Review Article

Microsomal prostaglandin E synthase-1 is involved in the brain ischemic injury

Yuri Ikeda-Matsuo
Laboratory of Pharmacology, School of Pharmaceutical Sciences, Kitasato University, Tokyo, Japan

Microsomal prostaglandin (PG) E synthase-1 (mPGES-1) is an inducible terminal enzyme in the synthetic pathway for PGE$_2$, which has been demonstrated to participate in many peripheral pathological inflammatory processes. Recently, we demonstrated that mPGES-1 also has a role in brain inflammation, such as that following cerebral ischemia. The expressions of mPGES-1 and cyclooxygenase-2 (COX-2) were induced and co-localized in neurons, microglia and endothelial cells in the cerebral cortex after transient focal ischemia. Using mPGES-1 knockout (KO) mice in which the postischemic PGE$_2$ production in the cortex was completely absent, we found that the ischemic injuries were reduced compared to those in wild-type (WT) mice. Furthermore, the ameliorated symptoms observed in KO mice after ischemia were reversed to almost the same severity as in WT mice by intracerebroventricular injection of PGE$_2$ into KO mice, suggesting the involvement of mPGES-1 in the exaggeration of ischemic injury through PGE$_2$ production. The induction and involvement of mPGES-1 in neurotoxicity were also observed in a glutamate-induced excitotoxicity model using rodent hippocampal slices. Glutamate increased the expression of mPGES-1 and production of PGE$_2$. The protective effect of NS-398, an inhibitor of COX-2, on the excitotoxicity observed in WT slices was completely abolished in mPGES-1 KO slices, which showed less excitotoxicity than WT slices. In the transient focal ischemia model, injection of NS-398 reduced not only ischemic PGE$_2$ production, but also ischemic injuries in WT mice, but not in mPGES-1 KO mice, which showed less dysfunction than WT mice. Our observations suggest that mPGES-1 is a critical determinant of postischemic neurological dysfunctions and that mPGES-1 and COX-2 are co-induced and co-localized by excess glutamate and act together to exacerbate stroke injury through excessive PGE$_2$ production. Considering that COX-2 inhibitors may non-selectively suppress the production of many types of prostanoids that are essential for normal physiological function of the brain and that a large number of epidemiological studies have provided evidence of an increased cardiovascular risk associated with the use of COX-2, an mPGES-1 inhibitor may prove to be an injury-selective inhibitor with fewer side effects. Thus, the results from mPGES-1 KO mice suggest that mPGES-1 is a promising novel target for the treatment of human stroke.

Correspondence should be addressed to:
Yuri Ikeda-Matsuo, Department of Pharmacology, School of Pharmaceutical Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan. Phone: +81-3-5791-6254; Fax: +81-3-3442-3875; e-mail: matsuoy@pharm.kitasato-u.ac.jp

Key words prostaglandin E$_2$, microsomal prostaglandin E synthase-1, cerebral ischemia, excitotoxicity, cyclooxygenase-2
Introduction

Stroke remains a major cause of death and neuronal disability worldwide. Although effective stroke treatments based on thrombolysis and restoration of blood flow have been developed\(^6\), these therapies are effective only during the first few hours after the onset of the stroke\(^5\). At later times after ischemia, many kinds of gene induction occur\(^5\), and some of these are known to be involved in brain inflammation, which is a major factor in the progression of the injury\(^4,5\).

Prostaglandin E\(_2\) (PGE\(_2\)), one of the most likely candidates for propagation of inflammation, is known to be accumulated at the lesion sites of the postischemic brain\(^5,7\). Among the PGE synthases (PGES), microsomal PGES-1 (mPGES-1) is an inducible terminal enzyme in the synthetase pathway for PGE\(_2\), and has been shown to participate in many peripheral pathological inflammatory processes\(^8\). Recently, the contribution of mPGES-1 has also been reported in several brain pathological processes, such as seizure\(^8\) and cerebral ischemia\(^9\). In this report, I review recent findings of the involvement of mPGES-1 in brain ischemic injury.

