Exp Brain Res (2002) 147:193–199
DOI 10.1007/s00221-002-1245-z

RESEARCH ARTICLE

Ibolja Cernak · Christine O'Connor · Robert Vink

Inhibition of cyclooxygenase 2 by nimesulide improves cognitive outcome more than motor outcome following diffuse traumatic brain injury in rats

Received: 21 March 2002 / Accepted: 31 July 2002 / Published online: 2 October 2002

Abstract Prostanoid synthesis is regulated by the enzyme cyclo-oxygenase (COX) that is present in at least two isoforms: COX-1, the constitutive form, and COX-2, the inducible form. Expression of COX-2 has recently been shown to be an important determinant of the cytotoxicity connected with inflammation following ischemic injury to the brain. The present study examines the temporal and spatial profiles of COX-2 expression following diffuse traumatic brain injury (TBI) in rats, and the effects of the COX-2 inhibitor nimesulide on cognitive and motor outcomes. Adult, male Sprague-Dawley rats were injured using the 2-meter impact acceleration model of diffuse TBI. At preselected time points after injury, animals were killed and the expression of COX-2 was measured in the hippocampus and parietal cortex by immunohistochemistry and Western blotting techniques. Effects of nimesulide (6 mg/kg daily over ten days) on cognitive and motor outcome was assessed in a separate group of animals using the Barnes circular maze and rotarod test, respectively. A highly significant up-regulation of COX-2 expression was found in the hippocampus as early as 3 h post-trauma and persisting for at least 12 days after TBI. In contrast, a slight but significant upregulation of COX-2 expression occurred in the cortex only at 3 days after trauma. Administration of the COX-2 inhibitor nimesulide resulted in a significant and substantial improvement in cognitive function compared to vehicle-treated controls, while motor deficits after injury was only improved at 24 h after injury. We conclude that COX-2 is involved in the development of functional deficits following diffuse TBI, particularly cognitive deficits, and that these can be improved by administration of COX-2 inhibitors.

Keywords Neurotrauma · COX · Cognition · Diffuse axonal injury

I. Cernak
Department of Neuroscience, Georgetown University, Washington DC, USA

C. O'Connor · R. Vink (✉)
Department of Pathology, The University of Adelaide, Adelaide SA 5005, Australia
e-mail: Robert.Vink@adelaide.edu.au
Tel.: +61-8-82223092
Fax: +61-8-82223093

Introduction

There is accumulating evidence suggesting that the brain damage produced by traumatic brain injury (TBI) develops over a significant period of time after the traumatic event. Indeed, post-traumatic functional outcome is now thought to be dependent not only on primary mechanisms of tissue destruction occurring at the time of injury, but also on complex secondary injury mechanisms initiated at the time of trauma and manifesting for minutes or even days after the traumatic insult. Accordingly, the identification of these secondary injury factors may present new opportunities for therapeutic strategies targeted at the late phase of the damage (Faden 1996).

One of the secondary injury processes that may promote delayed neuronal death is post-traumatic inflammation, which has been shown to increase blood-brain barrier permeability, cerebral edema and intracranial pressure, resulting in neuronal dysfunction after TBI (DeWitt and Prough 1998; McIntosh et al. 1998). Prostaglandins are among the pivotal mediators/modulators of this inflammation and, together with thromboxanes, are part of the prostanoid family that are synthesized from arachidonic acid (5,8,11,14-eicosatetraenoic acid) via cyclooxygenase (COX, or prostaglandin H synthase), which is present in at least two isoforms (Vane et al. 1998). COX-1 is a predominantly constitutive form and is involved in cellular homeostasis, while COX-2 is an inducible isoform up-regulated by reactive oxygen species (ROS), inflammatory cytokines and mitogens (Dubois et al. 1998; Lasa et al. 2000). The fact that COX-2 has been characterized as a representative of the immediate early genes (IEGs) that can directly influence cellular function (Yamagata et al. 1993) emphasizes its

potential importance in a number of pathophysiological processes. Induction of COX-2 has profound effects on the brain via the complex effects of prostanoids, which include, among others, modulation of glutamate release (Adams et al. 1996), cerebral vasoconstriction (Brian et al. 1998), induction of ROS release (Tardieu et al. 2000), and influence on neuroendocrine function (Parsadaniantz et al. 2000). Therefore, it is possible that COX-2 upregulation in injured neurons could contribute to neuronal death following brain injury. Consistent with this, inhibition of COX-2 activity has recently been demonstrated to protect against ischemic damage (Nakayama et al. 1998; Wakita et al. 1999), kainate-induced seizures (Candelario-Jalil et al. 2000), and neurotoxicity caused by chronic neuroinflammation (Willard et al. 2000).

