport 1984). The intracarotid infusion of hypertonic mannitol solutions is the most thoroughly investigated method to open the blood-brain barrier (Kroll et al. 1998; Rapoport et al. 1972), but it still has not found widespread acceptance in clinical neuro-oncology. This is due in part to the side effects observed after osmotic blood-brain barrier disruption (Neuwelt et al. 1983; Roman-Goldstein et al. 1991) and to the weaker effects on tumor tissue than on the normal tumor-free brain (Blasberg et al. 1990; Hiesinger et al. 1986; Inoue et al. 1987). A more specific increase in the permeability of brain tumor vasculature is produced by intra-arterial bradykinin (Nakano et al. 1996) or its analogue RMP-7 (Matsukado et al. 1996), but the

magnitude of increased delivery of chemotherapeutic agents to the tumor tissue is very limited (less than two-fold; Kroll et al. 1998). Other laboratory studies have recently described a blood-brain barrier modification using sodium dodecyl sulfate (Saija et al. 1997) or lysophosphatidic acid (Schulze et al. 1997). A new concept for improved drug delivery to the brain, however, has not yet been established.

Short-chain alkylglycerols are known to affect the physicochemical properties of biological membranes. Due to their lipophilia and rapid membrane permeation they were used in the cryopreservation of mononuclear cells (Schuff-Werner et al. 1988). Recently evidence has emerged showing that alkylglycerols increase the permeability of the blood-brain barrier (Erdlenbruch et al. 1999). The purpose of this study was to evaluate efficacy and structure-activity relationships of short-chain alkylglycerols on the permeability of the blood-brain barrier in order to find the most suited compounds for a new therapeutic approach of CNS diseases. We investigated the effects of several alkylmono-, alkyldi-, and alkyltriglycerols on the permeation of anticancer and antibiotic agents across the blood-brain barrier in normal and in C6 astroglioma bearing rats. A novel and promising concept for reversible, controllable and very effective increase in drug delivery both to the normal brain and to brain tumors is presented.

Materials and methods

Chemicals

Alkylmonoglycerols (propyl-, butyl-, isobutyl-, pentyl-, and hexyl-analogues) were obtained by alkylation of 1.2-isopropylidene-glycerol with alkylbromides in the presence of potassium *tert*-butylate. After acidic deprotection the compounds were purified by distillation or silica gel chromatography. The 1-*O*-alkyl-diglycerols (pentyl- and hexyldiglycerol) were prepared in a similar sequence of reactions via 2-*O*-benzyl-glycero-1.3′-(1.′2′-isopropylidene)-glycerol by alkylation with pentyl- or hexylbromide in the presence of potassium *tert*-butylate. After acidic deprotection, the benzyl group was removed by catalytic hydrogenolysis (5% Pd/C in tetrahydrofurane as solvent). The 2-*O*-alkyl-triglycerols (pentyl-, hexyl-, heptyl-, and octyl-) were prepared via 1-*O*-benzyl-glycero-3.1′-*O*-glycero-2′-*O*-benzyl-3′.1″-*O*-(2.″3″-isopropylidene)-glycerol as described above by alkylation with pentyl-, hexyl-, heptyl-, and octylbromide in the presence of potassium *tert*-butylate. Again, after acidic deprotection the two benzyl groups were removed by catalytic hydrogenolysis. The products were purified by silica gel chromatography. Purity of the alkyl-oligoglycerols was assessed by HPLC. No byproducts were observed. The following results were obtained in a more detailed analysis for contamination of alkyl-oliogoglycerols with positional isomers: In the case of the 1-*O*-alkylglycerols the purity of the compounds varied in the range of 98.5–99.5%, the difference to 100% being the 2-*O*-alkyl-isomer. The purity of the 2-*O*-alkyl-glycerols varied between 98.5–99.5% containing 0.5–1.5% of the 1-*O*-alkyl-isomer. The structural formulas of the substances are as follows: R_1-O-CH_2-CHOH-CH_2OH (R_1: propyl, butyl, *tert*-butyl, pentyl and hexyl), R_2-O-CH_2-CHOH-CH_2-O-CH_2-CHOH-CH_2OH (R_2: pentyl and hexyl) and HOH_2-CHOR_3-CH_2-O-CH_2-CHOH-CH_2-O-CH_2-CHOH-CH_2OH (R_3: pentyl, hexyl, heptyl and octyl). Concentrations of the alkylglycerols varied between 0.01 and 0.3 M. Osmolarity of the solutions administered was adjusted to 300 mosm/kg (260–350 mosm/kg).

