Original Article

Effect of indomethacin on motor activity and spinal cord free fatty acid content after experimental spinal cord injury in rabbits

R Pantović^{*1}, P Draganić², V Eraković³, B Blagović¹, Č Milin¹ and A Simonić²

¹Department of Chemistry and Biochemistry, Faculty of Medicine, University of Rijeka, Croatia; ²Department of Pharmacology, Faculty of Medicine, University of Rijeka, Croatia; ³PLIVA Research Institute Ltd, Zagreb, Croatia

Study design: Determination of functional and biochemical parameters as well as the effect of specific therapies on these parameters, in the experimental model of neurotrauma in rabbits.

Objective: To assess the effect of indomethacin (0.1–3.0 mg/kg for 9 days), a potent inhibitor of endogenous prostaglandin synthesis, on the motor activity and on the spinal cord tissue concentration of free palmitic, stearic, oleic, arachidonic and docosahexaenoic acids in an experimental model of a spinal cord injury in rabbits.

Setting: Faculty of Medicine, University of Rijeka, Croatia.

Methods: The animals were randomly divided into nine experimental groups, four sham and/or vehicle-treated and five indomethacin-treated (including one sham-operated and four injured groups). Laminectomy was followed by contusion of the spinal cord, using a modification of the technique of Albin. Motor activity was controlled daily during the course of the next nine postoperation days and scored using Tarlov's system. Spinal cord samples from the impact injury site were taken and frozen in liquid nitrogen. Total lipids were isolated and purified by a modification of the method of Folch. Free fatty acids (FFAs) were separated from the total lipid extract by preparative thin-layer chromatography, converted to the corresponding methyl esters and identified using gas chromatography, using nonadecanoic acid as the internal standard.

Results: The concentrations of all analysed free fatty acids were increased in the spinal cord after neurotrauma, in comparison to control tissues. Treatment of injured rabbits with indomethacin resulted in a significant decrease in spinal cord FFAs and exerted a positive effect on neurotrauma-induced motor impairment.

Conclusion: These results indicate a mechanism whereby indomethacin protects rabbits from the sequelae of neuronal damage caused by trauma, and suggests that it may be beneficial in the therapy of neurotrauma.

Sponsorship: This work was supported by the Croatian Ministry of Science and Technology (project 062019).

Spinal Cord (2005) 43, 519–526. doi:10.1038/sj.sc.3101763; published online 26 April 2005

Keywords: chromatography; free fatty acids; indomethacin; rabbit; spinal cord injury

Introduction

Spinal cord trauma is a serious disabling disease in the young and middle age population. Understanding the molecular mechanism of pathophysiology involved in nervous tissue damage opens up new research possibilities and may lead to the discovery of efficient therapies.

Major factors contributing to secondary tissue damage include release of neurochemicals, impairment of the microcirculation and its permeability and the formation of oedema.^{1,2} It seems likely that these

phenomena are interrelated in a complex way and that an early modification of any of these factors might influence the others.² With the exception of injuries that cause lacerations of the spinal cord, the primary tissue damage that occurs at the time of injury is minor when compared to secondary damage that develops over the next 48 h.³ The period after injury can be divided into three different therapeutic windows: biochemical and vascular events, starting at the time of injury and continuing for up to 48 h; the influx of inflammatory cells, starting within hours of injury and peaking 4 days after injury and axonal regeneration and lesion repair from 1 week after injury.³

*Correspondence: R Pantović, Department of Chemistry and Biochemistry, Faculty of Medicine, University of Rijeka, B Branchetta 20, HR-51000 Rijeka, Croatia

Release of free fatty acids (FFAs), due to the activation of membrane phospholipases (PL) and lipases,^{4–6} is one of the first pathophysiological events that follows primary trauma to the spinal cord, independently of the type of injury.^{7–10} FFAs are the components of membrane phospholipids, having an important role in maintaining the structure and functions of the cell membranes. Severe impact spinal cord injury results in decreased total phospholipid content for up to 3 days following injury,⁹ but no major class of phospholipids is selectively hydrolysed.^{7–9,11} Impact injury to the spinal cord is associated with a biphasic increase in FFA level in spinal cord tissue.⁹ The first increase, observed within 5 min of spinal cord trauma, is followed by a decline 30 min after the injury. The second increase, appearing 1 h after spinal cord trauma, is more persistent. It peaks 24 h post trauma and declines over the following 6 days.⁹

