

The effects of increasing PGE₂ on translocation of labeled albumin into rat brain

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Abstract

Under pathophysiological conditions, infiltration of leukocyte plays a key role in the progression of the neuroinflammatory reaction in the CNS. Prostaglandin E₂ (PGE₂) is known to accumulate at lesion sites of the post-ischemic brain. Although post-ischemic treatments with cyclooxygenase-2 inhibitors reduce blood-brain barrier (BBB) leukocyte infiltration, the direct effect of PGE₂ on BBB has not been fully implemented. Therefore, the direct effect of increasing PGE₂ infusion on translocation of labeled albumin into the brain was assessed. Under anesthesia rats were drilled stereo-taxically a burr hole in the right forebrain and PGE₂ was infused into the forebrain and the hole was occluded. The animals were then injected with fluorescent labeled albumin (FA), via internal right jugular vein and decapitated at different infusion time points. The forebrain was removed and each forebrain hemisphere was homogenized and fluorescence intensities were measured in the supernatant. The fluorescence intensities measured in the right and left forebrain hemispheres of the control group (0.0 µg PGE₂) were almost identical. Four hours after infusion of PGE₂ at doses higher than 250 µg, fluorescence intensity increased in the right forebrain supernatant, even if it was not statistically significant. The fluorescence intensity was detectable in the brain supernatant 4 h after infusion of PGE₂ in doses higher than 250 µg PGE₂. The highest fluorescence intensity was 16 h after infusion of 500 µg PGE₂, which returned to near control values after 48 h. Increased fluorescence intensity in the brain following PGE₂ infusion is concluded to be associated with disruption of the BBB.

Keywords: Blood-brain barrier; Ischemia; Neuroinflammatory; Prostaglandin

INTRODUCTION

Inflammation is one of the mechanisms known to participate in the progression of brain injury (1,2). A large number of studies indicate that blockade of the neuroinflammatory process considerably reduces ischemic brain injury (3-5). Prostaglandin E₂ (PGE₂), one of the most likely candidates for the manifestation of the inflammation, is known to be accumulated at the lesion sites of the post-ischemic brain (1,6,7). These observations have been confirmed by biochemical studies that reported a significant increase in the expression of the PGE₂ synthesizing enzyme, cyclooxygenase (COX-2), following ischemia (8-10). However, there is a debate on the specific role of PGE₂ in cerebral ischemia (1). A recent study has

found beneficial effects (11), another report claimed harmful (12), and an article reported PGE₂ did not affect ischemic brain injury (13). However, several lines of evidence indicated that after the onset of ischemia, there is a significant disruption of the blood-brain barrier (BBB), followed by a massive infiltration of leukocytes (3,14,15). These reports suggested that disruption of BBB might be the main cause of neuroinflammatory process in the brain. While it is becoming clear that increased expression of COX-2 is associated with ischemic brain, it is by no means clear that a cause-and-effect relationship exists between the increased PGE₂ and BBB permeability directly. This study examines the time course and direct effects of increasing PGE₂ infusion on translocation of labeled albumin into the brain.

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MATERIALS AND METHODS

Chemicals

PGE₂ (Prostin E₂, 10 mg/ml) was purchased from Upjohn (Puurs Belgium), fluorescein isothiocyanate isomer 1 (FITC), Sephadex G-25 and rat serum albumin were procured from Sigma (Poole, Dorset, UK). Unless stated, all reagents were of the highest grade and made up in double glass-distilled water.

Fluorescein-labeled rat serum albumin

Preparation of fluorescein-labeled rat serum albumin was carried out by the method of Nargessi and coworkers (16). Equal volumes of FITC solution (1 mg/ml) and rat serum albumin solution (0.25 mg protein/ml) were mixed and stirred overnight at 4 °C. A Sephadex G-25 column (1.2 × 20 cm) was then loaded by the FITC/serum albumin mixture.

The entire fluorescein labeled albumin (FALB) band was identified by its fluorescence under the UV light in a dark room. The FALB band eluted with 2 ml bicarbonate buffer (pH 9) and stored at -20 °C. Unconjugated FITC remained in the column. The pH of the labeled was adjusted to 7.4 and fluorescence intensity of FALB solution was measured using a Parkin-Elmer (Norwalk, CT) LSE spectrophotometer-fluorimeter with the excitation wavelength of 495 nm and an emission wavelength of 540 nm.

Animals and preparations

Eight-week-old male Wistar rats weighing 200-220 g were purchased from the Pasteur Institute (Tehran, Iran). The animals were maintained with respect to the animal welfare regulation in animal house (6 rats in each group) in a regulated environment (25 ± 1 °C; 50 to 55% relative humidity; 12 h light/dark cycle), with free access to food (Pars Dam Co., Iran) and water. All procedures were approved by the Ethical Committee of the Isfahan University of Medical Sciences, and conducted in accordance with the ‘Principles of Laboratory Animal Care’ (National Institutes of Health publication no. 86-23, revised 1985).