Chemotherapeutic and antibiotic agents known to have poor penetration into the brain tissue after parenteral administration were selected for coinjection at doses similar to those used in clinical practice. The antibiotics vancomycin and gentamicin were added into the study to investigate a broad spectrum of drugs with very different physicochemical properties. Additionally, enhanced delivery of drugs to the CNS is desirable not only in patients with malignant brain tumors but also in serious infectious diseases. Cisplatin (CDDP, 4 mg/kg body weight b.w.; Sigma, Deisenhofen, Germany) was dissolved in physiological saline while methotrexate (MTX, 5 mg/kg b.w.; Lederle Arzneimittel, Münster, Germany), vancomycin (10 mg/kg b.w.; Lilly, Homburg, Germany), and gentamicin (3 mg/kg b.w.; Merck, Darmstadt, Germany) were used as solutions manufactured for clinical treatment. All solutions for the intracarotid administration were heated to 37°C and sterilized by filtration immediately before use. Of the drug solution 800 µl was injected with a flow rate of 6 ml/min followed by rinsing with 400 µl isotonic saline. The total volume of 1.2 ml was injected within 12 s.

Animals and experimental procedure

Tumor-free rats

A total of 138 male Wistar rats weighing between 250 and 320 g were kept under conventional controlled conditions and had free access to a standard diet (Altromin) and tap water until the experiment. After intraperitoneal anesthesia by pentobarbital (50 mg/kg) the right external carotid artery was cannulated in a retrograde manner. Branches of the external carotid artery were ligated. Blood pressure and heart rate were recorded via the left femoral artery by a statham transducer (Gould). The test drugs were injected within 12 s either in the absence or in the presence of the alkylglycerol of interest. Antegrade blood flow was interrupted by clamping the common carotid artery during the injection. Blood samples were drawn 5 min after the injection, the left ventricle was cannulated, and organs were rinsed by isotonic saline and rapidly removed. Hematological and serum values were determined to evaluate acute toxicity of the alkylglycerols. In an additional series of experiments, CDDP or MTX was given separately after an interval of 3 and 15 min following the administration of the alkylglycerol. These experiments were also terminated 5 min after test drug injection.

Tumor experiments

C6 cells were injected stereotactically into the right putamen of male Wistar rats weighing 180–210 g as described by Erdlenbruch et al. (1998). Sixteen to 20 days after tumor implantation the animals showed signs of tumor manifestation, and MTX (5 mg/kg) was administered into the right internal carotid artery in the absence (n=6) or in the presence of 1-*O*-pentylglycerol (0.3 M, n=6) as described above. The current stipulations of the German law on the protection of animals were observed.

Analysis of drug concentrations

The tissue concentrations of the drugs were determined separately in the right hemisphere (ipsilateral to the injection), left hemisphere (contralateral), and cerebellum and given as picomoles/milligram of wet weight. Organs were minced and homogenized in aqueous acidic (perchloric acid 70%; CDDP), alkaline (NaOH 0.1 M; MTX), or neutral medium (H_2O; gentamicin and vancomycin). CDDP concentrations were analyzed by atomic absorption spectrometry at 2650°C by a GBC 904 AA spectrometer (Maassen, Ravensburg, Germany). Lower limit of detection was 0.16 µmol/l. MTX, gentamicin, and vancomycin concentrations were determined by fluorescence polarization immunoassay technology (Jolley et al. 1981). The immunoassay reagent systems

were purchased from Abbott Laboratories (Ill., USA) and analyses were performed according to the operation manuals. Calibration curves were established for tissue concentrations of the respective drugs.