Induction of mPGES-1 after cerebral ischemia

PGE\(_2\) is sequentially synthesized from arachidonic acid by cyclooxygenase (COX) and PGES (Fig.1). Among the COX isoforms, COX-2 is the only inducible form; however, it has been immunohistologically detected in neurons in the normal brain\(^11\). COX-2 has been demonstrated to be up-regulated after transient ischemia in neurons\(^12,13\) and has recently been identified in non-neuronal cells as well at some lesion sites, e.g., in microglia in the brains of patients with multiple sclerosis and chronic cerebral ischemia\(^14,15\). The genetic disruption and chemical inhibition of COX-2 have been shown to ameliorate neuronal death after transient forebrain ischemia, suggesting that the PGE\(_2\) accumulated through COX-2 induction mediates the toxic effects in the brain\(^7,12,16\).

Three major isoforms of PGES have been isolated: cytosolic PGES (cPGES), microsomal PGES (mPGES)-1, and mPGES-2. While cPGES and mPGES-2 are constitutively expressed in various cells and tissues, mPGES-1 is induced by proinflammatory stimuli and in various models of inflammation, and is functionally coupled to COX-2\(^17,18\). In the brain, mPGES-1 is reported to be induced in microglia by intra-nigral injection of lipopolysaccharide (LPS)\(^19\) or in endothelial cells by intraperitoneal injection of LPS\(^20\) and by intra-hippocampal injection of kainate\(^9\). The induction of mPGES-1 after transient focal ischemia has recently been reported in rat and mouse middle cerebral artery (MCA) occlusion models\(^20\). The immunostaining for mPGES-1 and COX-2 of rat brains after transient ischemia showed ipsilateral co-induction of mPGES-1 and COX-2 in the postischemic lesion site (Fig.2A). The Western blot analysis also demonstrated that mPGES-1 protein and COX-2 protein, but not mPGES-2, cPGES, or COX-1 protein, were potently induced in the ipsilateral cerebral cortex after transient ischemia. The increased mPGES-1 expression was maintained for at least 3 days after ischemia, whereas the enhanced expression of COX-2 was more transient, suggesting a difference in the mechanisms underlying the induction of mPGES-1 and COX-2. The mRNA of mPGES-1 was also significantly increased in the cortex and striatum after transient ischemia, indicating that the induction of mPGES-1 is regulated, at least in part, at the transcription level. The inductions of protein and mRNA of mPGES-1 were also observed by excitotox glutamate stimulation in rat and mouse hippocampal slices, which has been employed as an in vitro brain ischemia model\(^13\). Considering that an excess amount of extracellular glutamate is observed in the postischemic cortex\(^21\), glutamate may be one of the most potent inducers of mPGES-1 in the postischemic cortex. Indeed, two transcription factors, NF-\(\kappa\)B and Egr-1, which have been demonstrated to be involved in the transcriptional regulation of mPGES-1\(^22,24\), are known to be activated by glutamate and the glutamate receptor agonist N-methyl-
D-aspartate, as well as by MCA-occlusion\textsuperscript{24-28}.

Immunostaining of rat brain slices for mPGES-1 revealed co-induction and co-localization of mPGES-1 and COX-2 in the peri-infarct and core region of the postischemic cortex\textsuperscript{13}. In the peri-infarct region of the postischemic cortex, there were numerous mPGES-1-positive cells with moderate expression which were also positive for neuron-specific nuclear protein (Neu-N), a specific marker for neurons (Fig.2B). On the other hand, in the ischemic core region, there were fewer mPGES-1-positive cells with abundant expression. These cells were not positive for Neu-N, but were positive for CD-11b and von Willebrand factor (VWF), which are specific markers for microglia/macrophages/neutrophils and endothelial cells, respectively (Fig.2C,D). Thus, mPGES-1 is shown to be induced in neurons in the peri-infarct region and microglia/macrophages/neutrophils and vascular endothelial cells in the ischemic core region of the cortex.

