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"Short-Chain Alkylglycerols' Acute Impact on the Blood-Brain Barrier Characteristics of Cultured Brain Endothelial Cells"

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KEYWORDS

blood-brain barrier; brain endothelial cells; short-chain alkylglycerols; tight junctions; trans endothelial electrical resistance; permeability; claudin-5; electron microscopy.

ABSTRACT:

Patients with brain tumors cannot receive effective treatment because of the blood-brain barrier's (BBB) restriction on medicine penetration into the brain. Short-chain alkylglycerols (AGs) injected intraarterially open the blood-brain barrier (BBB) and enhance molecular transport to the mouse brain parenchyma in vivo. It is yet unknown what mechanism AG uses to modify BBB permeability. Here, we have examined how cultured brain microvascular endothelial cells' barrier characteristics are affected by AGs. Using an in vitro BBB model made up of primary cultures of rat brain endothelial cells co-cultured with rat cerebral glial cells, the effects of two AGs, 1-O-pentylglycerol and 2-O-hexyldiglycerol, were investigated. Claudin-claudin trans-interactions, immunostaining for junctional proteins, freeze-fracture electron microscopy, and functional assays were used to assess the integrity of the paracellular, tight junction-based permeation route AG therapy for five minutes altered BBB permeability for fluorescein and reversibly decreased trans endothelial electrical resistance, along with alterations in cell shape and β-catenin and claudin-5 immunostaining. Neither the trans-interaction of claudin-5 nor the inter-endothelial tight junction strand complexity changed in tandem with these transient modifications .The short-lived, reversible increase in brain endothelial paracellular permeability caused by AG did not impact the intricacy of tight junction strands. The involvement of the cytoskeleton in the action of AGs is indicated by changes in cell shape and redistribution of junctional proteins. These findings corroborate findings from rodent in vivo research that described AGs as adjuvants that temporarily breach the blood-brain barrier.

Introduction:

Treatment for brain illnesses including malignant brain tumors is hampered by the blood-brain barrier (BBB), which selectively controls the transcellular and paracellular transit of chemicals and cells between the blood and the central nervous system. Specific structures known as zonulae occludent, or tight junctions, are expressed in these closely spaced regions between brain endothelial cells (BECs). These structures' membranes attach to one another to form a nearly impermeable barrier, which significantly reduces the paracellular diffusion of hydrophilic substances through the vessel wall (Reese and Karnovsky, 1967). Transmembrane complexes called tight junctions are made up of branching networks of sealing strands formed by protein polymers. According to Bassig et al. (2006), each strand is made up of a row of transmembrane proteins implanted in both plasma membranes. Extracellular domains of these proteins engage through claudin-claudin trans- contacts with the tight junction proteins of the neighboring cell. Strand formation of the tight junction complexes (Furuse et al., 1998) and paracellular tightening (Piehl et al., 2010) are caused by claudin-claudin interactions. A weaker barrier is formed by zonae adherents, also known as adherents junctions (Rubin and Staddon, 1999). The Ca2+-regulated vascular endothelial protein cadherin, which facilitates cell-cell adhesion, is the main constituent of adherents junctions (Dejana et al., 2008). The cytoskeleton and cadherins are connected by a different group of proteins called catenin. Adherents junctions may be regulators of vascular permeability since they are necessary for the production of claudin-5 (Taddei et al., 2008). Apart from the reduced paracellular transport, the BBB restricts the transcellular movement of chemicals and cells into and out of the brain compartment due to the limited number of pinocytic vesicles. A very high transendothelial electrical

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resistance (TEER) is the result of the barrier functions. Generally speaking, a compound's size, or molecular weight, and its water solubility have an inverse relationship with its ability to penetrate the blood-brain barrier (BBB) (Pardridge, 2002). Accordingly, strengthening a drug's ability to pass through endothelial cells transcellularly has been achieved in part by making it more lipophilic (Reardon et al., 2006). Nevertheless, a number of substances that can cross the BEC membrane are efficiently returned to the capillary lumen by efflux transporters such P-glycoprotein and multidrug Inhibiting the efflux transporters has created another way for this efflux mechanism to increase drug entry into the brain. Therefore, medications that are substrates for P-glycoprotein will enter the brain more readily and be eliminated from it more slowly if P-glycoprotein is inhibited (Fellner et al., 2002; van Vliet et al., 2010; O'Brien et al., 2012). Coupled drug delivery to endogenous ligands of receptor- and carrier-mediated transport pathways, such as glucosamine (Dhanikula et al., 2008; also Deli, 2011) or angiopep-2 (Demeule et al., 2008), are further alternatives for better drug delivery to the central nervous system. There is currently no ideal treatment for CNS malignancies, either as primary tumors or metastases, despite much investigation. The cytostatic medications that are now on the market are either too big The use of tight junction modulators to open the epithelial or endothelial paracellular pathway is used to treat (high MW) or too water-soluble substances that cannot pass through blood vessel walls or biological barriers and access the brain (see Deli, 2009). The infusion of a hyperosmolar (1.37 M) mannitol solution (Neuwelt et al., 1985) is one previously described technique for increasing the paracellular diffusion rates of molecules across brain capillaries into the brain parenchyma. This opens the inter-endothelial tight junctions through the phosphorylation of the adherents junction protein, β-catenin, by Sac kinase (Rapoport, 2000; Farkas et al., 2005). In order to increase the efficacy of chemotherapy, osmotic BBB disruption has already been used in people with malignant brain tumors and in animal models (Doolittle et al., 2000). Osmotic BBB opening, however, was not well repeatable in clinical practice since it needed a modest therapeutic window and mannitol threshold concentration, and it lasted for roughly 6 hours (Siegal et al., 2000). This length of time may cause the increased BBB permeability to have harmful effects. Additionally, this approach requires interventional radiology, and its use has only been possible in a small number of clinics thus far. For over 25 years, short-chain alkylglycerols (AGs) have been recognized to enhance blood-brain barrier permeability (Unger et al., 1985). In the ipsilateral hemisphere of rodents, intraperitoneal administration of AGs resulted in a concentration-dependent enrichment of co-administered antibiotics and cytostatic medications (Erdlenbruch et al., 2000). AGs breached the blood-brain barrier in the surrounding brain and the tumor tissue in rats carrying gliomas (Erdlenbruch et al., 2003a). The therapeutic spectrum and efficiency of two AG derivatives, 1-O-pentylglycerol (PG) and 2-O-hexyldiglycerol (HG), have been demonstrated. After a bolus injection of PG (800 µL, 50 mM) and 5 mg·kg−1 methotrexate (MTX) into the rats' right internal carotid artery, the ipsilateral hemisphere of the brain contained approximately 1.2 pmol MTX per mg. In the opposite, that is, Merely 0.3 pmol MTX per mg brain was found in the untreated hemisphere with normal blood-brain barrier (Erdlenbruch et al., 2003b). It is even possible to greatly enhance the entry of high molecular weight substances like albumin and antibodies into the brain (Erdlenbruch et al., 2003a; Hülper et al., 2011). Depending on the AG concentration utilized, the BBB opening in vivo was reversible and lasted anywhere from a few minutes to approximately an hour (Erdlenbruch et al., 2000; 2002). These characteristics would be useful in a medical context. AGs were shown to be non-toxic and excreted from the body by the kidneys, according to the results of both in vitro and in vivo tests (Erdlenbruch et al., 2003b). There was no enrichment in the brain or other organs according to studies using radioactively labelled PG (Erdlenbruch et al., 2005). Following the addition of AG, the marker dye fluorescein may diffuse into the capillary lumen in newly isolated brain capillaries. Fluorescein was discovered in the paracellular cleft using confocal microscopy, suggesting that the paracellular diffusion barrier has opened (Erdlenbruch et al. In 2003a. Nevertheless, the manner in which AGs function and their impact on BECs remain unclear.