Statistical evaluation

Student's t test was used for statistical analysis. Mean values ±SD are presented unless otherwise indicated.

Results

The intra-arterial injection of CDDP, MTX, gentamicin, or vancomycin into the right internal carotid artery of rats in the absence of alkylglycerols resulted in low tissue concentrations of each drug with no regional differ-

ences between the right hemisphere, the left hemisphere and the cerebellum (Fig. 1A–D, $n=6$ in each group). After simultaneous administration with various alkylglycerol analogues, an accumulation of the drugs was found in the brain, predominantly in the ipsilateral right hemisphere. Using 1-O-pentylglycerol (0.3 M) the tissue concentrations in the ipsilateral hemisphere were increased 230-fold (MTX), 125-fold (CDDP), 15-fold (vancomycin) and 12-fold (gentamicin) compared to the injection in the absence of alkylglycerols (Fig. 1E–H, $n=6$ in each group).

The effect of the alkylglycerols on the blood-brain barrier was concentration dependent (Fig. 2A) and increased with the chain length of the alkyl group (Fig. 2B). Heptyl- and octyl derivatives were highly potent even at low doses, resulting in high concentrations of the coinjected drugs in the ipsilateral hemisphere and also to a lesser extent in the contralateral hemisphere and cerebellum. On the other hand, enhancement of drug delivery to
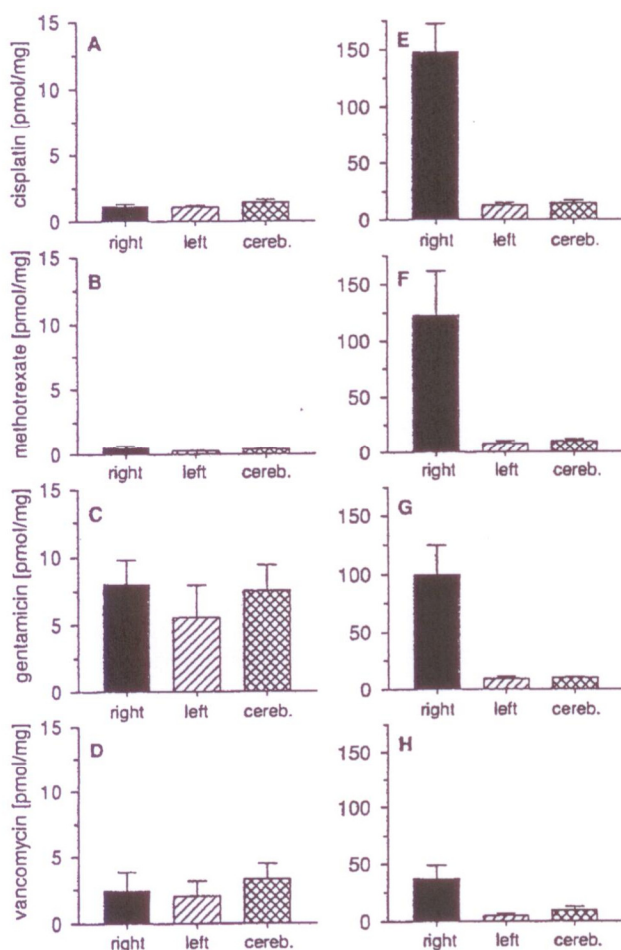

