Available online at www.sciencedirect.com
SciVerse ScienceDirect
www.elsevier.com/locate/brainres

Research Report

# Effects of selective and non-selective cyclooxygenase inhibition against neurological deficit and brain oedema following closed head injury in mice

*Haymen Girgis, Bruno Palmier, Nicole Croci, Mathieu Soustrat, Michel Plotkine, Catherine Marchand-Leroux**

*Laboratoire de Pharmacologie de la Circulation Cérébrale (EA 4475), Université Paris Descartes, Faculté des Sciences Pharmaceutiques et Biologiques, Paris, France*

ARTICLE INFO

*Article history:*
Accepted 25 October 2012
Available online 30 October 2012

*Keywords:*
Traumatic brain injury
Animal model
Cyclooxygenase
Behaviour
Brain oedema

ABSTRACT

The implication of cyclooxygenase (COX) type 2 in post-traumatic consequences is so far controversial. In experimental models of traumatic brain injury (TBI), genetic disruption or pharmacological inhibition of COX-2 has been shown to be neuroprotective, deleterious or without effect. Therefore, the aim of our study was to investigate the effect of COX-2 inhibition against neurological deficit and brain oedema after TBI that was induced by mechanical percussion in male Swiss mice. Despite the increased level and activity of COX-2, its inhibition either with nimesulide (12 mg/kg) or meloxicam (2 mg/kg) modified neither the neurological score nor the brain water content that were evaluated at 6 and 24 h after injury. Interestingly, the non-selective COX inhibition with indomethacin (5 mg/kg) significantly promoted neurological recovery at 6 and 24 h after trauma, without improving brain oedema. In conclusion, the present study yields considerable evidence that COX-2 may not solely constitute an interesting target for the treatment of TBI consequences. Our data point to a potentially deleterious role of COX-1 in the development of neurological impairment in brain-injured mice. However, the neuroprotective mechanism of indomethacin remains to be clarified.



## 1. Introduction

Traumatic brain injury (TBI) is the main cause of neurological impairment and handicap among young adults, who will be deprived of many years of potential productive life. Therefore, despite the remarkable progress in monitoring, clinical care and management of traumatised patients, TBI is still a major worldwide public health problem (Reilly, 2007).

TBI initiates an immediate mechanical brain damage, including skull fractures, tissue lacerations and intracranial haemorrhage. However, being a continuous dynamic process and not simply a traumatic incident (Masel and DeWitt, 2010), TBI also triggers a complex cascade of harmful mechanisms leading to a secondary injurious assault for brain parenchyma (Park et al., 2008; Werner and Engelhard, 2007). Both local (Cederberg and Siesjö, 2010; Schmidt et al., 2005; Ziebell and Morganti-Kossmann, 2010) and systemic (Lu et al., 2009) inflammatory reactions are thought to be the hallmark of several deleterious events that follow the TBI-induced acute insult and thus would play a major role in the enhancement of neuronal death.

*Corresponding author. Fax: +33 143 26 46 97.
E-mail address: catherine.marchand@parisdescartes.fr (C. Marchand-Leroux).

0006-8993/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.brainres.2012.10.049

Many reports have provided evidence for the implication of cyclooxygenase (COX) in the TBI-activated inflammation (Dash et al., 2000; Strauss et al., 2000), and hence, highly suggested its inhibition as a therapeutic strategy for brain injury (Hurley et al., 2002). COX is the enzyme regulating the metabolism of arachidonic acid and prostanoid synthesis. The latter is carried out by tissue-specific isomerases into prostaglandin (PG) $E_2$, $PGI_2$ (prostacyclin), $PGD_2$, $PGF_{2\alpha}$ and thromboxane $A_2$. COX-1 and COX-2 are the isoforms of COX that were initially identified. Both are constitutively expressed in the brain and thought to be essential for neuronal function and plasticity. However, it is known that COX-2 is highly inducible by a broad spectrum of mediators that are involved in inflammation (Hinz and Brune, 2002).

