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Review Article

The Experimental Basis for Early Pharmacological Intervention in Spinal Cord Injury

R. K. Simpson, MD, PhD, C. Y. Hsu, MD, PhD, M. R. Dimitrijevic, MD, DSc

Departments of Neurosurgery and Division of Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston, TX 77030, USA

The major impetus of basic spinal cord injury (SCI) research is that therapeutic interventions can be devised to prevent or reduce disability. For years, there was doubt about the prospect that any effective treatment could ever be developed. This view is based primarily on the notion that once spinal cord trauma has occurred, the damage is done and outcome is determined at the moment of impact. The potential for any therapeutic intervention to reverse the injury process or to reduce the damage has been, understandably, doubtful. Numerous therapeutic interventions had claimed to be successful in animal models, yet none had been confirmed in patients with SCI. Recently, however, the Second National Spinal Cord Injury Study (NASCIS II) has altered such pessimistic viewpoints.[1] This study is a landmark achievement in pharmacological intervention of SCI. For the first time, a multicentre placebo-controlled, double-blind trial, using a well designed protocol and sound statistical analysis, shows that an acute traumatic insult to the spinal cord is treatable if an appropriate therapeutic agent is administered within a therapeutic window. NASCIS II was based on earlier elegant investigations in animal models that showed methylprednisolone, a glucocorticoid, to be effective in ameliorating vascular, metabolic, pathological, and functional consequences of traumatic SCI.[2–9]

Confirmation of the animal studies in a human trial using a rigorously designed protocol has significant laboratory and clinical implications. It establishes the precedent that the therapeutic success of SCI models in animals can be duplicated in patients with a similar disorder. It also diminishes the pessimism concerning the applicability of animal models to human diseases affecting the central nervous system, in particular, central nervous system (CNS) trauma.[10, 11] The success of NASCIS II also legitimises therapeutic trials in animal models of SCI. Research projects exclusively devoted to therapeutic trials in animal models are usually not considered to be meritorious by review panels from various funding agencies. The

Correspondence to: R. K. Simpson, Jr., MD, PhD, Department Neurosurgery, 6560 Fannin, Suite 944, Baylor College of Medicine, Houston, Texas 77030, USA. Telephone: (713) 798-4696. Fax: (713) 798-3739.

perspective toward such projects by these panels in light of NASCIS II, however, may now be modified.

Extensive pathological studies in animal models have shown that tissue necrosis after impact injury to the spinal cord follows certain patterned events. The ultimate tissue loss is not apparent until 8 to 24 hours after injury.[12, 13] Secondary phenomena, notably vascular, metabolic, and other physiological events, appear to contribute to the eventual cord damage.[14–16] The premise that these secondary phenomena occur hours after the initial insult suggest that a therapeutic window may exist before the ultimate cord damage becomes irreversible. NASCIS II highlights the achievement of research efforts directed toward characterisation of secondary injury and the potential utility of early intervention during the 1980's, and heralds further success in the 1990's. One can not help but be cautiously optimistic at the beginning of the Decade of Brain that an era has been entered whereby the clinical outcome of traumatic SCI can be improved with aggressive early pharmacological intervention.

The efficacy of methylprednisolone, as detailed in NASCIS II, was proven significant based on the rigorous statistical analysis employed. The clinical improvement observed by health care professionals and patients themselves, however, is not substantial or striking. Can new pharmacotherapies currently being developed be more efficacious than that which has been shown with methylprednisolone in NASCSI II? Recent development of new regimens in experimental SCI appears promising indeed. The pharmacological basis of methylprednisolone as treatment for acute SCI is that high doses of this drug inhibits lipid peroxidation.[1, 17] Lipid peroxidation triggered by excessive free radical formation probably is a key event leading to progressive tissue injury following the initial insult.[18] Although methylprednisolone is a glucocorticoid, the observed therapeutic effect is postulated to be unrelated to the corticosteroid receptor mechanism.[1, 9] If inhibition of lipid oxidation is the key underlying mechanisms leading toward improved clinical outcome, then more promising results are likely with recently introduced 21-aminosteroids.[19] These compounds are more potent than methylprednisolone in inhibiting lipid peroxidation, as shown in recent in vitro studies.[18]