Although recent studies have demonstrated increased COX-2 expression in brain structures of rats following cortical contusion injury (Dash et al. 2000; Strauss et al. 2000), no studies have examined the role of COX-2 in models of traumatic brain injury that have diffuse axonal injury as a major component. This is despite the fact that diffuse axonal injury is thought to be a major component of severe clinical head injury and, in particular, the formation of the vegetative state. Accordingly, in this study we investigated whether COX-2 is expressed in the brain following diffuse TBI and, if so, whether its upregulation contributes to the secondary evolution of the damage and consequent development of cognitive and motor deficits.

Materials and methods

Animal preparation

All procedures in this study were performed in accordance with the NIH guidelines for the humane treatment of laboratory animals (NIH Publication No. 82–23, 1985) and the guidelines for the use of animals in experimental research as outlined by the Australian National Health and Medical Research Council. Traumatic brain injury was induced using the impact-acceleration model of diffuse traumatic brain injury (TBI) as previously described (Foda and Marmarou 1994; Marmarou et al. 1994; Heath and Vink 1999). Briefly, adult male Sprague-Dawley rats (n=83; 350–400 g) were anesthetized with sodium pentobarbital (50 mg/kg i.p.), intubated, and mechanically ventilated on room air using a Harvard Rodent Ventilator. Thereafter, rectal temperature was maintained at 37° C with a thermostatically controlled heating pad. The skull was then exposed by a midline incision and a stainless steel disc (10 mm in diameter and 3 mm in depth) was fixed rigidly with polyacrylamide adhesive to the animal's skull centrally between lambda and bregma. The rats were subsequently placed on a 10-cm foam bed and subjected to brain injury induced by dropping a 450-g brass weight a distance of 2 m onto the stainless steel disc. Such an injury has been previously shown to produce diffuse axonal injury of moderate severity (Foda and Marmarou 1994). Sham-operated controls (n=9) were surgically prepared, but were not injured.

Immunochemical techniques

Following TBI, animals were anesthetized and killed by decapitation at 3 h, 6 h, 12 h, 24 h, 2 days, 3 days, 4 days, 5 days, 7 days, or 12 days (n=6/ per time point) post-trauma. For immunoblotting assays, the brains were rapidly removed, and samples of parietal cortex and hippocampus were dissected. Extracts from brain samples were prepared by lysis in 100 mM HEPES buffer, pH 7.5, containing 10% sucrose, 1 mM EDTA, 1% Triton-X, 1 mM dithiothreitol (DTT), 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 μmg/ml leupeptin, 1 μmg/ml pepstatin A, and 5 μmg/ml aprotinin, and centrifuged at 10,000 g. Thirty micrograms of total protein of each sample was then loaded onto a 12% SDS-PAGE gel and after electrophoresis, protein was transferred to nitrocellulose membrane (Hybond-ECL, Amersham Pharmacia). Blots were blocked with 5% nonfat milk in TBST (10 mM Tris, pH 7.2, 150 mM NaCl, 0.05% Tween 20) and incubated with antibody directed against COX-2 (polyclonal murine; 1:1000; Cayman Chemical Company, Ann Arbor, USA) for 1 h at room temperature. After washing the blot three times in PBS containing 0.1% Tween 20, the secondary antibody (goat anti-rabbit IgG-HRP; 1:10,000; Sigma; St. Louis, Mo.) was applied for a further 1 h at room temperature. The blots were then washed three times in PBS containing 0.1% Tween before being incubated in commercial enhanced chemiluminescence reagents (Amersham, Arlington Heights, Ill.) and exposed to Kodak BioMax ML film (Eastman Kodak Company, Rochester, N.Y.). To assess changes in estimated proteins quantitatively, the bands were analyzed by densitometry and quantified by computer analysis.

For immunohistochemistry, brains were removed at 3 days post-trauma (n=3) and snap-frozen in liquid nitrogen and a cryostat used to obtain 8–10 μm coronal sections through the regions of interest. Slide-mounted slices were then incubated for 3 days at 4°C with polyclonal murine COX-2 antibody (1:2,000; Cayman Chemical, Ann Arbor, USA) dissolved in PBS containing 3% of normal rat serum. After washing the slices in PBS, slices were incubated with IgG-HRP conjugated secondary antibody (1:400; Sigma-Aldrich) for 1 h at room temperature, and the subsequent immunocomplex visualized using diaminobenzidine as a chromogen in a peroxidase reaction (Sigma-Aldrich). To assist in the determination of the cellular localization of the label, the sections were counterstained with hematoxylin (Sigma-Aldrich). After rinsing, the slides were examined in a Leica DML LB microscope. Control conditions to assess nonspecific labeling included omission of the primary antibody, preabsorption of the COX-2 antibody or omission of hydrogen peroxide to block endogenous peroxidase activity. In all cases, the controls were negative.

