How can we address the controversies surrounding the use of NSAIDs in neurodegeneration?

“We propose developing transgenic mouse and hybrid enzyme constructs as a research platform to help further our understanding of NSAIDs and their potential application in this pathology.”

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Neuroinflammation, which is regulated by arachidonic acid (AA) metabolites, is a key factor in the development of neurodegenerative disorders (NDD) [1–3]. Accordingly, anti-inflammatory agents, such as NSAIDs have been suggested as pharmacotherapy to prevent and treat several NDD, such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis [2–5]. However, the beneficial effects of these treatments have only exhibited in the preclinical phase, and they do not elicit similar effects in all patients with NDD [4]. Some clinical trial results even displayed detrimental effects on neuronal function, with few preventive benefits on neuronal degeneration [5–7].

The diverse end-active prostanoids including prostaglandin (PG) E_2 (PGE₂), prostacyclin (PGI₂), PGD₂, PGF₂ and thromboxane A₂ (TXA₂) are produced from the metabolism of AA by the coupling reactions of the upstream COX-1/-2 and downstream individual prostanoid synthases. In particular, the PGE₂ synthesized by inducible COX-2 coupled to microsomal PGE₂ synthase-1 (mPGES-1) activates its receptors to display neurotoxic activity [8–11]. However, the PGI₂ synthesized by both COX-1 and COX-2 coupled to prostacyclin synthase has neuroprotective activ-

ity [12–14]. NSAIDs inhibiting the upstream COX-1 and COX-2 to nonspecifically reduce all of the prostanoid biosyntheses are the most widely used drugs worldwide. For example, approximately 40,000 tons of aspirin, a type of NSAID, are produced globally per year as a therapeutic or preventive treatment for a variety of illnesses, including cardiovascular disease [15] and pain relief. Thus, it is important to understand the molecular and cellular mechanisms underlying toxic and beneficial effects of the NSAIDs on NDD, and also to develop pharmacotherapies which could improve the current NSAIDs through targeting more specific downstream enzymes to aid in the prevention and treatment of NDD.

Recent work by our lab showed that transgenic mice expressing an engineered hybrid enzyme showed altered AA metabolism, skewed toward PGI₂ production. This, in turn, reduced the conversion of AA metabolites to inflammatory PGE₂ and TXA₂ [14,16–19]. As adults, these animals exhibited improved cognitive performance in behavioral studies [14]. This model has also been shown to be effective in other diseases linked to ischemia and inflammation [14,18]. Additionally, preliminary data suggested that these mice have a longer

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life span. These results have led us to hypothesize that the conflicting evidence regarding the neuronal protection induced by NSAIDs is likely a consequence of non-specifically reduced COX-1 and -2 activities, effectively shutting down inflammatory PGE₂ production, as well as that of beneficial prostanoids, such as PGI₂. To help shed light on whether NSAID treatment and a resulting decrease in PGI₂ could precipitate neuroinflammation, the aforementioned transgenic mice, which express increased PGI₂ biosynthesis, can be used as a testing model. The neuroprotective effects of redirecting AA metabolism to PGI₂ production (thus reducing inflammatory PGE₂ and TXA₂ production), could be assessed *in vitro* through the use of a co-culture protocol. Primary neuronal cell cultures from transgenic mice and microglial cells could be grown together to simulate native tissue composition. β -amyloid peptide fragment could then be used to induce neuronal cell damage and death, with MAP-2 and CD-68 serving as markers for neurons and microglia, respectively. The degree of neurodegeneration could then be determined by calculating the percentage of viable neuronal cells postexposure [20].

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To determine whether the anti-neuroinflammatory effects seen in NSAID treatment are due to a reduction in PGE₂, two methods are under testing to reconstruct the inflammatory environment *in vitro*. One method is to apply PGE₂ directly to neuronal cells. The other method involved the use of newly created transgenic cell lines, which express the hybrid enzyme COX-2-linked to mPGES-1 hybrid enzyme. This hybrid enzyme would redirect AA metabolism to produce excess inflammatory PGE₂, thus mimicking the inflammatory environment. Again, neuronal viability could be measured to compare the fluctuation in neuroinflammatory response resulting from administration of PGE₂ and NSAIDs.

To address the potential benefit of PGI₂ in neurodegeneration *in vivo*, we propose establishing a mouse

strain through crossbreeding hybrid enzyme expressing transgenic mice with a standard Alzheimer's mouse strain.

The ideal drug to treat neuroinflammation would selectively reduce PGE₂ synthesis and maintain PGI₂ production. Such a drug is not currently available. This highlights the need to search for novel lead compounds that specifically inhibit the activity of downstream mPGES-1, which is a key enzyme responsible for the synthesis of inflammatory PGE₂, while retaining PGI₂ synthase activity. These compounds could serve to overcome the side effects of NSAID treatment regimens, inhibiting upstream COX enzymes. Lead compounds obtained through high-throughput screening could be used as a template to design and synthesize modified compounds exhibiting therapeutic activity. Our engineered hybrid enzyme which links COX-2 to mPGES-1 mimics the coupling of inducible COX-2 and mPGES-1, could thus be used as a target in lead compound optimization [21].

In this editorial, have discussed novel approaches to elucidate the controversies surrounding the use of NSAIDs in neuroinflammation and neurodegenerative disease. We propose developing transgenic mouse and hybrid enzyme constructs as a research platform to help further our understanding of NSAIDs and their potential application in this pathology. The platform will also be valuable in optimizing novel lead compounds targeting mPGES-1, which reduces inflammation with higher specificity. With these strategies, we hope to unlock the therapeutic potential of NSAIDs in neuroinflammation and neurodegenerative disease.

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