Review Article

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

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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.

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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[1], these therapies are effective only during the first few hours after the onset of the stroke[2]. At later times after ischemia, many kinds of gene induction occur[3], 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[6,7]. Among the PGE synthases (PGES), microsomal PGES-1 (mPGES-1) is an inducible terminal enzyme in the synthetic 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[9] and cerebral ischemia[10]. In this report, I review recent findings of the involvement of mPGES-1 in brain ischemic injury.

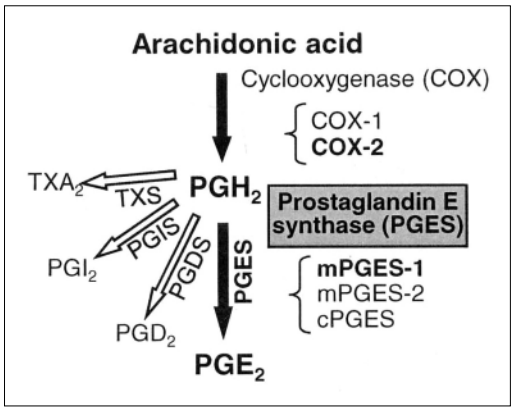

Fig.1 Biosynthetic pathway of PGE_2

Arachidonic acid is metabolized by cyclooxygenase (COX: COX-1 is the stable form and COX-2 is the inducible form) to the unstable endoperoxide PGH_2, and then metabolized by prostaglandin E synthase (PGES: cPGES and mPGES-2 are the stable forms and mPGES-1 is the inducible form) to PGE_2. PGH_2 is also metabolized to PGD_2, PGI_2 and TXA_2 by PGDS, PGIS and TXS, respectively.

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 immunohistochemically 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[10]. 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 excitotoxic glutamate stimulation in rat and mouse hippocampal slices, which has been employed as an *in vitro* brain ischemia model[21]. Considering that an excess amount of extracellular glutamate is observed in the postischemic cortex[22], glutamate may be one of the most potent inducers of mPGES-1 in the postischemic cortex. Indeed, two transcription factors, NF-κB and Egr-1, which have been demonstrated to be involved in the transcriptional regulation of mPGES-1[23,24], are known to be activated by glutamate and the glutamate receptor agonist N-methyl-

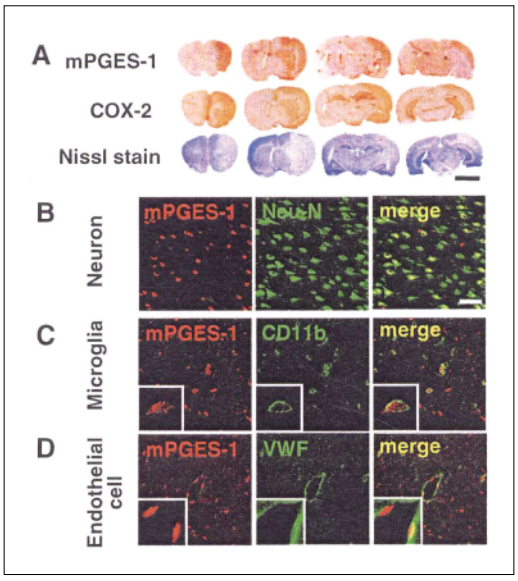

Fig.2 mPGES-1 induction in the rat brain after transient ischemia

(A) Immunostaining for mPGES-1 and COX-2 and Nissle 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).

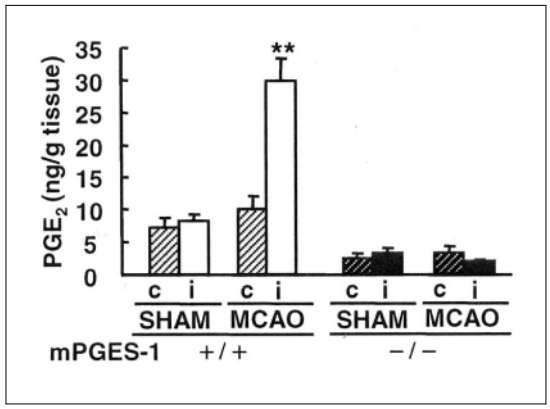

Fig.3 mPGES-1 is an essential component for PGE_2 production in the postischemic cortex

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).

D-aspartate, as well as by MCA-occlusion[25-28].

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[21]. 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.

Necessity of mPGES-1 for postischemic PGE_2 production

Because neuronal COX-2 induction has been reported in the peri-infarct region[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[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[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

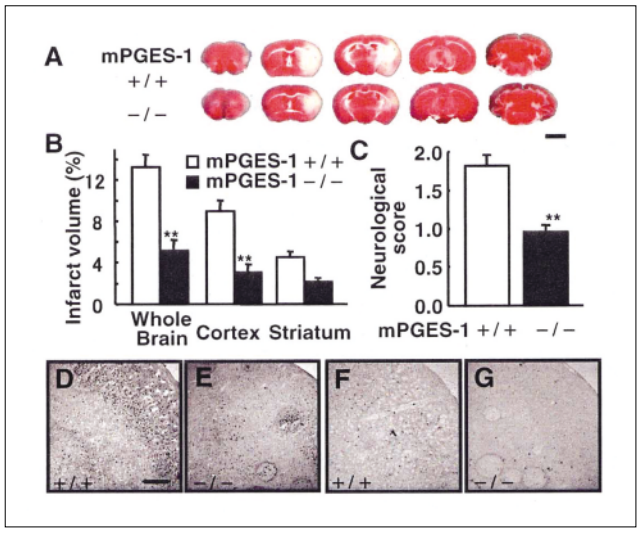

Fig.4 Deletion of the mPGES-1 resulted in marked amelioration of ischemic injury

(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).