Drug preparation and administration

N-(nitro-2-phenoxyphenyl)-methanesulfonamide (R805, marketed as Nimesulide, Cayman Chemical, Ann Arbor, USA) was suspended in DMSO and diluted into isotonic saline prior to administration. Animals were then administered nimesulide intraperitoneally (6 mg/kg, n=10) at 30 min after trauma and daily over a 10-day period. This dosage regimen is identical to that which has been used successfully in previous brain injury studies in rats (Wakita et al. 1999; Candelario-Jalil et al. 2000). The control group (n=10) received an equal volume of vehicle using the same administration regimen.

Assessment of cognitive performance

The Barnes circular maze (Barnes 1979) as modified and described in detail by Fox et al. (1998) was used to assess spatial reference memory following diffuse traumatic brain injury. Animals were trained to locate a hidden escape tunnel in response to an aversive light and sound stimulus. The tunnel was placed directly beneath one of 18 circular holes at the perimeter of a 1-m circular platform. Latency in seconds to find the escape tunnel from the center of the platform after initiation of aversive stimuli was assessed daily over a 10-day post-traumatic period.

Assessment of motor performance

Motor assessment was performed using the rotarod test, which has been described as being the most sensitive test to detect motor deficits in rodent brain injury (Hamm et al. 1994; Hamm 2001). This test has been successfully used previously to assess the effects of pharmacological agents on motor deficits following diffuse traumatic brain injury (Heath and Vink 1999). Briefly, the animals were placed on the rotarod device consisting of a motorized rotating ensemble of 18 rods. Rotational speed of the device was increased from 0 to 30 revolution per minute (rpm) in intervals of 3 rpm every 10 s and the latency to fall from the rotating bars or to grip the rods and spin two consecutive rotations was recorded for each rat. Animals were pretrained daily on the device for 1 week prior to injury and assessed daily for 10 days after injury.

Statistical analysis

Data are shown as means ±S.D. Statistical significance for Western blots was determined by analysis of variance (ANOVA), followed by Student-Newman-Keuls post hoc tests. For analysis of behavioral data, a repeated measure ANOVA was used to determine group main effects. Student-Newman-Keuls post hoc test was used to determine group differences on specific days after injury. The level of significance was set at $P<0.05$.

Results

COX-2 protein expression in brain structures following traumatic brain injury

Immunoblotting analyses of samples from hippocampus and cortex showed increases in COX-2 protein level following acceleration induced impact injury. Diffuse TBI induced a marked increase in hippocampal COX-2 immunoreactivity as early as 3 h after injury and achieved a maximum at 48 h to 3 days postinjury (F [10,55]=27.10; $P<0.001$; Fig. 1). The significant increase in expression of COX-2 in the hippocampus then persisted until the end of the 12-day observation period ($5.22<q<12.38$; $P<0.01$). In contrast, the cortex demonstrated a much smaller increase in immunoreactivity for COX-2 (F [10,55]=5.88; $P<0.001$) that was maximal at 3 days postinjury (148±16% as compared to sham-controls; Fig. 2). Only this 3-day timepoint was statistically significant from control ($q=4.08$; $P<0.01$).

Immunohistochemistry was performed at 3 days after injury in sham and injured animals. This time point was selected because immunoblotting analyses demonstrated that the maximal expression of COX-2 occurred at this time point after injury. Figure 3 shows that in the brain of sham-control rats, little immunoreactivity for COX-2 was observed in cerebral cortex and hippocampus. After diffuse TBI, there was a marked up-regulation of COX-2 immunoreactivity in the hippocampal pyramidal neurons. In the cortex, however, there was only a small increase in COX-2 immunoreactivity after TBI, with most of the increase occurring in the subcortical structures. Thus, both immunohistochemistry and immunoblotting confirmed a marked increase in COX-2 protein expres-