**Fig.2** mPGES-1 induction in the rat brain after transient ischemia

(A) Immunostaining for mPGES-1 and COX-2 and Nissl staining of a coronal brain slice 24 h after ischemia. Representative data from 6 animals are presented. Scale bar, 5 mm. (B-D) The double-immunostaining of mPGES-1 (red) and cell-type-specific marker proteins (green) in the peri-infarct (B) and core (C,D) regions of the cortex. Neurons (B), microglia (C), and endothelial cells (D) were recognized by antibodies for Neu-N, CD11b, and VWF, respectively. The insets show a highly magnified portion of each staining. Scale bar, 40 μm; insets, 10 μm. From ref.10.

**Fig.3** mPGES-1 is an essential component for PGE\(_2\) production in the postischemic cortex

The production of PGE\(_2\) in the ipsilateral (i) or contralateral (c) cortex of mPGES-1 KO (−/−) and WT (+/+) mice 24 h after MCA occlusion (MCAO) and sham operation (SHAM). n=8 animals per group. **p < 0.01 vs. another sample. From ref.10.

**Necessity of mPGES-1 for postischemic PGE\(_2\) production**

Because neuronal COX-2 induction has been reported in the peri-infarct region\textsuperscript{13}, the co-induction of mPGES-1 and COX-2 in neurons in the peri-infarct region may contribute to postischemic PGE\(_2\) production. Moreover, because microglial and endothelial COX-2 inductions after ischemia and multiple sclerosis have also been reported\textsuperscript{14,15}, PGE\(_2\) production through co-induction of mPGES-1 and COX-2 in microglia and endothelial cells is suggested to play an important role in postischemic PGE\(_2\) production. In fact, the postischemic PGE\(_2\) production observed in the ipsilateral cortex of WT mice was completely absent in that of mPGES-1 KO mice (Fig.3), indicating that mPGES-1 plays a predominant role in postischemic PGE\(_2\) production in the brain. Similarly, glutamate-induced PGE\(_2\) production observed in hippocampal slices from WT mice was completely absent in slices from mPGES-1KO mice\textsuperscript{21} again indicating a predominant role of mPGES-1 in ischemic PGE\(_2\) production. Thus, co-induction of COX-2 and mPGES-1 in neurons, microglia/macrophages/neutrophils and endothelial cells is suggested to be required for postischemic PGE\(_2\) production.

**Contribution of mPGES-1 to ischemic injury**

To investigate the role of mPGES-1 in postischemic insult, we carried out studies of transient focal ischemia-reperfusion in
Fig. 4 Deletion of the mPGES-1 resulted in marked amelioration of ischemic injury
(A) Representative TTC-stained coronal sections of mPGES-1 KO (-/-) and WT (+/+) mice. Scale bar, 5 mm. (B) The volume of infarcted brain tissue 24 h after ischemia was estimated and expressed as a percentage of the corrected tissue volume. n=10 animals per group, ** p <0.01 vs. WT mice. (C) Improvement of neurological dysfunction in mPGES-1 KO mice. The neurological score was measured 24 h after ischemia. n=21-22 animals per group. **p <0.01 vs. WT mice. (D-G) Representative results of TUNEL staining (D, E) and caspase-3 immunostaining (F, G) in the ipsilateral cortex of the mPGES-1 KO (E, G) and WT (D, F) mice. Scale bar, 100 μm. From ref.10).

Fig. 5 Effects of COX inhibitors on the excitotoxicity in mPGES-1 KO and WT mouse hippocampal slices
PI uptake was analyzed 24 h after 1 mM glutamate exposure for 30 min. (A) Representative confocal images of PI fluorescence 24 h after exposure to 1 mM glutamate (GLU) or vehicle (CTL) for 30 min in WT (+/+) mice and mPGES-1 KO (-/-) mice. Scale bar, 200 μm. (B) Quantitiated data from PI uptake analysis in the CA1 region with or without glutamate and indomethacin (IND, 1 μm) or NS-398 (1 μm) were scaled to a percentage of the glutamate response in slices from WT mice. n=6-11 slices per group. ** p <0.01, ##p <0.01, #p <0.05, vs. glutamate alone in WT slices; N.S. (not significant) vs. glutamate alone in mPGES-1 KO slices. From ref.21).