The current study used BEC cultures to investigate the direct, short-term effects of AGs on barrier properties and cell viability in an in vitro model of the blood–brain barrier. After an acute treatment with AGs, the paracellular barrier integrity of BECs was assessed using immunostaining for β-catenin and claudin-5 junctional proteins, measuring TEER and permeability for marker molecules (fluorescein and albumin), and analyzing the complexity of the inter-endothelial tight junction strands using freeze-fracture electron microscopy. A potential mechanism of action for AGs was also investigated by looking at the trans-interaction of claudin-5. For the first time, our in vitro results demonstrate that AGs could reversibly increase the flow of molecules through monolayers of brain endothelial cells without creating structural changes to the tight junction.

Culture of cells

Veszelka et al. (2007) provide a detailed description of how primary cultures of BECs were created from 2-

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week-old outbred Wistar rats (Harlan Laboratories, Indianapolis, IN, USA). Meninges were removed, and the grey matter was chopped into 1 mm3 pieces using a scalpel. The forebrains were then collected in ice-cold, sterile PBS, and digested for 1.5 hours at 37°C in DMEM using 1 mg·mL−1 collagenase CLS2 (Worthington Beachem., Lakewood, NJ, USA). The micro vessels containing myelin were separated by centrifugation (1000× g, 20 min) in 20% BSA–DMEM. They were then further digested for 1 hour in DMEM using 1 mg·mL−1 collagenase–dispose (Roche, Basel, Switzerland). Prior to plating on collagen, microvascular endothelial cell clusters were separated on a 33% continuous Percoll gradient (1000× g, 10 min), collected, and twice washed in DMEM. Dishes coated with type IV and fibronectin, multiwall plates (Fabcon, Becton Dickinson; Invitrogen, Carlsbad, CA, USA) or cell culture inserts (Transwell clear, 1 cm2; pore size of 0.4 µm; Corning Costar Co., Lowell, MA, USA) can be utilized. The cultures were kept in DMEM that was enhanced with 5% gentamicin, 20% bovine serum generated from plasma (First Link, Wolverhampton, UK), 1% basic fibroblast growth factor (Roche), and 100% heparin. Puromycin (4 µg·mL−1) was added to the growth medium for the first two days in order to specifically exclude P-glycoprotein-negative contaminating cells (Perrière et al., 2005). After a week, cultures acquired confluency and were put to use in tests. Rat cerebral glial cells were co-cultured with BECs in order to produce BBB properties (Deli et al., 2005; Veszelka et al., 2007). Glial cell primary cultures were created from 14-day-old Wistar rats. rats (Charles River, Sulzfeld, Germany, or Hannan Laboratories, Rossdorf, Germany). After the meninges were cut out, the cortical pieces were mechanically separated in DMEM with 10% FBS and 5 µg·mL−1 gentamicin. The mixture was then plated in 12-well dishes coated with poly-L-lysine and stored for at least three weeks before being used. 10% of the cells in confluent glia cultures were immunopositively for the microglia marker CD11b, whereas 90% of the cells were immunopositivity for the astroglia cell marker glial fibrillary acidic protein. Inserts were put into multiwall with astroglia at the bottom of the wells and endothelial culture media in both compartments for co-cultivation with BECs developed in cell culture. The culture media was supplemented with 550 nM hydrocortisone when the BECs were nearly confluent (Deli et al., 2005). Prior to beginning research, cells were treated with Ro 20-1724 (17.5 µM; Roche) and 8-(4-chlorophenylthio)-cAMP (CPT-cAMP; 250 µM) for 24 hours to tighten junctions and increase resistance (Deli et al., 2005; Perrière et al., 2005). Every experiment, with the exception of the claudin-claudin trans-interaction analysis, which were carried out using HEK293 cells and are detailed below, was carried out using primary rat BEC cultures.

Interventions

AGs were evaluated for five minutes on BECs at doses ranging from 0 to 100 mM. The sensitive BEC monolayers were cultured under physiological circumstances by incubating the cells with AGs dissolved in DMEM culture medium. As in earlier in vivo investigations, isotonic NaCl was employed for viability experiments (Erdlenbruch et al., 2003a,b; 2005). Treatment solutions were introduced to and taken out of the cells in each experiment very gradually so as not to disrupt the cell monolayers by medium changes.