Fig. 1 Drug concentrations in the brain tissue of normal rats after injection of cisplatin (4 mg/kg), methotrexate (5 mg/kg), gentamicin (3 mg/kg), and vancomycin (10 mg/kg) into the right internal carotid artery without (A–D) or in the presence of 0.3 M 1-O-pentylglycerol (E–H). Concentrations were determined in the right hemisphere, left hemisphere, and cerebellum and are given as means ±SEM (pmol/mg wet weight, $n=6$ in each experiment). Note different range of y-axis)
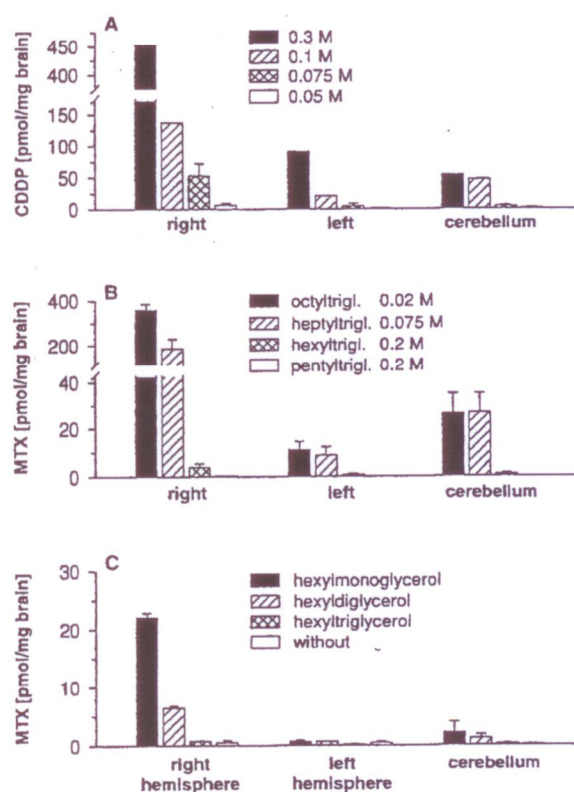

Fig. 2 Structure-activity relationships of the alkylglycerols on drug concentrations in the brain tissue. A Concentration dependence. Concentrations of cisplatin in the brain after intra-arterial coinjection with 1-O-hexyldiglycerol at concentrations increasing from 0.05 M to 0.3 M ($n=15$). B Effect of the chain length of the alkyl group. Concentrations of methotrexate in the brain after intra-arterial coinjection with different triglycerol derivatives ($n=15$). C Effect of polarity (number of glycerols). Concentrations of methotrexate in the brain after intra-arterial coinjection with 1-O-hexylmono-, 2-O-hexyldi-, and 2-O-hexyltriglycerol 0.1 M ($n=14$)

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Table 1 Effects of 1-*O*-pentylglycerol at the blood-tumor barrier. Concentration of MTX in the tumor and surrounding brain areas after a single intra-arterial injection of 5 mg/kg into the right inter-nal carotid artery in the absence or in the presence of 1-*O*-pentyl-glycerol (0.3 M). Tumors were situated in the right hemisphere

Solution injected	Tissue concentration of MTX (pmol/mg brain)			
	Tumor	Brain ipsilateral	Brain contralateral	Cerebellum
MTX	16±9.7	2.7±2.4	0.4±0.2	0.8±0.4
MTX + pentylglycerol	277±76*	76±57*	7.8±5.4*	14.7±9.8*

*$P<0.05$, MTX without vs. MTX in the presence of pentylglycerol
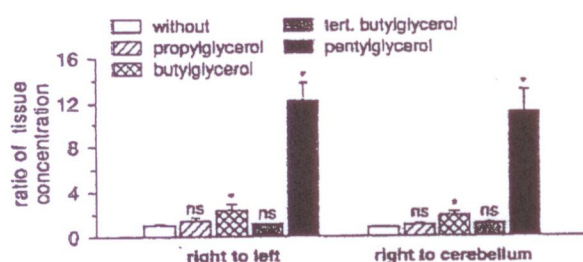