Free arachidonic acid (AA, 20:4), due to the specific phospholipid composition of neurons, was reported to be the primary fatty acid released as a result of injury-mediated tissue damage.¹² Phospholipase A₂ (PLA₂) and C are most likely to be responsible for liberation of FFAs from *sn*-1 and *sn*-2 positions, usually occupied by unsaturated fatty acids.^{7,9,13,14} Murphy *et al*¹² proposed the hypothesis that the release of FFAs, in particular AA, may not always involve general membrane degradation but rather an upregulation and/or overstimulation of PLA₂-linked membrane receptors.

FFAs, and AA in particular, may lead to secondary damage to spinal cord neurons by inducing oxidative stress or increasing intracellular calcium level.¹⁵ Additionally, AA plays a significant role in the cell trauma by being a precursor for prostaglandin and leukotriene synthesis.¹⁶ It has been reported that experimental spinal cord injury in rabbits induces significant production of leukotrienes that can further contribute to secondary central nervous system injury.¹⁷ Prostaglandins, on the other hand, may play an important role in the complex biochemical mechanisms occurring in the early period after spinal cord trauma leading to vascular permeability and oedema formation.¹⁸ Their increased presence in the spinal cord injury has been repeatedly reported.^{19–23}

Acute inflammation following spinal cord injury results in secondary injury and pathological reorganisation of the nervous tissue architecture. Cyclo-oxygenases

(COX) are key enzymes in the conversion of AA into prostanoids, which mediate immunomodulation, mitogenesis, apoptosis, blood flow, secondary injury (lipid peroxidation) and inflammation. Increased numbers of COX-1-positive cells have been found in the perivascular space and at the lesion site after spinal cord injury. Maximal cellular COX-1 expression in the perivascular space was observed on day 3. The maximal response at the site of the lesion was detected on the 7th day. Increased activation of COX-1-positive cells was detected in both regions up to the end of the observation period (day 28).²⁴ Indomethacin, an anti-inflammatory drug, inhibits prostaglandin synthesis by the inactivation of both the inducible and the constitutive forms of COX.¹⁵

The aim of this study was to evaluate the effect of indomethacin on the motor activity and the level of spinal cord FFAs, especially free AA, in an experimental model of spinal cord impact injury in rabbits.

Materials and methods

Animals

The study was carried out on adult rabbits, weighing 2.5–3.5 kg. Animals were maintained on a 12 h light–dark cycle and allowed free access to food and water. All experiments were performed between 10:00 and 12:00 hours in a silent room at a temperature of 22–24°C. The Faculty ethical committee approved all animal use procedures.

The animals were randomly divided into nine experimental groups (*n* = 6): Control SHAM (rabbits that were laminectomised, but without spinal cord injury), Control INJURY (laminectomized rabbits with spinal cord injury), Control SHAM-VEH (rabbits that were laminectomy, but without spinal cord injury, vehicle-treated), Control INJURY-VEH (laminectomized rabbits with spinal cord injury, vehicle-treated) and five groups of rabbits that were treated with indomethacin, one sham-operated group treated with 3.0 mg/kg and four injured groups treated with 0.1–3.0 mg/kg indomethacin (Table 1).

In animals, medial dorsal lumbar laminectomy was performed under pentobarbital sodium anaesthesia (30 mg/kg intravenously (i.v.)). The vertebral column was exposed in the upper lumbar region and a one-

Table 1 Distribution of animals to experimental groups (*n* = 6 per group)

	Control SHAM	Control INJURY	Control SHAM-VEH	Control INJURY-VEH	Indomethacin treatment (mg/kg per day)				
					0.1	0.3	1.0	3.0	3.0 SHAM
Vehicle i.v. for 9 days			+	+					
Indomethacin i.v. for 9 days					+	+	+	+	+
Laminectomy only	+	+	+	+	+	+	+	+	+
Laminectomy + spinal cord injury		+		+	+	+	+	+	

segment laminectomy was performed at the level of the second lumbar vertebrae (L 2). In some animals, laminectomy was followed by contusion of the spinal cord, provoked by a strike of 150 gcm using a modification of the technique of Albin *et al.*²⁵ Namely, a tapered weight weighing 12.5 g was dropped from a height of 120 mm onto the exposed spinal cord, striking the latter with a force of 150 gcm. After controlled spinal cord injury, the wound was closed by suturing the muscle and the skin. Each traumatised animal received 500 000 IU of benzyl penicillin, was individually housed and its bladder emptied by manual compression daily.