Assays for cell viability and apoptosis using cell cytotoxicity

The WST-1 test, which is predicated on mitochondrial dehydrogenases cleaving a tetrazolium salt, was used to assess cell viability. In 96-well flatbottom microtiter plates with 100 µL per well, about 5 × 103 BECs were seeded. After allowing the cells to adhere for 24 hours, they were treated with escalating concentrations of PG or HG for 5 minutes before being refed with culture media. After treatment, 10 µL of WST-1 reagent was added to each well at 45, 24, 48, and 72 hours to gauge cell viability. The absorbance was measured using a SUNRISE microplate reader (TECAN, Crailsheim, Germany) at 450 nm and a reference filter at 690 nm two hours after the WST-1 reagent was added. cells given a 1% Triton treatment Untreated cells were employed as the reference, while X-100 detergent (Tx-100) in serum-free media for six hours served as the positive control. An ELISA kit for detecting cell death was used to quantify apoptosis (Roche Molecular Biochemicals, Mannheim, Germany). 200 µL of cells were planted in each well of 96-well microtiter plates. Cells were treated with PG or HG at increasing concentrations for five minutes after 48 hours. To induce apoptosis, cells were treated with 85 µM Staropoli for one hour, serving as a positive control. The kit's DNA histone complex was also used as an ELISA technical control. In the sandwich ELISA, the cytosolic fraction of either treated or untreated cells served as the antigen source. samples and the anti-histone-biotin and anti-DNA-peroxidase combination are incubated on a streptavidin-coated microplate. Antibodies were carried out in compliance with the manufacturer's guidelines. Using a reference filter at 492 nm, the absorption was measured using a SUNRISE microplate reader (TECAN) at 405 nm.

An assessment of the integrity of the barrier

Using STX-2 electrodes, an EVOM resistance meter (World Precision Instruments Inc., Sarasota, FL, USA) assessed the permeability of tight junctions for sodium

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ions in culture conditions (TEER), which was then represented relative to the surface area of the endothelial monolayer (Ω × cm2). From the experimental values, the TEER of the cell-free inserts (90–100 Ω × cm2) was deducted. The mean TEER of rat primary BEC monolayers in co-culture was 305(±4) Ω × cm2 (±SEM; n = 72 trials from three different isolations). The TEER ranged from 250 to 400 Ω × cm2. As previously reported (Veszelka et al., 2007), the flux of Evans Bluebelles albumin (EBA) and sodium fluorescein (SF) across endothelial cell monolayers was measured. Inserts for cell culture after therapy and measurement of TEER, were moved to 12-well plates with 1.5 mL of Ringer-HEPES solution in the basolateral compartments (118 mM NaCl, 4.8 mM KCl, 2.5 mM CaCl2, 1.2 mM MgSO4, 5.5 mM D-glucose, 20 mM HEPES, pH 7.4). 500 µL Ringer–HEPES with 10 µg·mL−1 SF (MW = 376 Da) and 165 µg·mL−1 EBA (Evans blue bound to 0.1% BSA; MW = 67 kDa) was used in place of the culture medium in the apical chambers. At 20, 40, and 60 minutes, the implants were moved to a fresh well that held Ringer-Hepes solution. During permeability tests, a horizontal shaker (Biosan, Riga, Latvia) operating at 100 r.p.m. was utilized to reduce any unstirred water layers. Using a microplate reader, the concentrations of the marker molecules in samples from the upper and lower compartments were ascertained. (BMG Labtech, Ortenberg, Germany; EBA: absorbance at 620 nm; SF: excitation at 440 nm, emission at 525 nm; BMG Fluostar Optima). Additionally, flux via cell-free inserts was examined. The amount of transport was measured in microliters (µL) of donor (luminal) compartment volume, after the tracer has been totally removed. The method previously outlined for calculating the transendothelial permeability coefficient (Pe) (Deli et al., 2005; Veszelka et al., 2007). The volume (V) of the abluminal compartment (0.5 mL) and the concentration (C) of the tracer in the luminal and abluminal compartments were used to compute the cleared volume using the following.

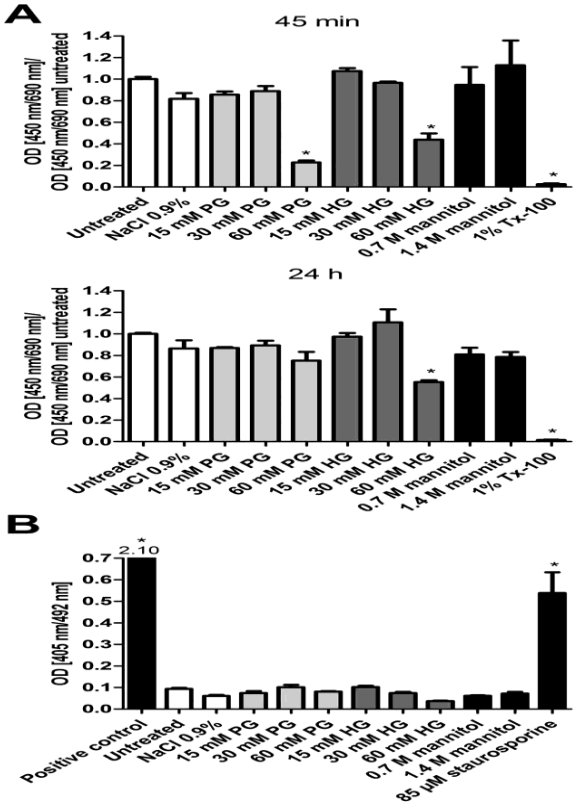

Figure 1: The effect of short-time AG treatment on primary rat BECs. Increas- ing concentrations of PG and HG were used to study the cytotoxic effects figure 1: on BECs. (A) WST-1 assay 45 min and 24 h after a 5 min AG treatment. Untreated cells served as negative control, cells treated with 1% Tx-100 for 6 h as positive control. For comparison, cells were treated with 0.7 and 1.4 M mannitol (M). All values presented are the means ± SEM, $n = 3$ for all groups. One-way ANOVA followed by Dunnett's multiple comparison test revealed statistically signifi- cant decrease (a) between treatment groups and NaCl-treated group. (B) Apoptosis ELISA 60 min after a 5 min AG treatment. Untreated cells served as negative control, DNA-histone complex served as positive control. All values presented are the means ± SEM, $n = 6$ for all groups. *P<0.05, significantly different from NaCl-treated group; one-way ANOVA followed by Dunnett's multiple comparison test.