Preparation for a multicentre, double-blind placebo-controlled trial of a potent 21-aminosteroid, tirilazed, is in the planning stage in the United States. This new project, NASCIS III, will compare tirilazed, virtually devoid of glucocorticoid, with methylprednisolone. The planned NASCIS III trial will provide crucial information concerning the relative significance of the conventional glucocorticoid action versus inhibition of lipid peroxidation in improving the clinical outcome of patients with SCI. If tirilazed is more effective than methylprednisolone, then the role of lipid peroxidation in traumatic myelopathy will be strengthened. Until NASCIS III is completed and has shown the anticipated superiority of tirilazed to methylprednisolone, one can not easily disregard the potential glucocorticoid effect of methylprednisolone in NASCIS II.[20] It may be true that methylprednisolone inhibits lipid peroxidation at high doses.[1] However, neither earlier animal studies,[3–9] nor NASCIS II[1] ruled out glucocorticoid action in the observed beneficial effect of methylprednisolone in SCI. One can argue the need for higher therapeutic levels of glucocorticoids for disorders affecting the central nervous system, as has been shown in certain neurological disorders such as multiple

sclerosis and transverse myelitis, before therapeutic benefits can be realised.[21]

Why does glucocorticoid action have a potentially significant beneficial role in acute SCI? Glucocorticoids are among the most potent anti-inflammatory agents ever discovered. Inflammation is a prominent feature after physical injury to tissues and organs.[22, 23] The inflammatory response can be arbitrarily divided into cellular and humoral components. Cellular reaction in acute inflammation consists of infiltration of polymorphonuclear leukocytes, deposition of platelets, and alteration of endothelial cell function. Numerous humoral features are synthesised in the locus of inflammation secondary to the interplay of leukocytes, platelets, and the endothelium.[24] The inflammatory mediators include metabolites of arachidonic acid (prostaglandins, thromboxane, leukotrienes, and hydroxyeicosatetraenoic acids), kinins, free radicals, interleukins, tissue necrosis factor, platelet-activating factor and others.[24–29] Proteolytic enzymes are also released.[26] The interaction of these cellular and humoral factors leads to increased vascular permeability, edema formation, and tissue destruction. To a large extent, tissue damage following inflammation is caused by proteolytic enzymes and free radicals.[26]

The mechanism of action of glucocorticoids in inflammation has not been fully characterised. Glucocorticoids, however, are known to suppress several key inflammatory processes. Glucocorticoids inhibit chemotaxis, phagocytosis, and release of proteolytic enzymes.[30] Glucocorticoids also reduce the production of key inflammatory mediators including arachidonic acid metabolites (prostaglandins, thromboxane, leukotrienes, and others).[25, 26, 28] Activation of arachidonic acid metabolism also contributes to free radical formation.[31] Glucocorticoids may reduce the production of these pro-inflammatory mediators by inhibiting phospholipase A2 activity.[32] Inhibition of phospholipase A2 by glucocorticoids is mediated by the induction of lipocortin synthesis.[33]

The cardinal features of acute inflammatory processes have been shown in experimental SCI in several animal models. Platelet deposition is seen early after impact injury to the spinal cord.[12, 34] Infiltration of acute inflammatory cells, notably polymorphonuclear leukocytes, into the site of injury has been shown in morphological and enzymatic studies.[12, 13, 35, 36] Alteration of endothelial cell function leading to increased vascular permeability and edema formation have been well documented.[37–39] Degradation of cytoskeleton including neurofilament by proteolytic enzymes was noted following impact injury.[40, 41] The progressive nature of endothelial injury leading to a step wise deterioration of spinal cord blood flow and delayed hypoperfusion constitutes a secondary injury process.[14–16] Another line of evidence supporting a secondary insult is the gradual increase in vascular permeability for hours after injury and the extension of the vascular changes beyond the segment of insult.[38–39] Accumulation of pro-inflammatory mediators including prostaglandins, thromboxane, and leukotrienes secondary to activation of arachidonic acid metabolism have been extensively documented in experimental SCI.[24, 26, 42, 43]

Post-traumatic activation of arachidonic acid metabolism may involve another inflammatory cascade, namely kininogen-kinin system. Recent studies in a rat SCI model indicate increased accumulation of kininogen and its conversion to pro-inflammatory kinins in the injured spinal cord.[44] In addition to their primary role as inflammatory mediators, kinins may also sustain the activation of phospholipases leading to a progressive accumulation of prostaglandins, thromboxane, and

leukotrienes.[31, 45] Kininogen has also been implicated in the secondary vascular changes in experimental traumatic head injury.[46]

The extensive documentation of an inflammatory reaction following traumatic injury to the spinal cord makes it difficult to disregard the potent anti-inflammatory action of glucocorticoids. The rationale for early therapeutic intervention is primarily based on the hypothesis that a secondary injury develops after the initial insult. The secondary injury processes are dominated by vascular changes which involve an inflammatory component. Future development of new therapeutic regimens should include strategies directed at limiting the post-traumatic inflammatory responses. Because of serious adverse effects, several potent anti-inflammatory regimens, including glucocorticoids, are not ideal for long term use in patients with chronic inflammatory disorders, such as rheumatoid arthritis. In traumatic SCI, the inflammatory reaction is short-lived. More aggressive treatment with potent anti-inflammatory agents, including glucocorticoids, is unlikely to cause serious adverse reaction as has been demonstrated in NASCIS II using high dose methylprednisolone.[1]

Highly relevant to anti-inflammatory strategies is the suppression of immune cells. The roles of immune cells in CNS injury other than autoimmune disorders have just begun to attract attention.[47] Immunosuppressive effects of glucocorticoids in SCI should be further explored. New therapeutic avenues for blocking receptors of inflammatory mediators, cytokines, inflammatory and immune cells and neutralising toxic free radicals are now possible. The Figure is a schematic illustration indicating that a number of possible approaches for pharmacological interventions can be directed at the variety of secondary events that occur after traumatic SCI.