The animal surgery was performed between 8-8:30 AM. Briefly rat was anesthetized by

intramuscular injection of ketamine (100 mg/kg) and xylazine (10 mg/kg) solution. The animals were then placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA). The scalp and underlying soft tissues were removed. The animal was drilled stereotaxically a burr hole in the right midline 3 mm anterior to bregma and 40 µl PGE₂ solutions (62.5, 125, 250, 500 µg PGE₂ diluted in normal saline) was infused slowly into the right forebrain by a fixed mycrosyringe for over 10 minutes, while only normal saline was infused into the right forebrain of the control group (sham treatment group). The hole was occluded and the animal was closely monitored for 60 min. The animal was then injected with 1 ml fluorescein labeled albumin (100 µg albumin/ml), via internal right jugular vein. The rats were killed by decapitation at different infusion time points: 4, 8, 16, 24 and 48 h. The forebrain was then rapidly removed and each forebrain hemisphere was weighed up and homogenized in 5 ml of ice-cold saline Triton X-100. The homogenate was centrifuged for 10 min at 16000 g and fluorescence intensity was measured in supernatant at the excitation wavelength of 495 nm and emission wavelength of 540 nm. Results were expressed relative to an arbitrary scale of fluorescence intensity.

Statistical analysis

Statistical analysis was performed using the Statistical Package of Social Science (SPSS) version 17. Data are presented as means ± standard deviation (SD). Experimental data from the right forebrain hemisphere are compared to their own left forebrain hemisphere values by pairing Student’s *t*-test. The level of significance was set at *P*<0.05. The corresponding parameters were also compared using analysis of variance (ANOVA).

RESULTS

Changes in BBB permeability to fluorescein-labeled albumin were measured at right and left forebrain hemispheres after infusion of the range concentrations of PGE₂ at different infusion time points. The results are summarized in Table 1.

Table 1. The effect of PGE₂ infusion on the translocation of fluorescein-labeled albumin into blood brain barrier.

Time/Brain Region	PGE ₂ Doses (μg)				
	0	62.5	125	250	500
4 h/ Right	275 ± 43	286 ± 28	302 ± 71	325 ± 65	369 ± 43
4 h/Left	241 ± 41	270 ± 23	281 ± 48	290 ± 62	300 ± 45
8 h/ Right	276 ± 45	324 ± 26	380 ± 51*	439 ± 58*#	460 ± 66*#
8 h/Left	245 ± 27	276 ± 16	309 ± 52	321 ± 41*	379 ± 78*
16 h/Right	271 ± 39	495 ± 43*#	536 ± 29*#	623 ± 88*#	699 ± 68*#
16 h/Left	239 ± 27	336 ± 24	374 ± 42*	409 ± 61*	485 ± 72*
24 h/Right	268 ± 45	511 ± 27*#	573 ± 80*#	658 ± 29*#	699 ± 98*#
24 h/left	231 ± 38	339 ± 58	383 ± 28*	422 ± 11*	496 ± 71*
48 h/Right	265 ± 40	334 ± 42	340 ± 60	384 ± 49#	421 ± 72*
48 h/Left	242 ± 52	296 ± 41	299 ± 34	230 ± 22	376 ± 67

Values are means of fluorescence intensities ± SD of 8 separate experiments. *; Differences are significant at $p<0.05$ as compared with corresponding sham values. #; Differences are significant at $p<0.05$ as compared with the left values.

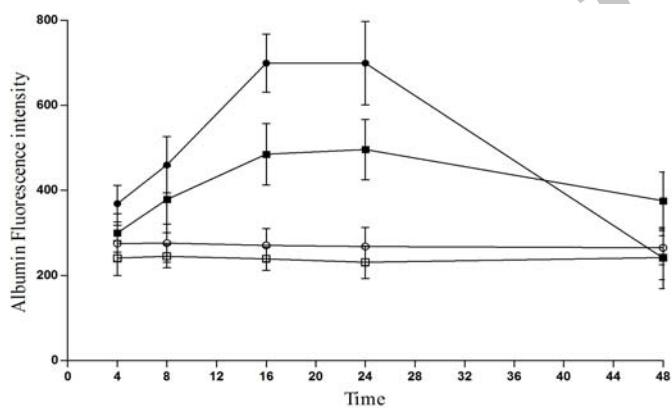


Fig. 1. The time course of translocation of fluorescent labeled albumin. Animals were injected with 1 ml fluorescein labeled albumin (100 μg albumin/ml), via internal right jugular vein and PGE₂ at doses of 500 μg was infused into the rat right brain hemisphere (see Method section). The fluorescence intensities were measured at infusion time points of 4, 8, 16, 24, and 48 h after infusion in right (-●-) and left (-■-) forebrain supernatant of treatment groups and right (-○-) and left (-□-) sham groups.