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Histopathological immunohistochemistry

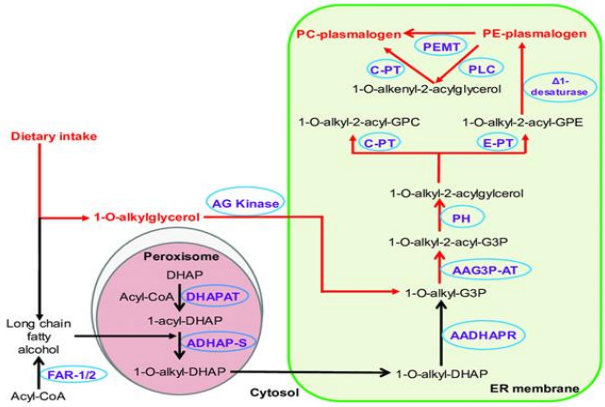
Confluent BEC monolayers were cultivated on inserts coated with fibronectin and collagen, treated with AGs and 1.4 M mannitol, and stained for adherens junction protein β-catenin and integral membrane tight junction protein claudin-5. Following a PBS wash, the cultures were fixed for 10 minutes at −20°C using ethanol (95 vol.%) and acetic acid (5 vol.%). After blocking the cells with 3% (BSA)-PBS, they were treated for an entire night with primary antibodies against β-catenin (a rabbit polyclonal antibody) and claudin-5 (a mouse monoclonal antibody; Zymed, South San Francisco, CA, USA). The secondary antibody (Invitrogen) Alexa Fluor-488-labelled anti-mouse IgG, Cy3-labelled anti-rabbit IgG, and Hoechst dye 33342 to stain the nuclei of the cells were incubated for one hour. Cells were rinsed three times with PBS in between incubations. Gel Mount was used to mount the membranes (Biomeda, Foster City, CA, USA) as well as staining were observed using a Nikon Eclipse TE2000 fluorescent microscope (Nikon, Tokyo, Japan), and images were captured using a Spot RT digital camera (Diagnostic Instruments, Campbell, CA, USA).

Materials

Unless stated otherwise, all reagents were bought from Sigma-Aldrich (St Louis, MO, USA). Enzyme Corporation provided the short-chain AGs, 2-O-HG and 1-O-PG. According to H. Eibl's personal communication, HG and PG do not form micelles at the applied concentrations; instead, they are dispersed molecularly in aqueous solutions. Use of distilled water and 0.9% (w/v) NaCl solution was required to create isotonic stock solutions of AGs. The value of physiological osmolarity was found.

Results:

Assay for cell viability At various times, the in vitro toxicity of PG and HG on primary rat BECs was investigated . The WST-1 test was run after 45 min, 24 h, 48 h, and 72 h to assess the immediate acute as well as long-term effects of a 5-min incubation with varying AG concentrations on the viability of the cells. As a negative control, untreated cells were utilized, and values from The unity of this control group was set. As anticipated, the vitality of cells treated with Tx-100 detergent was significantly decreased. Up to 30 mM, neither PG nor HG showed any harmful effects. Significant alterations were seen with increased AG concentrations at 45 minutes ($P < 0.0001$, d.f. = 10, $F = 16.09$) and 24 hours ($P < 0.0001$, d.f. = 10, $F = 25.42$) following the treatments. After 45 minutes, PG at a concentration of 60 mM dramatically ($P < 0.01$) decreased the viability of control cells to roughly 23%; however, endothelial cells recovered from this treatment in less than 24 hours. After 45 minutes, treatment with 60 mM HG significantly ($P < 0.05$) reduced the viability of the untreated cells to 44%.however, after a full day, there was no sign of recovery. The cells treated with 1.4 M mannitol for 5 minutes did not experience a decrease in viability. Comparing the 48- and 72-hour measurements to the 24-hour measurement, no additional changes were seen.



Dietary alkylglycerols can modulate endogenous plasmalogen content. Dietary AKGs can bypass the ratelimiting peroxisomal biosynthetic steps (red pathway). Metabolites are shown in red and black and enzymes are shown in violet. AADHAP-R: alkyl/acyl-DHAP-reductase, AAG3P-AT: alkyl/acyl-glycero-3-phosphate acyltransferase, ADHAP-S: alkyl DHAP synthase, AG kinase: alkylglycerol kinase, CoA: coenzyme A, C-PT: choline phosphotransferase, Δ1-desaturase: plasmany lethanolamine desaturase, DHAP: dihydroxyacetone phosphate, DHAP-AT: DHAP acyltransferase, E-PT: ethanolamine phosphor transferase, FAR-1/2: fatty acyl-CoA reductase 1 or 2, ER: endoplasmic reticulum, GPC: glycerophosp hocholine, GPE: glycerophosphoethanolamine; PC: phosphatidylcholine, PE: phosphatidylethanolamine, PEMT: phosphatidy lethanolamine N-methyl transferase, PH: phosphohydrolase, PLC: phospholipase C.

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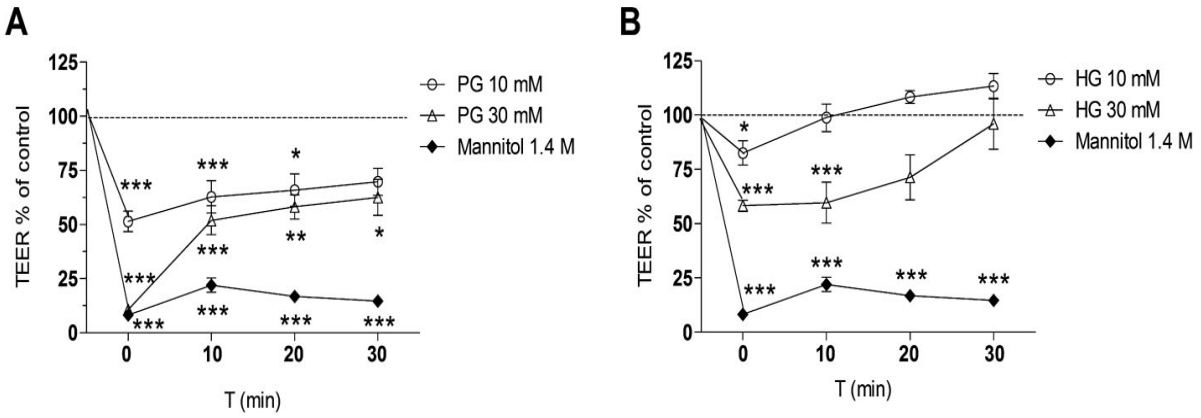

Figure 2

Changes in TEER were evaluated in primary rat brain endothelial cell monolayers treated with 10 and 30 mM PG (A), 10 and 30 mM HG (B), and mannitol (1.4 M) for 5 min. TEER was measured before treatment, directly after removal of AGs and mannitol (0 min), and following 10, 20 and 30 min of recovery in complete culture medium. All values presented are means ± SEM (n = 3). *P < 0.05, **P < 0.01, ***P < 0.001, significantly different from control group, at each time point; two-way repeated measures ANOVA followed by Bonferroni post test.