After this operation, hind limb motor activity was controlled daily over the course of the next 9 postoperating days and scored in accordance with Tarlov's system. The measures were performed blindly. Hind limb function was graded using a five-point ordinal scale as follows: 1 = complete paralysis; 2 = minimal functional movement; 3 = movement of legs, does not support weight; 4 = hopping, partially impaired; 5 = normal motor function.

Rabbits were monitored for 9 postoperative days due to the fact that a spinal shock lasts for 5–7 days.²⁶ At the end of the experiment, on the 9th postoperation day, animals were killed, the spinal cord was traversed and removed from the spinal canal, and, as soon as possible frozen in liquid nitrogen and prepared at -84°C for the further analysis.

Drug treatment

Animals in Control groups SHAM and SHAM-VEH were drug-naïve. Other rabbits with and without spinal cord injury were assigned randomly to treatment as follows: Controls SHAM-VEH and INJURY-VEH received vehicle (3 ml/kg of ethanol and water mixture 1:10, v/v), whereas other treated rabbits were given indomethacin in four different doses (0.1, 0.3, 1.0 or 3.0 mg/kg per day of treatment) (Figure 1). Vehicle or indomethacin was administered i.v. immediately after operation and daily during the 8 postoperational days.

Free fatty acids

Total lipids were extracted from spinal cord tissue using a modification of the method of Folch.²⁷ The frozen samples were weighed and homogenised in 10 ml of a

chloroform/methanol (2:1, v/v) mixture. Nonadecanoic acid (C19:0) was added as an internal standard to all samples and the antioxidant butylated hydroxy toluene (BHT) was added at 50 mg/l in all solvents. Homogenates were left overnight in the cold room at 4°C . After filtration, 2 ml of 0.034% MgCl_2 aqueous solution was added. The mixture was well mixed and left in the cold room at 4°C overnight. Water and organic phases were separated and the lower, organic phase was evaporated to dryness under N_2 gas. Total lipids were dissolved in 1 ml of the chloroform/methanol (2:1, v/v) mixture.

The FFAs were separated by preparative thin-layer chromatography using the following developing solvents: petroleum ether/ether/acetic acid (97:3:1, v/v/v) and petroleum ether/ether/acetic acid (80:20:1, v/v/v). Streaks on thin-layer plates were detected under a UV lamp and scraped off. FFA methyl esters were prepared by methylation with BF_3 -methanol (3.0 ml, 14% w/w) mixture (90 min, 100°C), water was added and the mixture was extracted with petroleum ether and quantified by gas chromatography using an internal standard.

Fatty acid gas chromatography analysis was performed on the Perkin-Elmer, Model 8410 gas chromatograph with an FID detector using a metal column (diameter 1/2 in, length 3 m) packed with 10% FFAP on Chromosorb W. H P. 80–100 mesh.

Statistical analysis

Values are expressed as means $\pm$ SEM of six rabbits. Analyses of variance (ANOVA) were performed at the $P \leq 0.05$ level to compare results between different treatments. The *post hoc* Tukey HSD test was used.

Results

Effect of spinal cord injury

Untreated, sham-operated rabbits (Control SHAM; laminectomy, without spinal cord injury) and the similar group of animals treated with vehicle (Control SHAM-VEH) exhibited mild and temporary disturbances in movement of their hind legs on the first and second postoperative days. These symptoms completely disappeared on the 3rd postoperative day (Figure 2). Spinal cord trauma of 150 gcm produced motor paralysis. As a result, a statistically significant difference during the whole testing period was observed between untreated, sham-operated rabbits (Control SHAM) and untreated rabbits with spinal cord trauma (Control INJURY). Similarly, Control SHAM-VEH (vehicle-treated laminectomised rabbits) differed significantly in motor activity from vehicle-treated rabbits with spinal cord injury (Control INJURY-VEH) during the whole testing period.