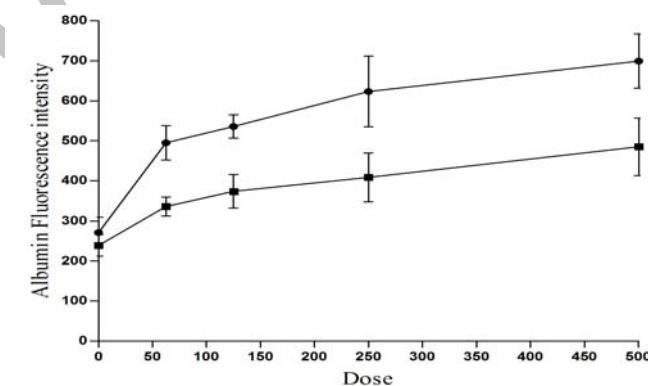


Fig. 2. Dose response curve of PGE₂ with respect to the fluorescence intensities. Animals were injected with 1 ml fluorescein labeled albumin (100 μg albumin/ml), via their internal right jugular vein and PGE₂ at dose ranges from 0 to 500 μg were infused into the rat right brain hemisphere (see Method section), and the fluorescence intensities were measured 16 h after infusion at right (-●-) and left (-■-) brain supernatant.

The fluorescence intensities measured in the right and left forebrain hemispheres of the control group (0.0 µg PGE₂) were almost identical. Four hours after infusion of PGE₂ at doses higher than 250 µg (see method section) fluorescence intensity increased in the right forebrain supernatant, even if it was not statistically significant. However, the fluorescence intensities in the right forebrain supernatant markedly increased 16 h after infusion of 500 µg PGE₂, which returned to near control values after 48 h. The fluorescence intensities in left forebrain supernatant increased compared to the sham operation group.

Fig. 1 shows the time course of translocation of FALB after 500 µg PGE₂ infusion. The values of fluorescence intensities increased in the treatment groups 16 and 24 h after infusion compared to corresponding sham values ($P<0.05$). Four hours after perfusion, the levels of tissue fluorescence intensities increased greatly in right forebrain compared with the left forebrain supernatant.

Fig. 2 compares the fluorescence intensities measured in the supernatants prepared from right and left brain hemispheres 16 h after infusion of different doses of PGE₂ (62.5, 125, 250 and 500 µg). As it can be seen the fluorescence intensities are significantly higher in the supernatants prepared from right forebrain ($P<0.05$). The results indicated that translocation of FALB is dose dependent with respect to infusion of PGE₂, noticeably FALB would be augmented with doses of PGE₂ higher than 125 µg.

DISCUSSION

The present study assessed for the first time the contribution of PGE₂ accumulation on BBB damage and infiltration of serum albumin in brain in an *in vivo* model. Furthermore, to the best of our knowledge, a detailed time course and dosages of PGE₂ function, and its relation to the development of BBB disruption had not been previously investigated. Post-ischemic inflammation has recently emerged as an important factor responsible for the development of the ischemic brain injury. In

this regard, the present findings indicate that increasing PGE₂ concentrations in right forebrain hemispheres resulted in BBB disruption and infiltration of the labeled albumin. The translocation of labeled albumin 24 h after infusion of PGE₂ is consistent with the observation of Krizanac-Bengez and coworkers who showed that the COX₂ inhibitor entirely protected BBB permeability in an *in vitro* BBB model using rat brain microvascular endothelial cells (17).

Our findings, however, is not in agreement with the suggestion made by McCullough and colleagues that the disruption of the PGE₂ receptors aggravated neuronal death after transient forebrain ischemia, proposing that PGE₂ has a neuroprotective effect on postischemic injury (18). In addition, the effect of PGE₂ in the *in vitro* studies has been controversial, with results showing both toxic and protective effects on neuronal survival (11). However, Interpretation of these results, is difficult in part due to the complexity and heterogeneity of the brain regions with multiple actions of PGE₂ receptors and treatment protocols. A potential link between PGE₂ expression and PGE₂ synthesis, which are involved in BBB damage, should be also considered as the main role of PGE₂-mediated mechanism involved in brain cell inflammatory conditions (19,21).

It is suggested that the restoration of cerebral blood flow after ischemia may provoke damage to the BBB, which may cause infiltration and aggravate brain edema (3). These observations shed more light into the specific role of the PGE₂ function in ischemic brain injury, and might have important implications for the potential use of COX inhibitors or agents modulating PGE₂ synthesis in different clinical stages of cerebral ischemia.

The model presented in this study demonstrated that PGE₂ accumulation in the brain is responsible for the expansion of BBB disruption frankly, which is well-known factor for brain damage after cerebral ischemia (21,22). This is interpreted as being consistent with the COX-2 activation after ischemic brain injury (23,24).

CONCLUSION

It is concluded that the cellular and microvascular response to PGE₂ action is mediated through changes in the ultrastructure of the brain accompanied by an increase in BBB permeability.

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