Measurements of permeability and TEER are used to assess barrier integrity.

The next tests were carried out utilizing primary rat BECs co-cultured with glial cells, a pertinent in vitro BBB model, in order to mimic the scenario in vivo, i.e., AG therapy by a single bolus injection (Veszelka et al., 2011). TEER measurements were used to first analyze the immediate and direct impact of AGs on the ion flux via endothelial cell monolayer's . We only utilized concentrations that the viability tests revealed to be non-toxic. To start the experiment (time −5 min), TEER was assessed in untreated cells (negative control) and set to 100%. Following a 5-minute incubation period with various AGs, additional measurements were taken right away (0 min) following the test substance's removal as well as 10, 20, and 30 minutes in full culture medium following recovery. The positive control cells were those that were exposed to 1.4 M mannitol. In comparison to the values assessed in the control group, there were significant changes in TEER seen during the observation period in both the PG-treated and HG-treated (column factor 97.71, P < 0.0001, d.f. = 3, F = 105.9; see Figure 2B) groups. TEER of BEC monolayer's was immediately reduced by 90% using 30 mM PG following the end of AG treatment When TEER was compared to the untreated control after 10 minutes, it was 48%, indicating that the cells had recovered and that the PG treatment was reversible. After 20 minutes of this, the TEER of the monolayer's increased much further. recuperation. Following treatment, TEER dropped by 50% at a lower 10 mM concentration of PG, however it eventually increased. Although less noticeable, the impact of HG was equally noteworthy. When compared to untreated cells evaluated immediately after treatment, TEER decreased by 58% and 29% following HG treatment at 30 and 10 mM concentrations. After 30 minutes, the barrier function for both concentrations had fully recovered. On the other hand, following 1.4 M mannitol therapy, TEER was measured and it fell below 25% of the control group, staying there for 30 minutes. Two test molecules, SF and EBA, were used to assess the permeability of brain endothelial monolayers in order to supplement the TEER findings exposure to the main A significantly higher flow of SF was also produced by subjecting rat BECs to 30 mM PG or HG, which resulted in reversible alterations in TEER . Permeability readings recovered to the level of the control group after a 15-minute interval. Unlike the outcomes with the lowMWSF (MW 376 Da), there was no discernible rise in albumin (MW 67 kDa) permeability in cell monolayers following treatment with30 mM HG (P = 0.2070, d.f. = 3, F = 1.907) or 30 mM PG (P =0.3235, d.f. = 3, F = 1.356) .

Discussion :

AG-mediated BBB opening may be used in a variety of ways to improve drug delivery to the brain, including as the treatment of primary brain tumors and the growing number of brain metastases patients. In this work, an in vitro model was used to study BBB opening and BECs' response to AG exposure. In vivo, rats and mice's BBBs were opened using PG and HG doses ranging from 50 to 200 mM. Rats and mice received the AGs as a single bolus injection over a period of 12 to 18 seconds into the internal carotid artery, while the common carotid artery was clamped during the injection. In this setup, the concentration of AG determined when the BBB

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opened (Erdlenbruch et al., 2000; 2003b), although the precise concentration and duration of AG's occupancy in brain capillaries cannot be predicted. Veszelka et al. (2007, 2011) found that the primary cell co-culture BBB model was a useful tool for investigating the endothelium barrier's opening in vitro with AGs. Every cell in cultures is exposed to the same AG concentration for the same amount of time, making thorough measurements under precise conditions possible, unlike the in vivo scenario where the actual AG concentration required for the barrier opening in a single capillary is unknown. Based on the outcomes of the viability tests and the incubation period, the AG concentrations employed in vitro were decreased from 50–100 mM to 10–30 mM relative to the initial in vivo conditions. Expanded from 18 seconds to almost 4.5 minutes per injection. The results of this model's permeability match those of the in vivo tests (Erdlenbruch et al., 2005). A reduction in TEER values was indicative of AG-mediated BBB opening. In line with the in vivo scenario (Erdlenbruch et al., 2003b), the permeability of BECs treated with PG reached the control level after a duration of 15 minutes. In the same concentration range, PG treatment was more successful than HG treatment in reducing the TEER value of endothelial monolayer's. Following AG treatment, TEER drop suggests that the tight junction-sealed par cellular diffusion barrier was breached (Madara, 1998). Integral membrane