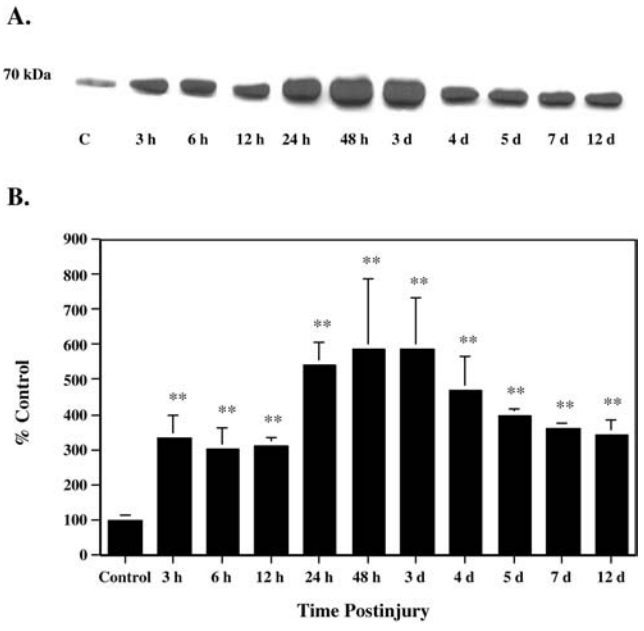

Fig. 1A, B COX-2 expression in hippocampus following diffuse traumatic brain injury. **A** Western blot analysis of COX-2 in the hippocampus isolated from control brains (*C*) or brains obtained after trauma at 3 h, 6 h, 12 h, 24 h, 48 h, 3 days, 4 days, 5 days, 7 days and 12 days. **B** Significant over-expression of COX-2 is shown starting at 3 h after trauma and persisting to the end of the 12-day study. Mean±SD; n=6/time point; $^{**}P<0.01$ vs control

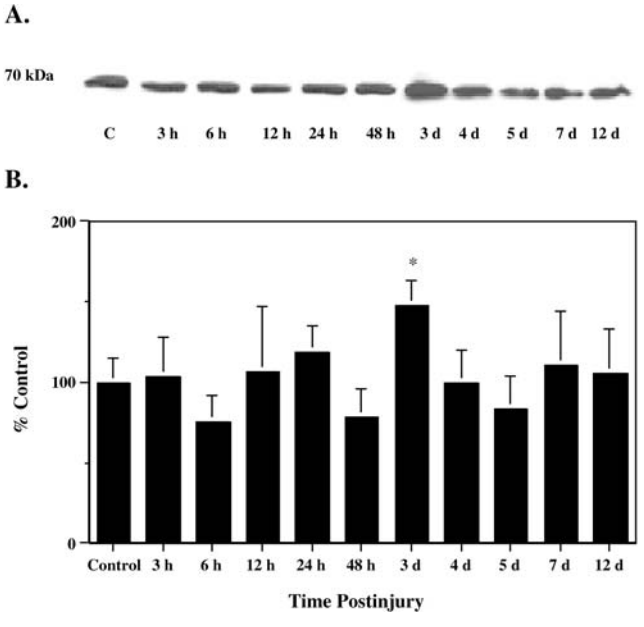

Fig. 2A, B COX-2 expression in cortex following diffuse traumatic brain injury. **A** Western blot analysis of COX-2 in the cortex isolated from control brains (*C*) or brains obtained after trauma at 3 h, 6 h, 12 h, 24 h, 48 h, 3 days, 4 days, 5 days, 7 days, and 12 days. **B** A significant change in expression of COX-2 in the cortex only occurred at 3 days after trauma. Mean±SD; n=6/time point; $^{*}P<0.05$ vs control

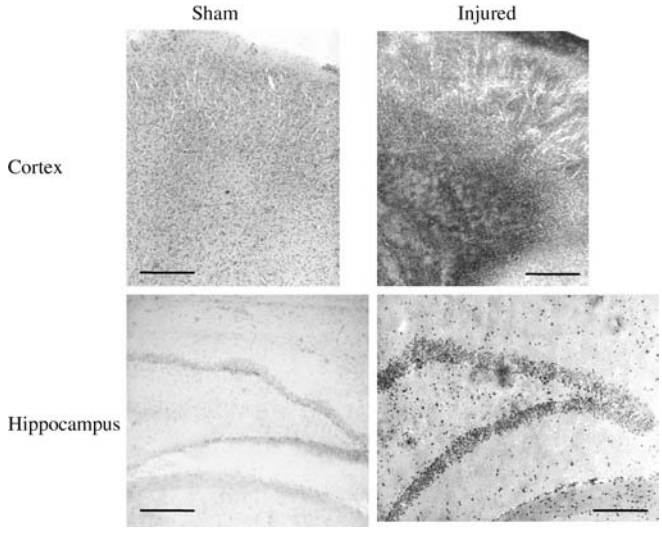

Fig. 3 COX-2 immunoreactivity in cortex and hippocampus. A montage of photomicrographs taken from brain structures from sham-operated animals and animals with diffuse traumatic brain injury 3 days following trauma. Increased COX-2 immunoreactivity can be clearly observed in the hippocampus whereas most of the increased COX-2 immunoreactivity in the cortical section occurs in the subcortical structures. Bar=250 μm

sion in the hippocampus, and a small increase in the cortex, following acceleration-induced diffuse TBI.