Fig. 6 Protective effect of NS-398 on ischemic injury in WT mice but not in mPGES-1 KO mice
NS-398 (10 mg kg⁻¹; i.p.) or vehicle was administered twice daily starting 10 min after MCA occlusion. (A) The volume of the infarcted region of the whole brain of WT (+/+) mice and mPGES-1 KO (-/-) mice 72 h after ischemia was estimated and expressed as a percentage of the corrected tissue volume. n=7-9 mice per group. (B, C) The effect of NS-398 on Evans Blue extravasation after ischemia. The Evans Blue contents of the ipsilateral and contralateral cortex of MCA-occluded (MCAO) or sham-operated (SHAM) WT (B) and mPGES-1 KO (C) mice injected with NS-398 or vehicle were measured 48 h after transient ischemia (n=6-7 mice per group). ** p <0.01 vs. the contralateral cortex of MCA-occluded mice or the ipsilateral cortex of sham-operated mice; ###p <0.01, N.S. (not significant) vs. the ipsilateral cortex of MCA-occluded vehicle-treated mice. Scale bar, 2 mm. From ref.21).
mPGES-1 KO mice. We observed a smaller infarct size in mPGES-1 KO mice compared with WT mice after ischemia (Fig.4A,B). The degree of edema of mPGES-1 KO mice was also significantly smaller than that of WT mice. Furthermore, the neurological dysfunction observed after MCA occlusion in mPGES-1 KO mice was significantly lower than that in WT mice (Fig.4C). To explore the nature of the differences in the infarct volume between mPGES-1 KO and WT mice, we examined the apoptotic reaction in the penumbra. In WT mice, significant TUNEL staining and anti-caspase-3 immunostaining were observed in the ipsilateral cortex after ischemia, while the level of staining was significantly lower in the mPGES-1 KO penumbra compared with the WT penumbra (Fig.4D-G). These results suggest that mPGES-1 exaggerates infarction, edema, neurological dysfunctions and neuronal apoptosis observed after ischemia. In hippocampal slice cultures, genetic deletion of mPGES-1 has also been shown to ameliorate the glutamate-induced excitotoxicity (Fig.5), suggesting that mPGES-1 contributes to exaggeration of ischemic injury through enhancement of the excitotoxicity induced by glutamate\(^{35}\). Taken together, these results suggest that mPGES-1 is a critical factor in the ischemic injury.

**Contribution of PGE\(_2\) to mPGES-1 neurotoxicity in ischemic injury**

Because posts ischemic PGE\(_2\) production was completely ablished in mPGES-1 KO mice (Fig.3), it is suggested that the improved symptoms in mPGES-1 KO mice after ischemia result from the absence of PGE\(_2\) production in the brain. Thus, we investigated the effect of intracerebroventricular injections of PGE\(_2\) on ischemic injury in mPGES-1 KO mice. The injections of PGE\(_2\) just before MCA occlusion significantly increased the infarct volume in the MCA-occluded KO mice to the same level as in the MCA-occluded WT mice\(^{36}\). Moreover, PGE\(_2\) significantly exaggerated the neurological dysfunctions in KO mice to the same level as in the MCA-occluded WT mice. The ICV injections of PGE\(_2\) caused no infarction or neurological dysfunction in sham-operated KO mice. These results suggest the involvement of PGE\(_2\) under induction of mPGES-1 in the expansion of posts ischemic brain injury. Because microglial and endothelial inductions of mPGES-1 and COX-2 after ischemia have been reported\(^{10,14,15}\), PGE\(_2\) production through co-induction of mPGES-1 and COX-2 in microglia and endothelial cells is suggested to play an important role in posts ischemic inflammation and microcirculatory disturbances, which in turn contribute to neuronal death and the expansion of cerebral infarction\(^{5,29}\).