Fig. 3 Accumulation of cisplatin in the ipsilateral (right) hemisphere after intra-arterial injection in the absence or in the presence of various monoglycerol derivatives. Modulation of the concentration ratio ipsilateral to contralateral and ipsilateral to cerebellum by structural variations of the alkyl chain ($n=31$)
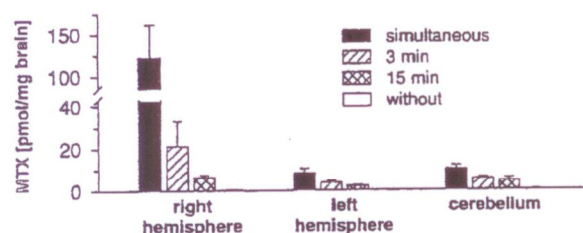

Fig. 4 Reversibility of the effect. Concentrations of methotrexate in the brain after simultaneous injection or 3 and 15 min after 1-*O*-pentylglycerol 0.3 M ($n=15$)

Hematological and serum analyses (sodium, potassium, calcium, glucose, total protein, aminotransferases, lactate dehydrogenase, bilirubin, and creatinine) revealed no acute toxic side effects of the monoglycerol analogues up to 0.3 M (hexylmonoglycerol was only injected up to 0.1 M). The diglycerol derivatives, however, caused hemolysis at concentrations exceeding 0.1 M (data not shown). 2-*O*-Heptyltriglycerol and 2-*O*-octyltriglycerol were hemolytic exceeding concentrations of 0.075 and 0.05 M, respectively.

The effect of 1-*O*-pentylglycerol was also evaluated at the blood-tumor barrier after coadministration of MTX to C6 rat astroglioma bearing rats (Table 1; Fig. 4). In the presence of 1-*O*-pentylglycerol (0.3 M) the concentrations of MTX were increased 18-fold in the tumor, 28-fold in the surrounding brain, 18-fold in the contralateral brain, and 19-fold in the cerebellum compared with MTX controls in the absence of pentylglycerol ($P<0.05$).

Discussion

Nearly all anticancer agents penetrate poorly into the brain after intravenous or intra-arterial injection (Donelli et al. 1992). The aims of the present study were to determine the extent of enhanced drug delivery to the brain by intra-arterial alkylglycerols, to evaluate structure activity relationships of these new compounds, and to assess the potential relevance of this procedure for chemotherapy of brain tumors. The intracarotid administration of short chain alkylglycerols resulted in a marked increase in the CNS concentrations of simultaneously given anticancer or antibiotic agents and allowed a precise regulation of drug amount delivered to the brain. The enhanced drug permeation across the blood-brain barrier could be adjusted by variations in the chemical structure and the concentration of the alkylglycerols as well as the dose of the coinjected drug (data not shown). Furthermore, lower molecular weight and higher lipophilia of the coinjected drug allowed higher CNS accumulation (Fig. 1). Due to a different predominance of the effect within the hemisphere ipsilateral to the intra-arterial injection the concentration ratio ipsilateral hemisphere to contralateral hemisphere and ipsilateral hemisphere to cerebellum could also be modified (Fig. 3). Moreover, the increase in barrier permeability was reversible within a few minutes.

the brain was weakened by the high polarity of the diglycerol and, in particular, the triglycerol analogues (Fig. 2C). Even small modifications in the chemical structure of the alkyl chain allowed to modulate the permeation of the therapeutics into the brain. The administration of CDDP together with 1-*O*-butyl- or 1-*O*-pentyl-glycerol caused a significant accumulation of CDDP in the right hemisphere ($P<0.05$), while the shorter 1-*O*-propyl- and the spheroid 1-*O*-tert-butylglycerol were ineffective (Fig. 3). Furthermore, the 2-*O*-alkyl-isomers were less effective than the respective 1-*O*-alkyl-isomers (see Fig. 2A, C) probably due to a decrease in lipophilia of the molecules. Separating the injection of alkylglycerol and MTX or CDDP by a time interval of 3 or 15 min led to an extensive attenuation (MTX, Fig. 3) or a complete loss (CDDP, data not shown) of the enhanced barrier permeability. Thus the effect of the alkylglycerols at the blood-brain barrier was reversible within a few minutes.