Motor and cognitive functional outcomes

To determine whether COX-2 expression contributes to functional deficits following TBI, we used the relatively selective COX-2 inhibitor nimesulide and assessed its effects on functional outcome. With respect to cognitive function, mean latency time for animals to locate the Barnes maze tunnel prior to injury was 8±1 s. Following injury, however, the latency to locate the tunnel in vehicle-treated animals increased significantly (F[10,99]=22.17; P<0.001) to a maximum of 70±24 s at 24 h post-injury (Fig. 4) and gradually improved thereafter, although never achieving preinjury values in the 10-day monitoring period. In contrast, animals treated with nimesulide demonstrated a mean latency of 11±4 s over the entire 10-day post-traumatic monitoring period, which was significantly better than that observed in the vehicle-treated controls (5.82<q<20.61; P<0.05).

With respect to motor outcome, mean preinjury rotarod score in all animals was 100±13 s (Fig. 5). In vehicle-treated control animals, a significant motor deficit was observed after diffuse TBI (F[10,99]=10.38; P<0.001) with the maximal deficit occurring at 24 h post-trauma (34±9 s). Thereafter, the motor performance gradually improved with time, although the motor performance never recovered to preinjury levels over the 10-day monitoring period. Administration of the COX-2 inhibitor, nimesulide, slightly attenuated the motor deficits after injury, although with the exception

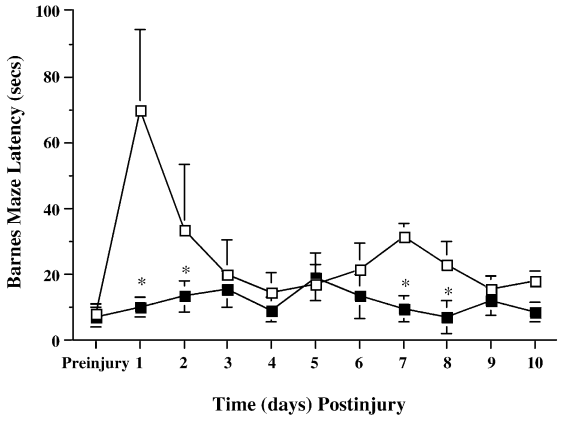

Fig. 4 Alterations in cognitive performance between animals treated with either nimesulide (COX-2 inhibitor; ■) or vehicle (❑) over a 10-day assessment period following diffuse traumatic brain injury in rats. Mean±SD; n=10/time point; *P<0.05 between treatment groups

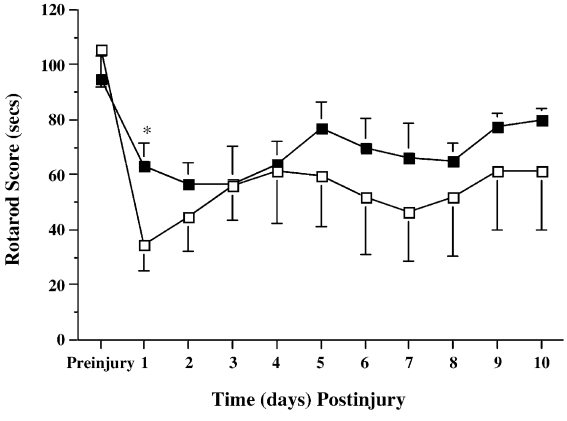

Fig. 5 Alterations in motor performance between animals treated with either nimesulide (COX-2 inhibitor; ■) or vehicle (❑) over a 10-day assessment period following diffuse traumatic brain injury in rats. Mean±SD; n=10/time point; *P<0.05 between treatment groups

of the 24 h time point (q=5.45; P<0.05), this motor performance was not significantly better than that observed in vehicle-treated controls.

Discussion

Increased prostanoid synthesis and release following brain injury has been reported to contribute to brain edema, cerebral vasoconstriction and increased intracranial pressure (DeWitt and Prough 1998). Consistent with this, inhibition of prostanoid synthesis has been demonstrated to be beneficial in experimental studies of focal ischemia in rats (Buccellati et al. 1998), fluid percussion-induced brain injury (Kim et al. 1989), concussive neurotrauma in cats (Wei et al. 1981), and clinical neurosurgery (Benedek et al. 1987). While previous studies have used non-selective inhibitors of cyclooxyge-

nase, recent investigations have targeted selective inhibition of COX-2. The use of a selective COX-2 inhibitor is based upon the hypothesis that the COX-2 isoenzyme is primarily responsible for the production of prostaglandins at sites of injury/inflammation. In this context, selective blockade of COX-2 will not inhibit the COX-1 isoform that is considered to be responsible for homeostatic functions, but only reduce the synthesis of prostaglandins that contribute to inflammation (Buttar and Wang 2000).