Among the cascade of events that occurs after an acute injury to the spinal cord is the entry of calcium into cell bodies and axons. In fact, calcium influx occurs quite rapidly after CNS trauma.[48] Once concentrations of free intracellular calcium become elevated, neuronal cells can die.[49] The mechanisms for aberrant calcium flux can be due to the immediate consequences of mechanical disruption of the cell membrane, or secondary to the release of endogenous neurochemical substrates. These substrates can act upon specific membrane receptors that mediate calcium transport or flux. Once within the cell, excessive calcium levels can interfere with normal metabolic processes and membrane functions, leading to cell death.[49 50] These processes include, and are in fact central to, activation of many of the cascades outlined in the Figure. These cascades, once activated, can trigger the complex, multiple components of the inflammatory response. In turn, these biochemical cascades can lead to lipid peroxidation and diminished spinal cord blood flow and further aggravate vascular permeability, edema formation, and tissue damage.[51] Pharmacological interventions are now being developed to interrupt elements responsible for triggering or mediating these biochemical cascades.

Among the endogenous substrates receiving intensive study are the amino acid neurotransmitters, particularly the excitatory agents glutamate and aspartate. Several well conducted studies have identified specific membrane receptors that when activated by these transmitters cause calcium influx to occur.[52] These receptors have been classified based on the effects of neurochemical agonists and electrophysiologic studies, and include N-methyl-D-aspartate (NMDA), kainate,

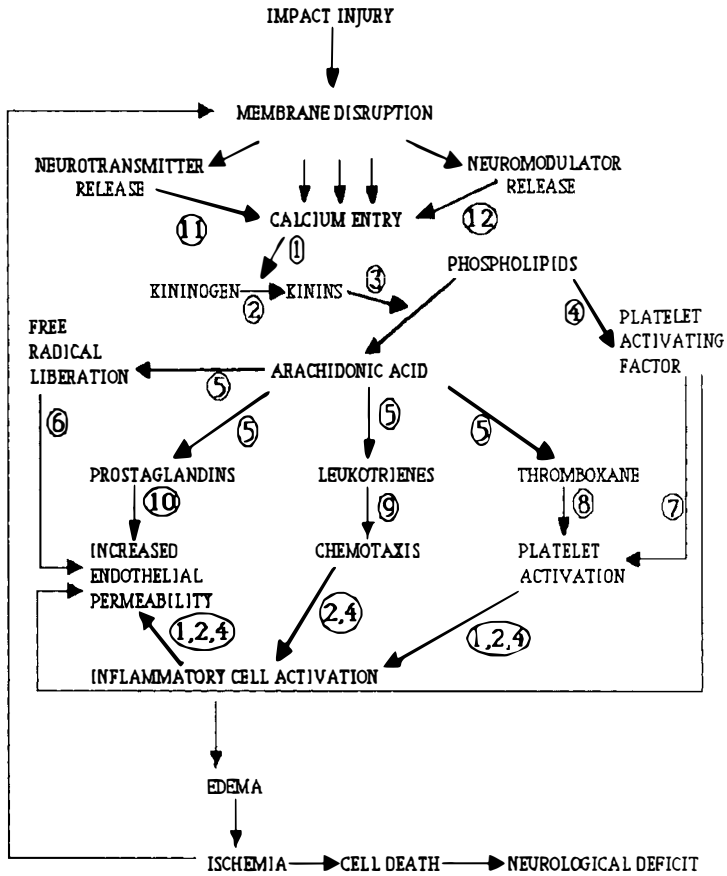

Figure A cascade of the biochemical events leading to delayed secondary injury after trauma to the central nervous system is illustrated. The points for potential pharmacological intervention are designated numerically. These include: 1=calcium channel blockers, 2=protease inhibitors, 3=kinin antagonists, 4=phospholipase and lipid peroxidation inhibitors, 5=cyclooxygenase and lipoxygenase inhibitors, 6=free radical scavengers, 7=platelet activating factor antagonists, 8=thromboxane synthetase inhibitors or blockers, 9=leukotriene antagonists, 10=prostaglandin synthesis inhibitors or blockers, 11=excitatory amino acid antagonists, and 12=opiate antagonists.