Since alkylglycerols failed to be effective after intravenous injections, it is most likely that high local concentrations in the brain capillaries were required to be effective. The lipophilia of the alkylglycerols allows a fast equilibrium between the cell membrane of the endothelium and the surrounding medium (Schuff-Werner et al. 1988). Due to the amphiphilic character of the alkylglycerols one can speculate that the rapid invasion of these molecules disturbs the arrangement of the membrane lipids. Therefore a short fluidization of the blood-brain barrier resulting in a transient destabilization of the barrier function can be expected. This was supported by the rapid and nearly complete restoration of the original impermeability within 3–15 min.

In contrast to the osmotic blood-brain barrier disruption showing a drastic and predominant effectiveness at the intact barrier of the tumor-free brain regions (Hiesinger et al. 1986), the administration of pentylglycerol to C6 tumor bearing rats revealed an equally potent increase in drug permeation across both the blood-tumor barrier and the intact blood-brain barrier. Thus a homogeneously enhanced delivery of anticancer drugs to both tumor tissue and surrounding normal brain was obtained with alkylglycerols without preference to the normal brain. In several animal brain tumor models the tumor vessels were less sensitive to osmotic manipulation than the microvasculature in the surrounding cortex (Nakagawa et al. 1984). The relative higher extent of drug accumulation in the tumor free brain was considered to increase neurotoxicity of anticancer agents after intracarotid infusion of hyperosmotic mannitol (Inoue et al. 1987). Consequently the homogeneous response of the tumor barrier and the barrier in surrounding normal brain to intracarotid alkylglycerols suggests that higher antitumor effects could be achieved without associated excessive neurotoxicity of the anticancer drugs. Moreover, the use of an alkylglycerol derivative with less marked effects in the contralateral hemisphere as 0.3 M pentylglycerol (Fig. 1) possibly enables a more specific drug accumulation within the tumor tissue. The high efficacy of the alkylglycerols at the tumor vessels also differed from bradykinin or RMP-7-mediated opening of the blood-tumor barrier, which resulted only in a moderate increase in the delivery of MTX to intracerebral tumors in rats (Kroll et al. 1998). To assess the clinical relevance of intra-arterial administration of alkylglycerols in brain tumor chemotherapy, however, enhanced neurotoxic effects of the coadministered cytotoxic drugs as MTX-induced leukoencephalopathy have yet to be excluded.

With regard to the dose-response curves, the hexyl derivatives allowed a stepwise increase in the drug concentrations in the brain (Fig. 2A) whereas the pentyl-derivatives showed a very marked accumulation only after exceeding a threshold concentration (data not shown). This might be of therapeutic interest because gradation and control of the effect would be a prerequisite for feasibility and safety in clinical use. The lack of abnormal hematological and clinical chemistry parameters is consistent with the low acute toxicity of the alkylglycerols

investigated, but long-term studies must evaluate toxicities of the most favorable derivatives. Ongoing toxicity studies in rats after intracarotid injection of 0.1 M 1-O-pentylglycerol revealed neither clinical nor histopathological signs of pentylglycerol-induced neurotoxicity within 2 and 4 weeks after a single bolus administration (data not shown). As short-chain alkylglycerols are not cleaved by O-alkylglycerol mono-oxygenase (Kötting et al. 1987), a rapid and unchanged elimination of the substances was predicted. Indeed, pharmacokinetic experiments using ^{14}C-labeled 1-O-pentylglycerol showed that 4.5 h after a single intracarotid bolus administration concentrations of pentylglycerol were very low in all brain areas, equal to the respective plasma levels. By then 75% of radioactivity had already been eliminated in the urine (unpublished data).

In conclusion, the intracarotid administration of alkylglycerols constitutes a novel and very promising principle to increase drug delivery to brain tissue in a well regulatable manner. The data presented emphasize the high efficacy of alkylglycerols in increasing cerebrovascular permeability in both normal and tumor tissue.

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