The results of the present study demonstrate that diffuse traumatic brain injury in rats enhances the expression of COX-2 protein, particularly in the hippocampus. Such an increase in expression in the hippocampus began as early as 3-h post-trauma and persisted for at least 12 days. In the cortex, there was a small increase in COX-2 expression, but this increase was only significant at 3 days after injury. Administration of a selective COX-2 inhibitor, nimesulide, during this time period significantly reduced the injury induced cognitive deficits and slightly improved motor performance, although as for the expression analysis, this improved motor outcome was not statistically significant compared to vehicle-treated controls for most time points after injury. Thus, the results of the protein expression analysis were consistent with the outcomes of our pharmacologic study where highly significant effects were observed with respect to the hippocampus and less so with respect to the cortex.

Many brain studies have now demonstrated increased COX-2 levels following various forms of injury (Brian et al. 1998; Iadecola et al. 1999; Dash et al. 2000; Strauss et al. 2000). Those that have examined COX-2 expression in traumatic brain injury have used the lateral cortical impact injury model and report increased COX-2 levels in both the ipsilateral cortex and hippocampus at 24 h to 3 days (Dash et al. 2000; Strauss et al. 2000). While our present results in a more diffuse model of TBI demonstrate similar trends, we only saw a transient increase in COX-2 expression at a single time point in the cortex. Furthermore, our COX-2 protein expression in the hippocampus was more profound and of a longer duration than those reported in the previous studies. These differences may be explained by the use of the more diffuse model of TBI in our studies. While the cortical impact injury model has been shown to result in focal ischemia at the cortical injury site (Hendrich et al. 1999), our diffuse injury model does not result in any cortical ischemia. Hence the increased COX-2 expression expected as the result of an ischemic component in the ipsilateral hemisphere of the cortical impact model would not occur in the diffuse traumatic brain injury model. As for the extended time course for COX-2 immunoreactivity in the hippocampus in our study, this may be accounted for either by a relatively long half-life of the protein, or its continued transcription. The latter is more likely given the lack of such an extended time course in the previous studies using the cortical impact model. The difference in COX-2 expression between the hippocampus and cortex in our study also suggests a regionally specific response to trauma. Clearly, if COX-2 upregulation following traumatic brain injury is regionally specific, the subsequent functional consequences will reflect such a structure-related pattern of enhanced COX-2 protein levels. This regional specificity of COX-2 expression was noted in our functional outcome studies. Rats treated with the COX-2 inhibitor, nimesulide, demonstrated a significant improvement in cognitive performance after diffuse TBI compared to vehicle-treated control animals. In contrast, the effects of the COX-2 inhibitor on motor performance were much less profound. This result was consistent with the COX-2 expression studies where profound expression in the hippocampus would be associated with formation of profound cognitive deficits, whereas the limited expression of COX-2 in the cortex would contribute little to the observed motor deficits in this model.

COX-2 expression following brain injury is thought to contribute to brain injury through a number of mechanisms. Selective inhibition of COX-2 is therefore expected to prevent or at least attenuate these events and consequently reduce post-traumatic neurologic deficits. However, not all studies of COX-2 inhibitors have yielded positive results. Koyfman et al. (2000) reported that nimesulide reduced tissue prostaglandin (PGE_2) production after closed head injury, but did not attenuate brain tissue edema formation or motor deficits. Moreover, Dash et al. (2000) demonstrated that celecoxib, another specific COX-2 inhibitor, did not improve cognitive outcome following lateral cortical impact injury in rats and, in fact, exacerbated motor deficits. Although the explanation for these contradictions is unknown, there are several possibilities. First, the regional specificity of COX-2 expression in the different models as discussed above may impact on the efficacy of COX-2 inhibitors. It is important that the regional expression and the temporal profile of the protein be described in the different models and taken into account. Second, different models of TBI have varying degrees of ischemia contributing to the injury cascade. It is important to understand the contribution of ischemia in each of the different experimental models of TBI. Third, as shown in the present study, functional deficits are unlikely to be accounted for by a single factor. While there was some increased expression of COX-2 in the cortex observed in the present study, the motor deficits were only slightly attenuated by treatment with a COX-2 inhibitor. Clearly, a number of factors contribute to the formation of the deficit. Finally, there is the question of drug specificity. Willoughby et al. (2000) suggested that there may be a third isoform of the COX family, named COX-3, whose structure is similar to that of COX-2. However, COX-3, unlike COX-2, does not produce proinflammatory prostanoids, but rather anti-inflammatory members of that mediator-family. The authors postulated that COX-3 has an important role in the third, resolution phase of inflammation, while COX-1 and COX-2 are involved in the onset and peak stages of such an inflammatory response. Consistent with this hypothesis, Gilroy et al. (1999) reported that a selective COX-2 inhibitor inhibited inflammation at 2 h following carrageenin-induced pleurisy in rats, but significantly