Figure 3 shows the effect of spinal cord injury on the level of FFAs in spinal cord tissue in untreated rabbits. In comparison to sham-operated animals (Control SHAM), spinal cord injury (Control INJURY) was

Figure 1 Schematic representation of the experimental protocol for the injured rabbits

Figure 2 Motor activity (Tarlov's units) in animals of four control groups: Control SHAM, Control INJURY, Control SHAM-VEH and Control INJURY-VEH during 9 post-operative days. * $P < 0.05$: Significantly different Control SHAM

Figure 3 The level of spinal cord tissue free palmitic (C16:0), stearic (C18:0), oleic (C18:1), arachidonic (C20:4) and docosahexaenoic (C22:6) acids (mg/g tissue) in laminectomized rabbits without (Control SHAM) and with spinal cord injury (Control INJURY) 9 days postsurgery. Each column represents mean $\pm$ SEM; $n = 6$ for each group. * $P < 0.05$: Significantly different from Control SHAM

associated with a statistically significant increase in free palmitic (16:0), stearic (18:0), oleic (18:0), arachidonic (20:4 $n = 6$) and docosahexaenoic acids (22:6 $n = 3$).

In sham-operated animals, a slight, but in the case of free palmitic, stearic and arachidonic acids, significantly higher concentration was seen in spinal cord tissue from the vehicle-treated Control SHAM-VEH group than in the merely laminectomized Control SHAM group (Figure 4). A similar moderate, but significant effect of vehicle administration causing an increase in palmitic,

stearic, oleic, and a significant decrease in docosahexaenoic acids was also observed in spinal cord-injured animals (Control INJURY-VEH) in comparison to the non-vehicle-treated, spinal cord-injured (Control INJURY) group (Figure 6).

Effect of indomethacin

Indomethacin (3.0 mg/kg per day) treatment did not influence the motor activity of sham-operated rabbits (data not shown), whereas it caused a significant increase in free palmitic ($F(1,8) = 5.56$; $P < 0.05$) and arachidonic acid ($F(1,8) = 10.56$; $P < 0.05$) in the same group of animals (Figure 4).

Figure 5 presents motor activity of vehicle- and indomethacin-treated rabbits with spinal cord injury. ANOVA revealed a significant beneficial effect of indomethacin treatment ($F(4,25) = 10.1$; $P \leq 0.05$). *Post hoc* analysis showed that motor activity was improved in all groups receiving indomethacin in comparison to the relevant vehicle-treated group (Control INJURY-VEH) in a dose-related manner.

Administration of indomethacin also significantly altered the spinal cord tissue levels of free palmitic (16:0) ($F(1,25) = 338.05$; $P \leq 0.05$), stearic (18:0) ($F(1,25) = 159.38$; $P \leq 0.05$), oleic (18:1) ($F(1,25) = 103.54$; $P < 0.05$), arachidonic (20:4) ($F(1,25) = 49.46$; $P < 0.05$) and docosahexaenoic acid (22:6) ($F(1,25) = 61.92$; $P < 0.05$). Lower FFAs levels were detected in all indomethacin-treated groups in comparison to the vehicle-treated Control INJURY-VEH group, and also in comparison to the Control INJURY group (Figure 6).

Discussion

In this study, as expected, spinal cord injury resulted in motor disturbances and accumulation of FFAs at the site of injury. The data correlate well with earlier published reports.^{7-9,12,28-30} Loss or decrease of motor activity is the most prominent and the most functionally relevant result of the spinal cord injury.³¹ Motor disturbances observed in the rabbits that experienced trauma in response to the force of 150 g cm were not the result of the laminectomy procedure. The symptoms of the injured animals differed significantly from the mild and temporary symptoms observed in the group that was only laminectomized.

Following impact trauma, the spinal cord undergoes a progressive series of autodestructive pathological changes.^{7,8,32} A significant loss of motor function occurs in severe cases as a result of haemorrhagic necrosis of central grey matter that begins within minutes of the injury. The degeneration of long tracts of the white matter is evident within 24–36 h. A very likely site of post-traumatic molecular damage is the cell membrane that undergoes marked alterations of integrity and function.^{7,8} Demediuk *et al.*⁹ who followed the 7 day postinjury period, discovered that traumatic spinal cord injury results in early, transient, postinjury membrane