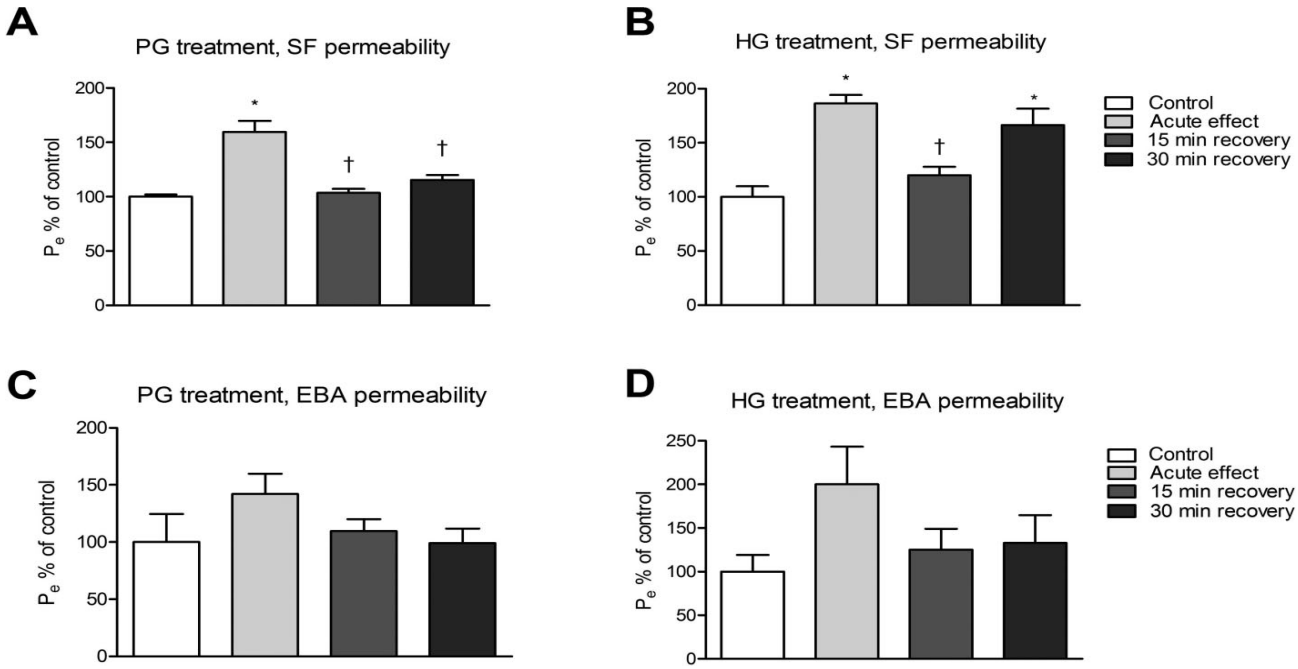

Figure: 3 Changes in endothelial permeability (Pe) for the paracellular permeability marker SF (A, B) and the transendothelial permeability marker EBA (C, D) in primary rat brain endothelial cell monolayers treated with 30 mM of PG (A–C) or HG (B–D) for 5 min (acute effect). After removal of AGs, 15 and 30 min recovery was allowed in complete culture medium. Pe was calculated as described. Data are shown as percentage of control (means ± SEM; $n = 3$). *$P <$ 0.05, significantly different from control group, $^{\dagger}P < 0.05$, significantly different from acute values; one-way ANOVA followed by Neuman–Keuls test.

tight junction proteins, particularly those belonging to the claudin family, are the primary factors limiting par cellular permeability (Krause et al., 2008). They control the ions' and molecules' physiological par cellular permeability of less than 4 Å (Anderson and Van Itallie, 2009). Per prior research (Greenwood et al., 1988; Farkas et al., 2005), a decrease in TEER was also seen following mannitol administration, which is consistent with our current observations. Since fluoresce in is a tiny, water-soluble molecule, its greater transit through BEC monolayer's following cell exposure to AGs suggests that a par cellular channel across tight junctions has been opened, akin to the TEER modifications. This is consistent with Erdlenbruch et al.'s findings (2003a). They used AGs and fluorescein to incubate recently isolated brain capillaries, and they saw fluoresce in paracellularly entering the lumen of the capillaries. As seen with the TEER variations, the elevated permeability for Fluorescein might be reversed. Unlike fluorescein, albumin permeability in BECs did not increase statistically significantly after treatment with AGs, even though albumin flux did tend

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to rise. Under healthy circumstances in vivo, albumin transport across the blood-brain barrier occurs transcellularly and at a very low level (Abbott et al., 2006). Transport through this system can rise several times in pathological situations, and this increase can occur independently of the paracellular pathway opening. In cultivated BECs, hypoxia was shown to selectively promote vesicular albumin transport (Plateel et al., 1997). For high MW molecules the size of albumin, the par cellular pathway via intact tight junctions can also be opened (Artursson et al., 1993; Knipp et al., 1997). Variations in fluorescein and TEER Its albumin flow together suggest that a selective impact on the paracellular cleft and tight junctions between BECs may be the primary mechanism by which AGs breaks the blood-brain barrier. Apart from the noted functional modifications, primary BECs' β- and claudin-5 immune staining revealed that AG treatment also had an impact on the cell junctions. The results of Erdlenbruch et al. (2003a), who conjectured that AGs primarily boost the paracellular pathway at the BBB, are all consistent with these findings. Cytoskeletal disruption has the ability to modify the par cellular route (Bruewer et al., 2004; Ivanov et al., 2005), and cytoskeletal dynamics appear to regulate the tightness of tight junctions (Lai et al., 2005; Hartsock and Nelson, 2008). β-Catani is a According to Noda et al. (2010), adherents junction protein connects the cytoskeleton to the integral membrane functional proteins. The observed morphological changes in BECs upon exposure to AGs may suggest that actin-junctional anchoring plays a role in the mechanism of AGs. In light of these finding and potential cytoskeleton modifications, it is plausible that AGs changed the structure or intricacy of tight junction strands, resulting in heightened par cellular permeability. The control of the par cellular pathway in BECs is mostly dependent on tight junction strands (Wolburg et al., 1994; Rubin and Staddon, 1999). According to Wolburg et al. (1994), BECs have more P-face associated tight junction strands than non-BECs do, and 16–24 hours of cultivation resulted in a significant loss of P-face attachment of these strands in tight junctions. The ability of the brain microenvironment to create and sustain a high P-face connection of tight junction strands was understood to be the cause of this. Endothelial cells were treated with forskolin for two days in order to mimic the brain milieu; this increased the intricacy of tight junctions and P-face interaction (Wolburg et al., 1994). Even though cell cultures in the current study were only exposed to AGs for five minutes, this brief treatment had a considerable impact on the par cellular permeability as shown by functional assays. On the other hand, there was no difference in the tight junction architecture between the control and AG-treated BECs in terms of complexity or E/P-face relationship. In both the treated and control samples, at the The tight connections on the E-face looked like pearl chain strands. Our findings suggest that structural changes at the electron microscopic level did not coincide with the functional changes induced by AGs. Therefore, the answer to the commonly questioned question of whether functional modifications of the barrier would always occur concurrently with strand alterations must be, at least in vitro, "no." This conclusion is reinforced by a recent revelation that structural rearrangement of the tight junction strands can regulate barrier permeability without causing any morphologically noticeable alterations (Piehl et al., 2010). Phosphorylation allows tight junctions to rapidly change in function. Permeability might be observed to decrease as quickly as 10 minutes following cAMP. care in BECs that have been grown (Deli et al., 1995) and tight junction protein turnover is very quick; claudin-5, for instance, has a membrane half-life of 33 seconds (Piontek et al., 2011). A significant tight junction protein in BECs, claudin-5 interacts with the equivalent extracellular loops of the neighboring endothelial cell's claudins to close the intercellular cleft (see Krause et al., 2009). Independent of ZO-1 or cytoskeleton anchoring, hemophilic Claudine trans-interaction is demonstrated by cell-contact enrichment in HEK293 cells expressing fluorescent claudin-5 (Piontek et al., 2008). Using this model, the direct impact of AGs on these Claudine trans-interactions was investigated. We discovered that at non-toxic concentrations utilized for functional testing of par cellular permeability, HG had no effect on the trans-interaction of claudin-5. Just the The greatest dosage of HG changed the trans-interaction and membrane localization of claudin-5, which also caused membrane breakdown and was hazardous in other experiments. This suggests that AGs may have more of an impact on a cell's plasma membrane than on the claudin-5 trans-interaction directly. The intensity of claudin-5 immune staining in BECs treated with AGs rose in the cytoplasm but declined at the cell boundaries, suggesting a redistribution of the tight junction protein. These findings suggest that AGs may not directly impact Claudine interactions, but rather that the effect may be mediated by additional pathways including Claudine cytoskeleton anchoring. Prostanoids, which are metabolites of arachidonic acid, and leukotrienes are examples of biologically active lipids that affect BBB permeability. Lately, an antagonist of the prostanoid EP1 receptor was demonstrated to stop brain ischemia-induced BBB leakage (Fukumoto et al., 2010). While this area remains unexplored experimentally, it is impossible to rule out the possibility that short-chain AGs affect the BBB through lipid receptors. AGs may also influence the tight junctions of brain endothelial cell monolayer’s selectively by altering the lipid content and/or fluidity of rafts carrying integral membrane tight