exacerbated inflammation at 48 h. These authors postulated that COX-2 may be proinflammatory during the early inflammatory phase, but may aid resolution of the later stage associated with anti-inflammatory prostaglandins. Whatever the explanation for the contradictory result observed in the studies of COX-2 in traumatic brain injury, clarification of these issues may help in the development of new neuroprotective drugs targeted at reducing cyclo-oxygenase induced damage following traumatic brain injury.

In conclusion, the present study has demonstrated that diffuse traumatic brain injury resulted in an increased expression of COX-2 protein in the hippocampus with little response in the cortex. The increase in COX-2 protein expression after TBI was associated with the development of functional deficits. Indeed, selective inhibition of COX-2 by nimesulide significantly reduced the injury-induced cognitive deficits, with little effect on motor performance. The findings of this study encourage further work to clarify the therapeutic utility of selective COX-2 inhibitors in neurotrauma.

Acknowledgement This study was supported, in part, by a grant from the Neurosurgical Research Foundation of Australia to RV and IC.

References

Adams J, Collaco-Moraes Y, de Belleroche J (1996) Cyclooxygenase-2 induction in cerebral cortex: an intracellular response to synaptic excitation. J Neurochem. 66:6–13

Barnes CA (1979) Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. J Comp Physiol Psychiat 93:74–104

Benedek G, Toth-Daru P, Janaky J, Hortobagyi A, Obal FJ, Colner-Sasi K (1987) Indomethacin is effective against neurogenic hyperthermia following cranial trauma or brain surgery. Can J Neurol Sci 14:145–148

Brian JEJ, Moore SA, Faraci FM (1998) Expression and vascular effects of cyclooxygenase-2 in brain. Stroke 29:2600–2606

Buccellati C, Folco GC, Sala A, Scelsi R, Masoero E, Poggi P, Govoni S, Favalli L, Rozza A (1998) Inhibition of prostanoid synthesis protects against neuronal damage induced by focal ischemia in rat brain. Neurosci Lett 257:123–126

Buttar NS, Wang KK (2000) The "aspirin" of the new millennium: cyclooxygenase-2 inhibitors. Mayo Clin Proc 75:1027–1038

Candelario-Jalil E, Ajamieh HH, Sam S, Martinez G, Leon Fernandez OS (2000) Nimesulide limits kainate-induced oxidative damage in the rat hippocampus. Eur J Pharmacol 390:295–298

Dash PK, Mach SA, Moore AN (2000) Regional expression and role of cyclooxygenase-2 following experimental traumatic brain injury. J Neurotrauma 17:69–81

DeWitt DS, Prough DS (1998) Vasoactive prostanoids and traumatic brain injury. Crit Care Med 26:819–821

Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, Van De Putte LB, Lipsky PE (1998) Cyclooxygenase in biology and disease. FASEB J 12:1063–1073

Faden AI (1996) Pharmacologic treatment of acute traumatic brain injury. JAMA 276:569–570

Foda MAA, Marmarou A (1994) A new model of diffuse brain injury in rats; Part II: morphological characterization. J Neurosurg 80:301–313

Fox GB, Fan L, LeVasseur R, Faden AI (1998) Effect of traumatic brain injury on mouse spatial and nonspatial learning in the barnes circular maze. J Neurotrauma 15:1037–1046

Gilroy DW, Colville-Nash PR, Willis D, Chivers J, Paul-Clark MJ, Willoughby DA (1999) Inducible cyclooxygenase may have anti-inflammatory properties. Nature Med 5:698–701

Hamm RJ (2001) Neurobehavioral assessment of outcome following traumatic brain injury in rats: an evaluation of selected measures. J Neurotrauma 18:1207–1216

Hamm RJ, Pike BR, O'Dell DM, Lyeth BG, Jenkins LW (1994) The rotarod test: an evaluation of its effectiveness in assessing motor deficits following traumatic brain injury. J. Neurotrauma 11:187–196