**Requirement of mPGES-1 activity for COX-2 to cause neuronal damage in ischemic injury**

In recent studies, COX-2-deficient mice showed attenuated ischemia-induced neurotoxicity and DNA fragmentation as well as PGE\(_2\) production\(^{7,18}\). The phenotype in the posts ischemic insult of mPGES-1 KO mice resembles that of COX-2-deficient mice. This suggests that coordinate induction of mPGES-1 and COX-2 is required for posts ischemic PGE\(_2\) production, which causes inflammation and then ischemia-induced neuronal death. However, COX-2-derived PGH\(_1\) could be a precursor of not only PGE\(_2\) but also thromboxane A\(_2\) (TXA\(_2\)), PG\(_{12}\) and PGD\(_2\) (Fig.1). In addition to prostanoids, COX-2 also generates free radical species\(^{30}\). Therefore, not only PGE\(_2\), but also other prostanoids and/or reactive oxygen species have been suggested to mediate the toxic effects of COX-2 in brain ischemia\(^{31,32}\).

Recently, using a COX-2 inhibitor, NS-398, and mPGES-1 KO mice, we demonstrated that mPGES-1 activity is required for COX-2 to enhance excitotoxicity, and thus that COX-2 and mPGES-1 coordinately contribute to the exacerbation of stroke injury through PGE\(_2\) production\(^{33}\). The protective effect of NS-
318 on the glutamate-induced excitotoxicity observed in hippocampal slices from WT mice was completely abolished in slices from mPGES-1 KO mice, which showed less excitotoxicity than WT slices (Fig.5). Furthermore, in a transient focal ischemia model, the peritoneal injection of NS-318 after reperfusion significantly decreased not only ischemic PGE2 production, but also infarction, neurological dysfunctions, and the blood-brain barrier (BBB) disruption in WT mice, but not in mPGES-1 KO mice, which showed less dysfunction than WT mice (Fig.6). Taken together, these results suggest that mPGES-1 is needed for COX-2 to exert its effects, which contribute to the glutamate-induced excitotoxicity and ischemic injury, and that mPGES-1 is functionally coupled with COX-2 to exacerbate excitotoxicity and stroke injury through PGE2 production (Fig.7). In other words, among the COX-2 reaction products, PGE2 made the greatest contribution to mediation of the neurotoxicity of COX-2 in some experimental ischemia models.[21,33]

Contribution of EP receptors to ischemic injury

How, then, is the toxicity of posts ischemic PGE2 mediated? PGE2 can efflux by simple diffusion after synthesis and activate four receptor subtypes (EP1-4) via quite different signaling cascades. The various roles of the PGE2 receptors in the neuronal death induced by excitotoxicity and ischemic stroke have been clarified by genetic deletion and selective inhibition of each EP receptor — e.g., deletion of the EP2 receptors exaggerates ischemic injury through cAMP signaling[34], while deletion and inhibition of the EP4 receptors partially reduces the neuronal damage caused by excitotoxicity and ischemic stroke[35]. Interestingly, a recent study showed that genetic deletion of the EP3 receptors reduces the neuronal damage caused by oxygen/glucose deprivation or ischemic stroke[36]. We have also confirmed the amelioration of excitotoxicity and stroke injuries by inhibition and deletion of EP3 receptors (under preparation for submission). Thus, EP3 and/or EP1 could be critical effectors for the neurotoxicity of PGE2 (Fig.7).

Conclusion

In this review, I have shown that mPGES-1, when co-induced with COX-2, plays a critical role in the excitotoxicity and ischemic injury through PGE2 production. Accumulating reports have shown that COX-2 inhibitors attenuate ischemia-induced neurotoxicity and injury[37,38]. These findings have important implications for the therapeutic potential of COX-2 inhibitors in the treatment of stroke. The study of mPGES-1 suggests that not only COX-2 inhibitors, but also mPGES-1 inhibitors may have therapeutic potential in the treatment of stroke. Considering that COX-2 inhibitors may non-selectively suppress the production of many types of prostanooids that are essential for normal physiological functions of the brain[11] and that a large number of epidemiological studies have provided evidence of an increased cardiovascular risk associated with the use of COX-2[39,40], an mPGES-1 inhibitor may prove to be an injury-selective inhibitor with fewer side effects. Thus, mPGES-1 is a promising novel target for the treatment of human stroke[41]. Further studies will be needed to discover potent, specific and BBB permeable inhibitors of mPGES-1, and to identify the protective effect of mPGES-1 inhibitors on stroke injury.

References
USA, 103: 11790-11795, 2006.