and quisqualate receptors.[53] Excitatory amino acids released into the extracellular space as a result of tissue injury can overstimulate these receptors, particularly the NMDA receptor, leading to a massive influx of calcium ions and subsequent delayed cell damage and death.[52] Several antagonists to these receptors have been developed with varying degrees of specificity and have been shown to exert a protective influence on neurons exposed to high levels of excitatory amino acid neurotransmitters. The recently developed non-competitive, selective NMDA receptor antagonist MK801 has been found to protect the spinal cord from delayed, secondary SCI in animals.[54] This compound has the additional advantage of readily crossing the blood brain barrier. Inhibitory amino acid neurotransmitters, such as glycine, have also been shown to contribute to delayed neuronal injury through NMDA receptor activation.[55] Protection of injured neural tissue from the effects of elevated concentrations of amino acid neurotransmitters, or 'excitotoxins' deserves further investigation.

Endogenous opiates have also been suggested to be part of the complex mechanism for delayed spinal cord damage. The NASCIS II study showed that blockade of opiate receptors with the non-specific opiate antagonist naloxone, at the dosage and regimen utilised, did not significantly alter neurological outcome.[1] These findings were in contrast to results reported from many animal investigations and the NASCIS I trial of naloxone administration in cases of human SCI.[56, 57] Several mechanisms have been proposed to explain beneficial effects of opiate antagonists including increasing spinal cord blood flow and blockade of calcium influx. Although the results from the NASCIS II trial, with regard to naloxone, are extremely valuable, specific opiate receptor blockade with other, more selective compounds may prove to be of significant clinical utility. Several unique opiate receptors have been biochemically defined within the spinal cord and include mu, sigma, epislon, delta and kappa.[58] Selective kappa receptor antagonists that block the paralysis inducing activity of the opiate agonist, dynorphin, can reduce delayed neurological deterioration in animals.[59] The influence of naloxone on the kappa receptor is considerably less (5–10%) than for other opiate receptor subtypes.[58] Furthermore, these protective influences may be the result of non-opiate receptor function mediating calcium flux.[59] Investigations directed toward the role of opiate peptides in delayed secondary SCI should not be abandoned since further study may elucidate the mechanisms of receptor mediated neuronal damage, if not becoming immediately applicable as a potential method of therapy.

Central to the deleterious effects of endogenous opioids and, in particular, the excitatory amino acids is the role of membrane receptor induced calcium influx. Over the past decade, several calcium channel blockers have been developed with a high degree of specificity.[51, 60, 61] It would seem reasonable to assume that if effective calcium channel blockade could be achieved, the deleterious events precipitated by increased free intracellular calcium might be limited or reduced. The results of several studies are encouraging, however, a clear consensus of opinion regarding their effect is lacking. This confusion is primarily due to the influence this class of drugs has on the cardiovascular system.

Several calcium channel antagonists have been studied to date and include verapamil, nifedipine, nimodipine, and diltiazem.[51, 60, 61] The method by which calcium channel antagonists can potentially reduce secondary SCI are, however, unclear. Augmentation of spinal cord blood flow after injury may be the primary mechanism of neuronal protection.[51] When blood pressure is carefully controlled, calcium channel blockers have been shown to protect the spinal cord from secondary injury, as determined by both electrophysiological studies and clinically measured functional recovery in animal models.[60, 62] However, several investigations have found no change in either of these parameters following administration of these drugs after SCI.[51, 61] Because these drugs can influence blood pressure, hence spinal cord perfusion, failure to adequately determine the beneficial effects from calcium channel blockers may be secondary to their vasodilatory properties and subsequent hypoperfusion.[63] Select pharmacological properties of these highly complex drugs need to be carefully defined and applied to injured neuronal tissue. In order to achieve this goal, additional laboratory investigations are essential. A specific antagonist to calcium channels of neuronal tissues would be highly desirable.

Strategies directed toward the successful medical management of acute SCI

should include prevention of initiation of the deleterious cascade of secondary events such as calcium influx, as well as limiting the sequela of these events once they are in progress such as ischemia and the inflammatory response. Several key steps in the cascade of events leading to delayed secondary damage have been identified and can be interrupted in order to reduce neurological injury. Our role is to develop the pharmacological agents by which these interruptions can be successfully implemented. Clearly, the NASCIS II trial was a major advance in the treatment of acute SCI and certainly justifies additional laboratory studies of prevention, interruption, or limitation of the variety of events that lead to secondary damage. Promising new agents such as excitatory amino acid inhibitors, novel optiate antagonists, and selective calcium channel blockers should be explored, and support should be given to provide the necessary environment for these investigations to be effectively carried out.

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