Figure 4 The spinal cord tissue level of free palmitic (C16:0), stearic (C18:0), oleic (C18:1), arachidonic (C20:4) and docosahexaenoic (C22:6) acids (mg/g tissue) in the sham-operated groups without (Control SHAM) and following treatment with vehicle (Control SHAM-VEH) or indomethacin (dose 3.0 mg/kg body weight) 9 days after laminectomy. Each column represents mean $\pm$ SEM; $n = 6$ for each group. * $P < 0.05$: Significantly different from Control SHAM. ** $P < 0.05$: Significantly different from Control SHAM-VEH

Figure 5 Motor activity (Tarlov's units) of traumatised animals treated with vehicle (Control INJURY-VEH) or different doses of indomethacin during the 9 postoperative days. * $P < 0.05$: Significantly different from Control INJURY-VEH

phospholipid hydrolysis, the magnitude of which is relatively independent of the severity of injury. In the second wave, more delayed and sustained lipid hydrolysis occurs, the magnitude of which is related to the severity of injury.⁹ The changes in lipid metabolism may play an important role in the delayed secondary tissue damage.⁹

Murphy *et al*¹² reported a significant elevation of free AA during the early postinjury period, reaching a

20-fold increase after 24 h. Under our experimental conditions, samples were taken 9 days after injury and we still observed a six-fold increase in free AA.¹⁴ This is in line with the gradual spontaneous wound-healing process that follows spinal cord injury and has been reported by other authors.^{9,30}

Vehicle administration did not influence motor activity. However, it increased levels of FFAs in the examined segment of the spinal cord, both in the control, laminectomised animals, and in the animals with spinal cord injury. An explanation could be found in its ethanol content. Both acute and chronic administration of ethanol induces functional changes in neuronal membranes.³² Chronic ethanol exposure is known to affect deacylation–reacylation of membrane phospholipids. It leads to a progressive increase in membrane PLA₂ activity and changes in the fatty acid composition of membrane phospholipids.³² Our findings are in an agreement with the work of Halt *et al*³³ who found higher levels of FFAs after spinal cord injury associated with acute alcohol intoxication.

Indomethacin, a nonsteroidal antiinflammatory drug, has several known pharmacological actions. Being an inhibitor of COX, it inhibits formation of prostaglandins and thromboxanes.³⁴ Several reports have been published on indomethacin as a PLA₂ inhibitor in human neutrophils,^{34,35} but the expected decrease in AA release has never been observed in humans.³⁴ Recent reports have shown that indomethacin inhibits proteinases, in particular cathepsin B and calpain, and attenuates degradation of basic myelin and neurofilament proteins in the spinal cord.³⁶ Additionally, indomethacin reduces cerebral blood flow by preventing the formation of cerebral vasodilating prostaglandins, inducing hyperventilation and vasoconstriction of cerebral vessels.³⁷

Figure 6 The effect of indomethacin (0.1, 0.3, 1.0 and 3.0 mg/kg body weight) on spinal cord tissue levels of free palmitic (C16:0), stearic (C18:0), oleic (C18:1), arachidonic (C20:4 n-6) and docosahexaenoic (C22:6 n-3) acids (mg/g tissue) 9 days after experimental spinal cord injury in rabbits, in comparison to injured animals either treated (Control INJURY-VEH) or not (Control INJURY) with vehicle. Each column represents mean $\pm$ SEM; $n = 6$ for each group. [#] $P < 0.05$: Significantly different from Control INJURY. * $P < 0.05$: Significantly different from control group (Control INJURY). ** $P < 0.05$: Significantly different from control group treated with vehicle (Control INJURY-VEH)

Indomethacin treatment of rabbits in our experiment, which were laminectomised but not traumatised, did not alter motor performance but caused an increase in the free FFA level. It has been reported that laminectomy by itself markedly perturbs lipid metabolism in the spinal cord^{8,38} and the detected 10-fold increase in free AA on day 9 in our model could be the final result of the described changes due to laminectomy and indomethacin-induced inhibition of AA COX metabolism.