Many years of experimental research came up with conflicting reports about the contribution of COX-2 in the neuroinflammatory process. In models of TBI using adult rats, selective inhibition of COX-2 with rofecoxib had no effect on neuronal death (Kunz et al., 2006). Celecoxib, another highly selective COX-2 inhibitor, worsened post-traumatic functional deficit (Dash et al., 2000). In the developing rat, the use of SC58125 as a COX-2 inhibitor neither reduced lesion volume nor improved cognitive deficit (Hickey et al., 2007). Similarly, the genetic disruption of COX-2 was not shown to be neuroprotective in mice (Ahmad et al., 2008). On the contrary, other studies suggested a detrimental role of COX-2 in TBI consequences. It was shown that COX-2 inhibition in traumatised rats attenuated cognitive deficit (Cernak et al., 2001, 2002), functional impairment and neuronal death (Gopez et al., 2005), blood-brain barrier dysfunction, oxidative stress and neuroinflammation (Hakan et al., 2010). Furthermore, Thau-Zuchman et al. (2012) have recently shown that COX-2 inhibition by carprofen after closed head injury (CHI) in mice was associated with better functional and histopathological outcomes, in addition to anti-inflammatory and anti-oedematous effects. In other models of brain injury, the role of COX-2 is also controversial. Although selective inhibition of COX-2 with NS-398 had no influence on infarct volume (Hara et al., 1998), a beneficial effect was induced by nimesulide, another COX-2 inhibitor, after ischaemic stroke in gerbils (Candelario-Jalil et al., 2002) and rats (Candelario-Jalil et al., 2007a, 2005, 2004). The post-ischaemic lesion volume was also significantly reduced in COX-2-deficient mice (Iadecola et al., 2001). Following intracerebral haemorrhage in rats, while inhibition of COX-2 with NS-398 had no effect on brain water and ion contents, and did not affect cerebral blood flow (Gong et al., 2001), celecoxib reduced sensorimotor deficit, neuronal death and brain oedema (Chu et al., 2004). In view of these inconsistent data, the implication of COX-2 in neuroinflammation is so far unclear, and thus, still constitutes the topic of a widespread scientific debate.

Therefore, our aim was to investigate the potential relevance of an early and a short-term inhibition of COX-2 with meloxicam or nimesulide for the management of TBI-induced consequences. In order to further evaluate the utility of COX inhibition, the effect of these preferential inhibitors of COX-2 was compared with that of indomethacin, a non-selective COX inhibitor. In spite of its increased level and activity following brain insult, the inhibition of COX-2 did not alter any of our studied variables. Interestingly, indomethacin was shown to be neuroprotective against post-traumatic neurological deficit, without affecting brain oedema.

## 2. Results

### 2.1. *Time-course studies of COX-2 expression and 6-keto $PGF_{1\alpha}$ production after TBI*

COX-2 enzyme level and activity were assessed at different time points in our murine model of CHI. The immunoblotting analysis showed that COX-2 protein was detected in naïve mice (Fig. 1A), reflecting its constitutive expression in the brain. The quantification of band intensities showed a 3-fold

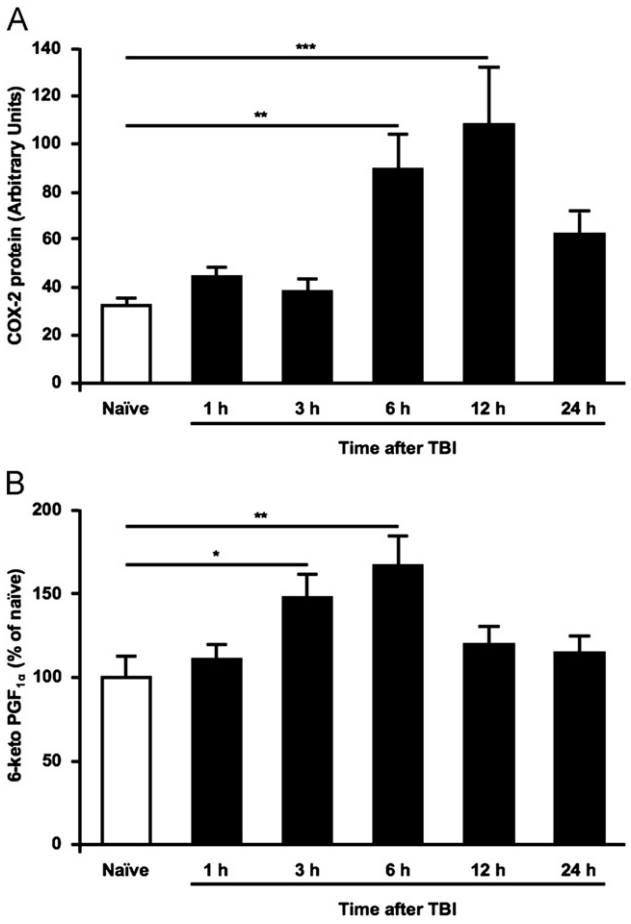


**Fig. 1 – Time-course study of the brain content of COX-2, and COX *in vivo* activity in naïve and TBI mice: 1, 3, 6, 12 and 24 h after injury. (A) Histogram presenting the post-traumatic time evolution of COX-2 expression in brain parenchyma, as revealed by Western blot. The results are expressed in arbitrary units and presented as the mean ± SEM ($n$ = 13–16 per group). (B) Histogram presenting the post-traumatic time evolution of 6-keto $PGF_{1\alpha}$ synthesis in brain parenchyma, as determined by enzyme immunoassay. Data are expressed in percentage of the mean of the naïve mice group and presented as the mean ± SEM ($n$ = 13–16 per group). Differences between groups were analysed by ANOVA one-way analysis of variance, followed by the Bonferroni test. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ *versus* naïve.**