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junction proteins (Dodelet-Devillers et al., 2009). To clarify which molecules are involved in AGs' opening of the par cellular transport pathway, more research is required.

Conclusion:

When combined, our study's findings showed that cultured BECs can be briefly treated with short-chain AGs at non-toxic doses to reversibly open the par cellular barrier without experiencing long-term damage. There were no changes in the tight junction strand complexity to go along with the rise in the penetration rate of water-soluble compounds across BECs. These in vitro findings show that exposure to AGs does not result in the disruption of BBB functions and shape, and that recovery of the monolayer integrity following AG treatment is feasible. The work indicates that the characteristics of AGs may be appropriate for opening the BBB to treat brain cancers and validates the findings of earlier in vivo experiments on rats and mice. The increase in brain endothelial par cellular permeability produced by AG was transient reversible and had no effect on the intricacy of the tight connection strand. The involvement of the cytoskeleton in the action of AGs is indicated by changes in cell shape and redistribution of junction proteins. These findings corroborate findings from rodent in vivo research that described AGs as adjutants that temporarily breach the blood-brain barrier.

References:

1. Deli MA (2009). Potential use of tight junction modulators to reversibly open membranous barriers and improve drug delivery. Biochim Biophys Acta 1788: 892–910.
2. Deli MA (2011). Drug transport and the blood-brain barrier. In: Tihanyi K, Vastag M (eds). Solubility, Delivery, and ADME Problems of Drugs and Drug-Candidates. Washington: Bentham Science Publ. Ltd., pp. 144–165.
3. Deli MA, Dehouck MP, Ábrahám CS, Cecchelli R, Joó F (1995). Penetration of small molecular weight substances through cultured bovine brain capillary endothelial cell monolayers: the early effects of cyclic adenosine 3',5'-monophosphate. Exp Physiol 80: 675–678.
4. Deli MA, Ábrahám CS, Kataoka Y, Niwa M (2005). Permeability studies on in vitro blood-brain barrier models: physiology, pathology, and pharmacology. Cell Mol Neurobiol 25: 59–127.
5. Demeule M, Regina A, Che C, Poirier J, Nguyen T, Gabathuler R et al. (2008). Identification and design of peptides as a new drug delivery system for the brain. J Pharmacol Exp Ther 324: 1064–1072.
6. Dhanikula RS, Argaw A, Bouchard JF, Hildgen P (2008). Methotrexate loaded polyether-copolyester dendrimers for the treatment of gliomas: enhanced efficacy and intratumoral transport capability. Mol Pharm 5: 105–116.
7. Dodelet-Devillers A, Cayrol R, van Horssen J, Haqqani AS, de Vries HE, Engelhardt B et al. (2009). Functions of lipid raft membrane microdomains at the blood-brain barrier. J Mol Med (Berl) 87: 765–774.
8. Doolittle ND, Miner ME, Hall WA, Siegal T, Jerome E, Osztie E et al. (2000). Safety and efficacy of a multicenter study using intraarterial chemotherapy in conjunction with osmotic opening of the blood-brain barrier for the treatment of patients with malignant brain tumors. Cancer 88: 637–647.
9. 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: 417–422.
10. Erdlenbruch B, Jendrossek V, Kugler W, Eibl H, Lakomek M (2002). Increased delivery of erucylphosphocholine to C6 gliomas by chemical opening of the blood-brain barrier using intracarotid pentylglycerol in rats. Cancer Chemother Pharmacol 50: 299–304.
11. Erdlenbruch B, Alipour M, Fricker G, Miller DS, Kugler W, Eibl H et al. (2003a). Opening of the blood-brain barrier to small and large fluorescence markers in normal and C6 glioma bearing rats and isolated capillaries using intracarotid short chain alkylglycerols. Br J Pharmacol 140: 1201–1210.
12. Erdlenbruch B, Schinkhof C, Kugler W, Heinemann DEH, Herms J, Eibl H et al. (2003b). Intraarterial short chain alkylglycerols for increased delivery of methotrexate to the rat brain. Br J Pharmacol 139: 685–694.
13. Erdlenbruch B, Kugler W, Schinkhof C, Neurath H, Eibl H, Lakomek M (2005). Blood-brain barrier opening with alkylglycerols: biodistribution of 1-O-pentylglycerol after intravenous and intracarotid administration in rats. J Drug Target 13: 143–150.
14. Farkas A, Szatmári E, Orbók A, Wilhelm I, Wejksza K, Nagyo˝szi P et al. (2005). Hyperosmotic mannitol induces Src kinase-dependent phosphorylation of beta-catenin in cerebral endothelial cells. J Neurosci Res 80: 855–861.
15. Fellner F, Bauer B, Miller DS, Schaffrik M, Fankhänel M, Spruß T et al. (2002). Transport of paclitaxel (Taxol) across the blood-brain barrier in vitro and in vivo. J Clin Invest 110: 1309–131
16. Greenwood J, Luthert PJ, Pratt OE, Lantos PL (1988). Hyperosmolar opening of the blood-brain barrier in the energy-depleted rat brain. Part 1. Permeability studies. J Cereb Blood Flow Metab 8: 9–15.

