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BRAIN RESEARCH

Research Report

Endothelial-monocyte-activating polypeptide II increases blood–tumor barrier permeability by down-regulating the expression levels of tight junction associated proteins

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ABSTRACT

This study was performed to determine whether endothelial-monocyte-activating polypeptide (EMAP) II increases the permeability of the blood–tumor barrier (BTB) in the rat model of C6 glioma, and whether EMAP II opens the BTB by affecting tight junction (TJ) associated proteins zonula occluden-1 (ZO-1), occludin and claudin-5. The rats were divided into eight groups randomly: control group, EMAPII 0 h group, EMAPII 0.5 h group, EMAPII 1 h group, EMAPII 2 h group, EMAPII 3 h group, EMAPII 6 h group and EMAPII 12 h group. The BTB permeability was assessed by Evans blue extravasation. The mRNA and protein expressions of ZO-1, occludin, and claudin-5 were determined by reverse transcriptase–polymerase chain reaction, western blot, and immunohistochemistry assays. The BTB permeability significantly increased after EMAP II injection in different doses (40 ng/kg, 80 ng/kg and 160 ng/kg). The BTB permeability started to increase from 0.5 h, reached a peak at 1 h, and finally returned to the level of EMAP II 0 h group after EMAP II injection at dose of 80 ng/kg. The mRNA and protein expression levels of ZO-1, occludin and claudin-5 were significantly decreased after EMAP II injection. This study demonstrates for the first time that EMAP II increases the permeability of BTB selectively, and the possible mechanism is associated with the down-regulation of ZO-1, occludin and claudin-5.

1. Introduction

Blood–tumor barrier (BTB), similar to blood–brain barrier (BBB), is located between brain tumor cells and microvessels formed by highly specialized endothelial cells (ECs). It limits the paracellular diffusion of hydrophilic molecules to tumor tissue by an intricate network of complex endothelial tight junction (TJ) (Wolburg and Lippoldt, 2002; Wolburg et al., 2003). Although the permeability of BTB is slightly higher than that of BBB (Yuan et al., 1994), it still restricts access of chemotherapeutic

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Abbreviations: BTB, blood–tumor barrier; BBB, blood–brain barrier; EC, endothelial cell; TJ, tight junction; EMAP, endothelial-monocyte-activating polypeptide; TNF, tumor necrosis factor; ZOs, zonula occludens; HUVEC, human umbilical vein endothelial cell; NOS, nitric oxide synthase; EB, Evans blue; RT-PCR, reverse transcription-polymerase chain reaction; IDV, integrated density value; PBS, phosphate-buffered saline; ANOVA, analyses of variance

[1] The first two authors contributed equally to this work.

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drugs to brain tumor tissue. Some studies show that if the local drug concentration is elevated by two fold, the efficacy to kill the brain tumor cells can be increased by ten fold (Samoto et al., 2001). Therefore, efficient delivery of drugs to tumor cells is crucial for effective chemotherapy of glioma.

Endothelial-monocyte-activating polypeptide (EMAP) II is a proinflammatory cytokine originally isolated from the supernatants of methylcholanthrene A-induced fibrosarcoma cells. The mature EMAP II is proteolytically cleaved from its precursor proEMAP. It has pleiotropic effects on ECs, monocytes, and neutrophils. EMAP II has been shown to induce apoptosis of ECs and inhibit tumor angiogenesis. Thus far, it has mainly been used for experimental research on tumor therapy (Berger et al., 2000; Shalak et al., 2007). Several studies have indicated that low-dose EMAP II increases the effect of tumor necrosis factor (TNF)-α on tumor microvascular EC by regulating the TNF receptor 1 on the surface of EC (Crippa et al., 2008). TNF-α is known to not only inhibit tumor growth and invasion but also to a certain extent increase the permeability of the BBB (Farkas et al., 2006; Mckenzie and Ridley, 2007).