Heath DL, Vink R (1999) Optimisation of magnesium therapy following severe diffuse axonal brain injury in rats. J Pharmacol Exp Ther 288:1311–1316

Hendrich KS, Kochanek PM, Williams DS, Schiding JK, Marion DW, Ho C (1999) Early perfusion after controlled cortical impact in rats: quantification by arterial spin-labeled MRI and the influence of spin-lattice relaxation time heterogeneity. Magn Reson Med 4:673–681

Iadecola C, Forster C, Nogawa S, Clark HB, Ross ME (1999) Cyclooxygenase-2 immunoreactivity in the human brain following cerebral ischemia. Acta Neuropathol (Berl) 98:9–14

Kim HJ, Levasseur JE, Patterson JLJ, Jackson GF, Madge GE, Povlishock JT, Kontos HA (1989) Effect of indomethacin pretreatment on acute mortality in experimental brain injury. J Neurosurg 71:565–572

Koyfman L, Kaplanski J, Artru AA, Talmor D, Rubin M, Shapira Y (2000) Inhibition of cyclooxygenase 2 by nimesulide decreases prostaglandin E2 formation but does not alter brain edema or clinical recovery after closed head injury in rats. J Neurosurg Anesthesiol 12:44–50

Lasa M, Mahtani KR, Finch A, Brewer G, Saklatvala J, Clark AR (2000) Regulation of cyclooxygenase 2 mRNA stability by the mitogen-activated protein kinase p38 signaling cascade. Mol Cell Biol 20:4265–4274

Marmarou A, Foda MAA, Van den Brink W, Campbell J, Kita H, Demetriadou K (1994) A new model of diffuse brain injury in rats. I. Pathophysiology and biomechanics. J Neurosurg 80:291–300

McIntosh TK, Saatman KE, Raghupathi R, Graham DI, Smith DH, Lee VM, Trojanowski JQ (1998) The Dorothy Russell Memorial Lecture. The molecular and cellular sequelae of experimental traumatic brain injury: pathogenetic mechanisms. Neuropathol Appl Neurobiol 24:251–267

Nakayama M, Uchimura K, Zhu RL, Nagayama T, Rose ME, Stetler RA, Isakson PC, Chen J, Graham SH (1998) Cyclooxygenase-2 inhibition prevents delayed death of CA1 hippocampal neurons following global ischemia. Proc Natl Acad Sci USA 95:10954–10959

Parsadaniantz SM, Lebeau A, Duval P, Grimaldi B, Terlain B, Kerdelhue B (2000) Effects of the inhibition of cyclo-oxygenase 1 or 2 or 5-lipoxygenase on the activation of the hypothalamic-pituitary-adrenal axis induced by interleukin-1beta in the male rat. J Neuroendocrinol 12:766–773

Strauss KI, Barbe MF, Marshall RM, Raghupathi R, Mehta S, Narayan RK (2000) Prolonged cyclooxygenase-2 induction in neurons and glia following traumatic brain injury in the rat. J Neurotrauma 17:795–711

Tardieu D, Jaeg JP, Deloly A, Corpet DE, Cadet J, Petit CR (2000) The COX-2 inhibitor nimesulide suppresses superoxide and 8-hydroxy-deoxyguanosine formation, and stimulates apoptosis in mucosa during early colonic inflammation in rats. Carcinogenesis 21:973–976

Vane JR, Bakhle YS, Botting RM (1998) Cyclooxygenases 1 and 2. Annu Rev Toxicol 38:97–120

Wakita H, Tomimoto H, Akiguchi I, Lin JX, Miyamoto K, Oka N (1999) A cyclooxygenase-2 inhibitor attenuates white matter damage in chronic cerebral ischemia. Neuroreport 10:1461–1465

Wei E, Kontos H, Dietrich W, Dietrich W, Povlishock J, Ellis E (1981) Inhibition by free radical scavengers and by cyclooxygenase inhibitors of pial arteriolar abnormalities from concussive brain injury in cats. Circ Res 48:95–103

Willard LB, Hauss-Wegrzyniak B, Danysz W, Wenk GL (2000) Inhibition by free radical scavengers and by cyclooxygenase inhibitors of pial arteriolar abnormalities from concussive brain injury in cats. Circ Res 48:95–103

Willoughby DA, Moore AR, Colville-Nash PR (2000) COX-1, COX-2, and COX-3 and the future treatment of chronic inflammatory disease. Lancet 355:646–648

Yamagata K, Andreasson KI, Kaufmann WE, Barnes CA, Worley PF (1993) Expression of a mitogen-inducible cyclooxygenase in brain neurons: regulation by synaptic activity and glucocorticoids. Neuron 11:371–386