According to Guven *et al.*³⁴ the administration of 3.0 mg/kg indomethacin immediately after spinal injury induced significant lipid peroxidation and led Guven *et al.*³⁴ to suggest the possibility that early post-traumatic indomethacin treatment may be harmful in spinal cord injury. However, when they examined the early post-injury period, the detected changes were rather small. Later, according to other authors, more prominent changes have not been reported.³⁹

In our experiment, indomethacin at all tested doses improved motor activity and attenuated FFA accumulation in the injured spinal cord. Improvement of motor activity was exhibited in a dose-dependent manner, the highest dose of 3.0 mg/kg having the most pronounced effect. The lowest dose of indomethacin used, 0.1 mg/kg, successfully decreased FFA levels to the normal range, suggesting a potential protective efficacy of even lower doses of indomethacin. Efficacy in our experiment does not overrule or contradict Guven's data since we monitored the final result of tissue necrosis and wound healing 9 days after spinal cord injury. This is first time up to our knowledge that somebody has demonstrated influence of indomethacin on FFA level and cell

membrane integrity in SCI model. Guth *et al.*³⁵ have discovered beneficial influence of LPS and indomethacin combination on Tarlov's score in SCI in cats. Indomethacin alone was not tested. Our results are in an agreement with published data reporting that indomethacin significantly improves neurological function and reduces the severity of histopathological changes in rat experimental cord injury model.⁴⁰ Winkler *et al.*⁴¹ have demonstrated a beneficial effect of indomethacin on trauma-induced spinal cord evoked potential changes. Pretreatment with indomethacin attenuated the spinal cord evoked potential changes immediately after trauma and resulted in a marked reduction in oedema formation, the blood-spinal cord barrier permeability and blood flow changes at 5h.³⁹ The anti-inflammatory drug eliminated spinal cord oedema in the experimental rat model of neoplastic spinal cord compression,^{22,23} as well as in the spinal cord injury rat model.^{18,41,42} It also inhibited prostaglandin E2 production.^{22,23} Additionally, indomethacin reduced dye extravasation in paraplegic rats harbouring an epidural neoplasm and in rats with spinal cord injury. Permeability in the control groups treated with indomethacin was not changed.^{2,43} Progressive necrosis might be initiated and maintained in spinal cord injury model by inflammatory mechanisms.⁴⁴ Numerous recent papers on heterotopic ossification after spinal cord injury have demonstrated a beneficial effect of indomethacin.⁴⁵⁻⁴⁷

In all cited publications, animals were pretreated with indomethacin, whereas in our experiment we have treated rabbits after SCI. Post-treatment could have greater relevance to clinical practice.

Conclusion

Treatment with specific anti-inflammatory agents modulates various components of the pathological process thereby selectively attenuating neurological damage and encouraging wound healing. Indomethacin, repeatedly given during the longer post-traumatic period, demonstrated a final neuroprotective ability to regulate the dynamic balance between the destructive and reparative process. Therefore, we suggest its usage in patients who suffer spinal cord injury.

Acknowledgements

We express our gratitude to Professor Michael Parnham for a critical reading of the paper and valuable suggestions. We also acknowledge Mrs Katka Georgiū and Mr Milorad Ljubačev for their excellent technical assistance.