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JCHR (2023) 13(4s), 914-924 | ISSN:2251-6727

17. Hartsock A, Nelson WJ (2008). Adherens and tight junctions: structure, function and connections to the actin cytoskeleton. Biochim Biophys Acta 1778: 660–669.
18. Hülper P, Dullin C, Kugler W, Lakomek M, Erdlenbruch B (2011). Monitoring proteins using in vivo near-infrared time-domain optical imaging after 2-O-hexyldiglycerol-mediated transfer to the brain. Mol Imaging Biol 13: 275–283.
19. Ivanov AI, Hunt D, Utech M, Nusrat A, Parkos CA (2005). Differential roles for actin polymerization and a myosin II motor in assembly of the epithelial apical junctional complex. Mol Biol Cell 16: 2636–2650.
20. Kemper EM, Boogerd W, Thuis E, Beijnen JH, van Tellingen O (2004). Modulation of the blood-brain barrier in oncology: therapeutic opportunities for the treatment of brain tumors. Cancer Treat Rev 30: 415–423.
21. Knipp GT, Ho NF, Barsuhn CL, Borchardt RT (1997). Paracellular diffusion in Caco-2 cell monolayers: effect of perturbation on the transport of hydrophilic compounds that vary in charge and size. J Pharm Sci 86: 1105–1110.
22. Krause G, Winkler L, Mueller SL, Haseloff RF, Piontek J, Blasig IE (2008). Structure and function of claudins. Biochim Biophys Acta 1778: 631–645.
23. Krause G, Winkler L, Piehl C, Blasig I, Piontek J, Müller SL (2009). Structure and function of extracellular claudin domains. Ann N Y Acad Sci 1165: 34–43.
24. Lai CH, Kuo KH, Leo JM (2005). Critical role of actin in modulating BBB permeability. Brain Res Brain Res Rev 50: 7–13.
25. Madara JL (1998). Regulation of the movement of solutes across tight junctions. Annu Rev Physiol 60: 143–159.
26. Neuwelt EA, Barnett PA, McCormick CI, Frenkel EP, Minna JD (1985). Osmotic blood-brain barrier modification: monoclonal antibody, albumin, and methotrexate delivery to cerebrospinal fluid and brain. Neurosurgery 17: 419–423.
27. Noda K, Zhang J, Fukuhara S, Kunimoto S, Yoshimura M, Mochizuki N (2010). Vascular endothelial-cadherin stabilizes at cell-cell junctions by anchoring to circumferential actin bundles through alpha- and beta-catenins in cyclic AMP-Epac-Rap1 signal-activated endothelial cells. Mol Biol Cell 21: 584–596.
28. O'Brien FE, Clarke G, Fitzgerald P, Dinan TG, Griffin BT, Cryan JF (2012). Inhibition of P-glycoprotein enhances transport of imipramine across the blood–brain barrier: microdialysis studies in conscious freely moving rats. Br J Pharmacol 166: 1333–1343.
29. Pardridge WM (2002). Drug and gene targeting to brain with molecular Trojan horses. Nat Rev Drug Discov 1: 131–139.
30. Perrière N, Demeuse PH, Garcia E, Regina A, Debray M, Andreux JP et al. (2005). Puromycin-based purification of rat brain capillary endothelial cell cultures. Effect on the expression of blood-brain barrier-specific properties. J Neurochem 93: 279–289.
31. Piehl C, Piontek J, Cording J, Wolburg H, Blasig IE (2010). Participation of the second extracellular loop of claudin-5 in paracellular tightening against ions, small and large molecules. Cell Mol Life Sci 67: 2131–2140.
32. Piontek J, Winkler L, Wolburg H, Müller SL, Zuleger N, Piehl C et al. (2008). Formation of tight junction: determinants of homophilic interaction between classic claudins. FASEB J 22: 146–158.
33. Piontek J, Fritzsche S, Cording JD, Richter S, Hartwig J, Walter M et al. (2011). Elucidating the principles of the molecular organization of heteropolymeric tight junction strands. Cell Mol Life Sci 68: 3903–3918.
34. Plateel M, Teissier E, Cecchelli R (1997). Hypoxia dramatically increases the nonspecific transport of blood-borne proteins to the brain. J Neurochem 68: 874–877.
35. Rapoport SI (2000). Osmotic opening of the blood–brain barrier: principles, mechanisms, and therapeutic applications. Cell Mol Neurobiol 20: 217–230.
36. Reardon DA, Rich JN, Friedman HS, Bigner DD (2006). Recent advances in the treatment of malignant astrocytoma. J Clin Oncol 24: 1253–1265.
37. Reese TS, Karnovsky MJ (1967). Fine structural localization of a blood-brain barrier to exogenous peroxidase. J Cell Biol 34: 207–217.
38. Rubin LL, Staddon JM (1999). The cell biology of the blood-brain barrier. Annu Rev Neurosci 22: 11–28.
39. Siegal T, Rubinstein R, Bokstein F, Schwartz A, Lossos A, Shalom E et al. (2000). In vivo assessment of the window of barrier opening after osmotic blood – brain barrier disruption in humans. J Neurosurg 92: 599–605.
40. Taddei A, Giampietro C, Conti A, Orsenigo F, Breviario F, Pirazzoli V et al. (2008). Endothelial adherens junctions control tight junctions by VE-cadherin-mediated upregulation of claudin-5. Nat Cell Biol 10: 923–934.
41. Unger C, Eibl H, von Heyden HW, Kirsch B, Nagel GA (1985). Blut-Hirn-Schranke und Penetration von Zytostatika. Klin Wochenschr 63: 565–571.
42. Veszelka S, Pásztói M, Farkas AE, Krizbai I, Ngo TK, Niwa M et al. (2007). Pentosan polysulfate

protects brain endothelial cells against bacterial lipopolysaccharide-induced damages. Neurochem Int 50: 219–228.

43. Veszelka S, Kittel A, Deli MA (2011). Tools of modelling blood-brain barrier penetrability. In: Tihanyi K, Vastag M (eds). Solubility, Delivery, and ADME Problems of Drugs and Drug-Candidates. Washington: Bentham Science Publ. Ltd., pp. 144–165.
44. Vliet EA, Zibell G, Pekcec A, Schlichtiger J, Edelbroek PM, Holtman L et al. (2010). COX-2 inhibition controls P-glycoprotein expression and promotes brain delivery of phenytoin in chronic epileptic rats. Neuropharmacology 58: 404–412.
45. Wolburg H, Neuhaus J, Kniesel U, Krauss B, Schmid EM, Öcalan M (1994). Modulation of tight junction structure in blood-brain barrier endothelial cells. Effects of tissue culture, second messengers and cocultured astrocytes. J Cell Sci 107: 1347–1357.