Some studies illustrate that TJ is an important structural and functional basis of maintaining the BBB integrity in vivo. It is a complex consisting of complicated proteins including transmembrane proteins of occludin and claudins, peripheral membrane protein family of zonula occludens (ZOs), adhesion molecules, etc. The alteration of their structure plays an essential role in modulating the function of TJ (Wolburg and Lippoldt, 2002; Gloor et al., 2001). Studies demonstrate that EMAP II induces remodeling of actin cytoskeleton and increases both the density of actin stress fibers and the number of focal adhesion plaques in human umbilical vein endothelial cells (HUVECs) (Tandle et al., 2005; Keezer et al., 2003). Moreover, the enhancement of actin stress fibers and focal adhesion plaques could induce TJ opening and the increase in the BBB permeability through affecting TJ associated proteins and cytoskeleton protein (Laia et al., 2005; Lee et al., 2004).

The purpose of this study was to determine whether EMAP II increases the permeability of BTB in C6 glioma rats, and the possible underlying mechanism including the involvement of TJ.

2. Results

2.1. EMAP II increased the permeability of BTB selectively

Effect on BTB permeability for EB extravasation showed that the brain tumor tissue of rat C6 glioma model was stained in blue after EMAP II injection at different doses, while no visible staining was found in normal brain tissue. The EB content of tumor-bearing brain significantly increased after EMAP II injection at different doses (40 ng/kg, 80 ng/kg and 160 ng/kg), while there was no change in the EB content of contralateral brain (Fig. 1). Furthermore, the EB content of tumor-bearing brain was significantly higher in 80 ng/kg and 160 ng/kg groups than in 40 ng/kg group (Fig. 1). There was no significant difference between the EMAP II 80 ng/kg and 160 ng/kg groups. Therefore we chose 80 ng/kg as the optimal dosage to use in the subsequent experiments.

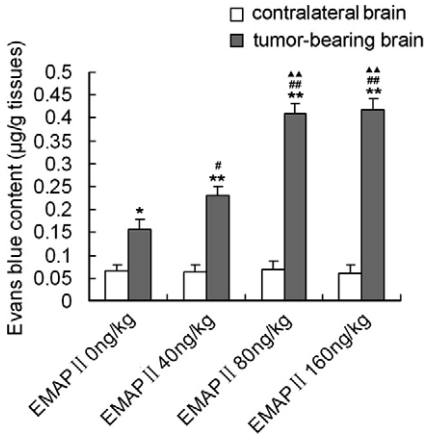

Fig. 1 – Content of EB in tumor-bearing brain and contralateral brain after EMAP II injection at different doses. Values present means ± SD (n = 5, each group). $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. contralateral brain of each group, respectively. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. tumor-bearing brain of EMAP II 0 ng/kg group. $^{\blacktriangle\blacktriangle}P < 0.01$ vs. tumor-bearing brain of EMAP II 40 ng/kg group.

As shown in Fig. 2, the EB content of tumor-bearing brain started to increase from 0.5 h, reached a peak at 1 h, and then decreased and finally returned to the level of EMAP II 0 h group after injection of EMAP II at a dose of 80 ng/kg.

2.2. EMAP II decreased the mRNA and protein expressions of TJ related proteins

We determined the mRNA and protein expression levels of ZO-1, occludin, and claudin-5 by RT-PCR and western blot assays. After EMAP II injection, the mRNA and protein expression levels of ZO-1, occludin and claudin-5 in tumor microvessels were decreased by 0.5 h. The lowest level appeared at 1 h and enhanced thereafter, and then the mRNA and protein expression levels of the three proteins nearly restored to the

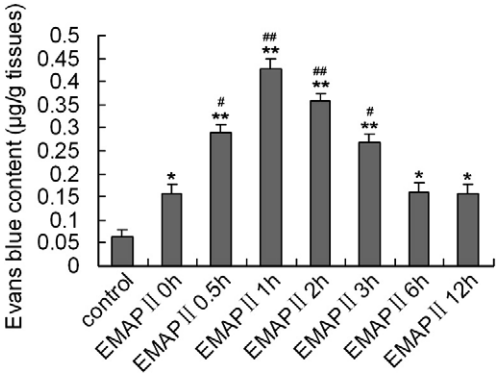

Fig. 2 – Content of EB in tumor-bearing brain at different time points after injection of 80 ng/kg EMAP II. In control group, normal rats were treated with saline for 1 h. In EMAP II groups, tumor-bearing rats were treated with 80 ng/kg EMAP II for 0, 0.5, 1, 2, 3, 6 and 12 h, respectively. Values present means ± SD (n = 5, each group). $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. control group. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. EMAP II 0 h group.