References

- 1 Tator CH, Fehlings MG. Review of the secondary injury theory of acute spinal cord trauma with emphasis on vascular mechanisms. *J Neurosurg* 1991; **75**: 15–26.
- 2 Sharma HS, Olsson Y, Persson S, Nyberg F. Trauma-induced opening of the blood–spinal cord barrier is reduced by indomethacin, an inhibitor of prostaglandin biosynthesis. Experimental observations in the rat using [13 J]-sodium. Evans blue and lanthanum as tracers. *Restor Neurol Neurosci* 1995; **7**: 207–215.
- 3 Olby N. Current concepts in the management of acute spinal cord injury. *J Vet Intern Med* 1999; **13**: 399–407.
- 4 Farooqui A, Horrocks LA. Excitatory amino acid receptors, neural membrane phospholipid metabolism and neurological disorders. *Brain Res Rev* 1991; **16**: 171–191.
- 5 Farooqui AA, Yang HC, Rosenberger TA, Horrocks LA. Phospholipase A2 and its role in brain tissue. *J Neurochem* 1997; **69**: 889–891.
- 6 Toborek M et al. Nicotine attenuates arachidonic acid-induced overexpression of nitric oxide synthase in cultured spinal cord neurons. *Exp Neurol* 2000; **161**: 609–620.
- 7 Demediuk P, Saunders RD, Clendenon NR, Means ED, Anderson DK, Horrocks LA. Changes in lipid metabolism in traumatized spinal cord. *Prog Brain Res* 1985a; **63**: 211–226.
- 8 Demediuk P, Saunders RD, Anderson DK, Means ED, Horrocks LA. Membrane lipid changes in laminectomized and traumatized cat spinal cord. *Proc Natl Acad Sci USA* 1985b; **82**: 7071–7075.
- 9 Demediuk P, Daly MP, Faden AI. Changes in free fatty acids, phospholipids, and cholesterol following impact injury to the rat spinal cord. *J Neurosci Res* 1989; **23**: 95–106.
- 10 Anderson DK, Hall ED. Pathophysiology of spinal cord trauma. *Ann Emerg Med* 1993; **22**: 987–992.
- 11 Yoshijara Y, Watanabe Y. Translocation of phospholipase A2 from cytosol to membranes in rat brain induced by calcium ion. *Biochem Biophys Res Commun* 1990; **170**: 484–490.
- 12 Murphy EJ, Behrmann D, Bates CM, Horrocks LA. Lipid alterations following impact spinal cord injury in the rat. *Mol Chem Neuropathol* 1994; **23**: 13–26.
- 13 Župan G et al. Accumulation dynamics of the brain total free fatty acids and free arachidonic acid in the models of cerebral hypoxia and epilepsy in rats. *Croat Chem Acta* 1995; **68**: 485–490.
- 14 Pantović R, Draganić P, Blagović B, Eraković V, Simonić A, Milin C. Effect of indomethacin on the level of arachidonic acid in an experimental model of neurotrauma. *Period Biol* 2002; **104**: 95–97.
- 15 Toborek M, Malecki A, Garrido R, Mattson MP, Henning B, Young B. Arachidonic acid-induced oxidative injury to cultured spinal cord neurons. *J Neurochem* 1999; **73**: 684–692.
- 16 Bazan N, Rodriguez de Turco E, Allan G. Mediators of injury in neurotrauma. Intracellular signal transduction and gene expression. *J Neurotrauma* 1995; **12**: 791–814.
- 17 Moreland DB, Soloniuk DS, Feldman MJ. Leukotrienes in experimental spinal cord injury. *Surg Neurol* 1989; **31**: 277–280.
- 18 Olsson Y, Sharma HS, Pettersson A, Cervos-Navarro J. Release of endogenous neurochemicals may increase vascular permeability, induce edema and influence cell changes in trauma to the spinal cord. *Prog Brain Res* 1992; **91**: 197–203.
- 19 Hsu CY, Halaushka PV, Hogan EL, Banik NL, Lee WA, Perot Jr PL. Alteration of thromboxane and prostacyclin levels in experimental spinal cord injury. *Neurology* 1985; **35**: 1003–1009.
- 20 Hsu CY, Halaushka PV, Hogan EL, Cox RD. Increased thromboxane level in experimental spinal cord injury. *J Neurol Sci* 1986; **74**: 289–296.
- 21 Jonsson HT, Daniell HB. Altered levels of PGF in cat spinal cord tissue following traumatic injury. *Prostaglandins* 1976; **11**: 51–61.
- 22 Siegal T, Shohami E, Shapira Y, Siegal T. Indomethacin and dexamethasone treatment in experimental neoplastic spinal cord compression: Part 2. Effect on edema and prostaglandin synthesis. *Neurosurgery* 1988a; **22**: 334–339.
- 23 Siegal T, Siegal T, Shohami E, Shapira Y. Comparison of soluble dexamethasone sodium phosphate with free dexamethasone and indomethacin in treatment of experimental neoplastic spinal cord compression. *Spine* 1988b; **13**: 1171–1176.
- 24 Schwab JM, Brechtel K, Nguyen TD, Schluesener HJ. Persistent accumulation of cyclooxygenase-1 (COX-1) expressing microglia/macrophages and upregulation by endothelium following spinal cord injury. *J Neuroimmunol* 2000; **111**: 122–130.
- 25 Albin MS, White RH, Acosta-Rua G. Study of functional recovery produced by delayed localized cooling after spinal cord injury in primates. *J Neurosurg* 1968; **29**: 113–120.
- 26 Ducker TB, Kindt GW, Kemple LG. Pathological Findings in acute experimental spinal cord trauma. *J Neurosurg* 1971; **35**: 700–708.
- 27 Folch J, Lees M, Sloane-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 1957; **226**: 497–509.
- 28 Bingham WG, Goldman H, Friedman SJ. Blood flow in normal and injured monkey spinal cord. *J Neurosurg* 1975; **43**: 162–171.
- 29 Viser WM, Yashon D, Hunt W. Mechanisms of norepinephrine accumulation within sites of spinal cord injury. *J Neurosurg* 1974; **40**: 77–82.
- 30 Faden AI, Chan PH, Longar S. Alterations in lipid metabolism, Na⁺, K⁺-ATPase activity, and tissue water content of spinal cord following experimental traumatic injury. *J Neurochem* 1987; **48**: 1809–1816.