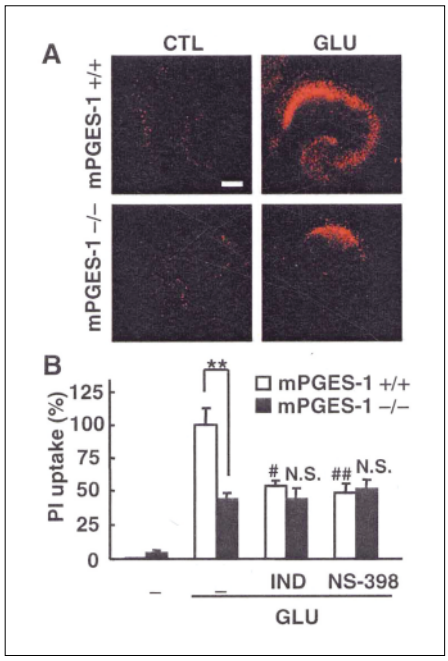

Fig.5 Effects of COX inhibitors on the excitotoxicity in mPGES-1 KO and WT mouse hippocampal slices

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) Quantitated 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).

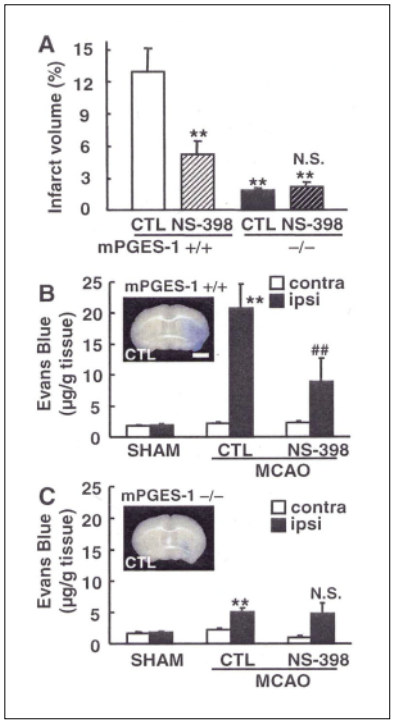

Fig.6 Protective effect of NS-398 on ischemic injury in WT mice but not in mPGES-1 KO mice

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).

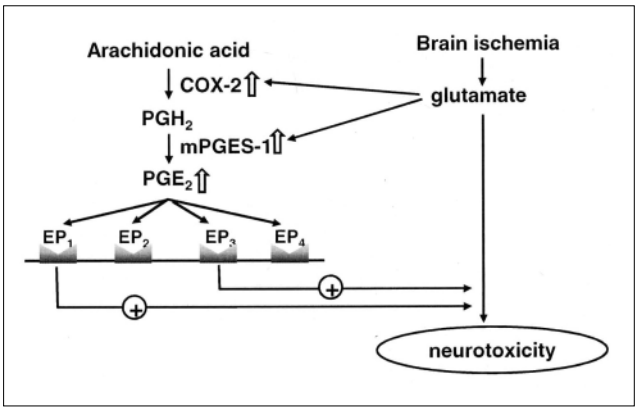

Fig.7 Pathway for mPGES-1-induced exaggeration of stroke injury

The ischemic PGE_2 produced by induction of COX-2 and mPGES-1 by excess glutamate exaggerates excitotoxicity and ischemic injury through EP_1 and/or EP_3 receptors.

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 infarction 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[21]. 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 postischemic PGE_2 production was completely abolished in mPGSE-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[10]. 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 postischemic 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 postischemic 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[7,16]. The phenotype in the postischemic 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 postischemic PGE_2 production, which causes inflammation and then ischemia-induced neuronal death. However, COX-2-derived PGH_2 could be a precursor of not only PGE_2 but also thromboxane A_2 (TXA_2), PGI_2 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[21]. The protective effect of NS-

398 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-398 after reperfusion significantly decreased not only ischemic PGE_2 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 PGE_2 production (Fig.7). In other words, among the COX-2 reaction products, PGE_2 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 postischemic PGE_2 mediated? PGE_2 can efflux by simple diffusion after synthesis and activate four receptor subtypes ($EP_{1\text{-}4}$) via quite different signaling cascades. The various roles of the PGE_2 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 EP_2 receptors exaggerates ischemic injury through cAMP signaling[34], while deletion and inhibition of the EP_1 receptors partially reduces the neuronal damage caused by excitotoxicity and ischemic stroke[35]. Interestingly, a recent study showed that genetic deletion of the EP_3 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 EP_3 receptors (under preparation for submission). Thus, EP_3 and/or EP_1 could be critical effectors for the neurotoxicity of PGE_2 (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 PGE_2 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 prostanoids 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[8]. 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.

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