- 31 Van Hedeman LS, Shellenberger MK, Gordon JH. Studies in experimental spinal cord trauma. Part 1: Alterations in catecholamine levels. *J Neurosurg* 1974; **40**: 37–43.
- 32 Zheng ZH, Barkai AI, Hungund BL. Effects of ethanol on the incorporation of free fatty acids into cerebral membrane phospholipids. *Neurochem Int* 1996; **28**: 551–555.
- 33 Halt PS, Swanson RA, Faden AI. Alcohol exacerbates behavioral and neurochemical effects of rat spinal cord trauma. *Arch neurol* 1992; **49**: 1178–1184.
- 34 Guven MB, Cirak B, Yuceer N, Ozveren F. Is indomethacin harmful in spinal cord injury treatment? An experimental study. *Pediatr Neurosurg* 1999; **31**: 189–193.
- 35 Guth L, Zhang Z, DiProspero NA, Joubin K, Fitch MT. Spinal cord injury in the rat: treatment with bacterial lipopolysaccharide and indomethacin enhances cellular repair and locomotor function. *Exp Neurol* 1994; **126**: 76–87.
- 36 Banik NL, Matzelle D, Terry E, Gantt-Wilford G, Hogan EL. Inhibition of proteolysis by a cyclooxygenase inhibitor, indomethacin. *Neurochem Res* 2000; **25**: 1509–1515.
- 37 Slavik RS, Rhoney DH. Indomethacin: a review of its cerebral blood flow effects and potential use for controlling intracranial pressure in traumatic brain injury patients. *Neurol Res* 1999; **21**: 491–499.
- 38 Horrocks LA et al. The degradation of phospholipids, formation of metabolites of arachidonic, and demyelination following experimental spinal cord injury. *Cent Nerv Syst Trauma* 1985; **2**: 115–120.
- 39 Sharma HS, Winkler T. Assessment of spinal cord pathology following trauma using early changes in the spinal cord evoked potentials: a pharmacological and morphological study in the rat. *Muscle Nerve* 2002; **11**: 583–591.
- 40 Simpson RK, Baskin DS, Dudley AW, Bogue L, Rothenberg F. The influence of long-term nifedipine or indomethacin therapy on neurologic recovery from experimental spinal cord injury. *J Spinal Disord* 1991; **4**: 420–427.
- 41 Winkler T, Sharma HS, Stalberg E, Olsson Y. Indomethacin, an inhibitor of prostaglandin synthesis attenuates alteration in spinal cord evoked potentials and edema formation after trauma to the spinal cord: an experimental study in the rat. *Neuroscience* 1993; **52**: 1057–1067.
- 42 Sharma HS, Olsson Y, Cervos-Navarro J. Early perifocal cell changes and edema in traumatic injury of the spinal cord are reduced by indomethacin, an inhibitor of prostaglandin synthesis. Experimental study in the rat. *Acta Neuropathol* 1993; **85**: 145–153.
- 43 Siegal T, Siegal T, Lossos F. Experimental neoplastic spinal cord compression: effect of anti-inflammatory agents and glutamate receptor antagonists on vascular permeability. *Neurosurgery* 1990; **26**: 967–970.
- 44 Zhang Z, Krebs CJ, Guth L. Experimental analysis of progressive necrosis after spinal cord trauma in the rat: etiological role of the inflammatory response. *Exp Neurol* 1997; **143**: 141–152.
- 45 Banovac K, Williams JM, Patrick LD, Haniff YM. Prevention of heterotopic ossification after spinal cord injury with indomethacin. *Spinal Cord* 2001; **39**: 370–374.
- 46 Shehab D, Elgazzar AH, Collier BD. Heterotopic ossification. *J Nucl Med* 2002; **43**: 346–353.
- 47 Yin KS, James J, Lew K, Little JW. Refractory heterotopic ossification with complications. *J Spinal Cord Med* 2001; **24**: 119–122.