Neuroprotective Drugs in Traumatic CNS Injury

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Abstract: Despite extensive experimental research, the numbers of neuroprotective drugs that have proven efficacy following treatment of patients with traumatic CNS injuries still remain meager. It would be worthwhile to emphasize that majority of the victims are mostly in the second or third decades of their lives. A survey on the neuroprotective molecules that has been tested experimentally and subsequently tried clinically has been found somewhat beneficial. In the present review, we consolidated the updates on a number of such drugs, which hold promise for therapy of traumatic CNS injuries. Two such agents, endogenous molecules estrogen and melatonin have been under investigation in our laboratory for their efficacy in experimental spinal cord injury in rats.

Keywords: Neuroprotective agents, spinal cord injury, estrogen, melatonin.

INTRODUCTION

Traumatic Central Nervous System (CNS) injuries include a wide array of devastating and life threatening injuries. The CNS (the brain and the spinal cord) when stressed or damaged, can lead to the impairment of many organs and parts of the body and in many cases even lead to paralysis and death. Since, the structure-function relationships within CNS are so complex, any amount of damage or injury to a specific area can result in a loss of homeostasis and/or induce entropy within the system. Traumatic CNS injuries are largely heterogeneous, but may be broadly grouped as Traumatic Brain Injury (TBI), Spinal Cord Injury (SCI) and Stroke [1, 2]. The data in Table **1** surmises the financial burden associated with the annual incidence of such injuries worldwide.

Treatment following traumatic CNS injuries offers a major biomedical challenge. Attempts are made to address such trauma by surgical interventions, however, limited corrective surgeries can be done, and often the damage due to CNS trauma is impossible to reverse. Thus, the ultimate functional revival may largely depend on the pharmacological intervention or neuroprotective regimen provided. Several neuroprotectants have been tested in experimental research and some of them proceeded to clinical trials. Despite, enormous efforts there is paucity of neuroprotective molecules that benefit the survivors of a traumatic CNS injury. A major cause of such discrepancy lies in the heterogeneity and multi-factorial nature of such injuries. Consequently, it has been difficult for pharmaceutical companies to develop agents for pharmacological intervention. Mono-therapy or drugs with limited action fail to provide the adequate protection in CNS trauma. A long-standing focus in our laboratory has been to explore the therapeutic efficacy of drugs with multi-active properties or combination of drugs as therapy following SCI.

Present review is organized under three major headings: the subset of neuroprotective agents for CNS trauma, clinical trials on selective neuroprotectants for CNS trauma and the quest for neuroprotective agents following SCI in our laboratory with reference to others in the field.

NEUROPROTECTIVE AGENTS FOR CNS TRAUMA

Neuroprotective drugs include an enormous array of potential moieties of diverse origins - biologically active natural products, plant extracts, endogenous peptides that are beneficial for preservation of the structural-functional integrity of the CNS. They may be anti-oxidative, anti-nitrasative, anti-inflammatory, immunosuppressive, ion channel modulators, angiogenesis-promoters, neurotrophic agents or even a combination of many such homeostasis promoting agents; however, only a selective subset of them can benefit CNS trauma. There have been organized attempts to harness the benefits of such neuroprotective agents, presented in a series of scientific conference, whose proceedings have been published in select volumes of Annals of New York Academy of Sciences [3-9]. Table **2** depicts the focus of these proceedings and converges on the selective list of neuroprotective agents deemed useful for CNS trauma.

CLINICAL TRIALS ON NEUROPROTECTIVE AGENTS FOR CNS TRAUMA

A major advancement occurs in any drug-discovery as an experimental drug enters the phases of clinical trial. Completion of these phases is a pivotal landmark for any

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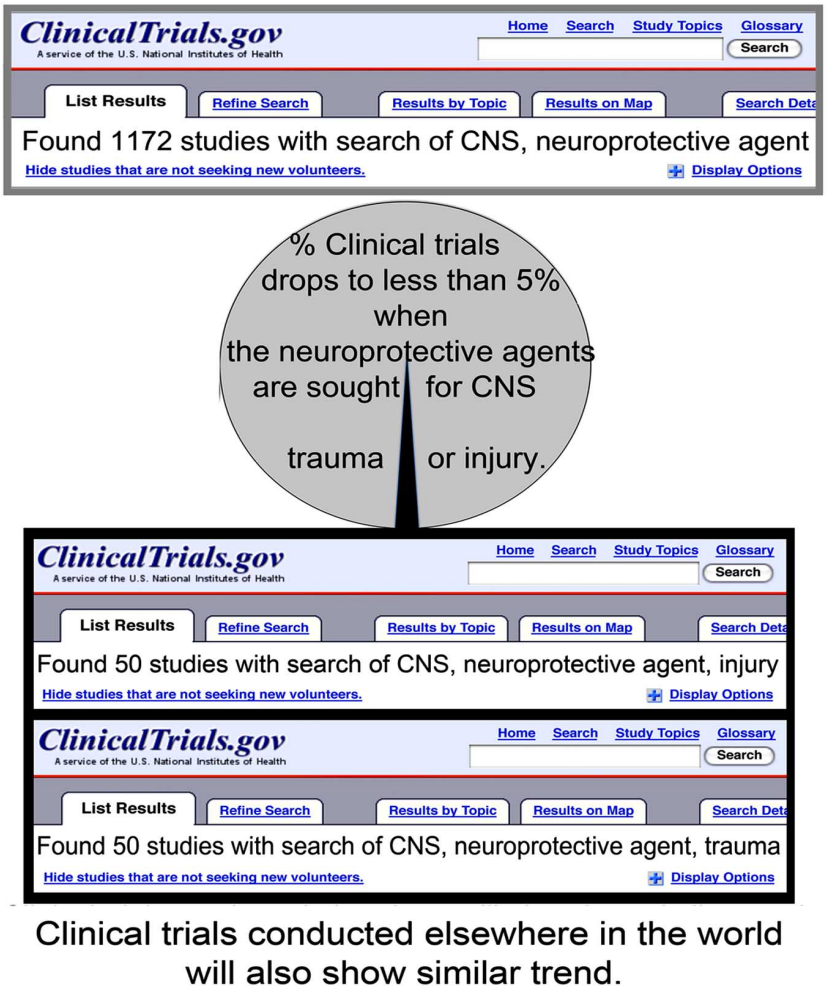

Fig. (1). A schematic representations of the organized trials to consolidate the neuroprotective agents is presented, outlining the selective neuroprotective agents for CNS trauma.

neuroprotective agent. A current search for neuroprotective agents under clinical trials in the website of National Institute of Health (NIH) retrieved 838 queries which reduced to just 41 (≈ 5%) when further refined with CNS injury/trauma criterion. Thus, this finding substantiates the fact that not all neuroprotective agents can benefit CNS trauma due to the multi-factorial nature of CNS injury. The sketch in Fig. (**1**) represents the fact that only a marginal fraction of the neuroprotective agents are useful in CNS injury. Furthermore, an itemized presentation of these selective drugs that may render neuroprotection following CNS trauma as per clinical trials under NIH is presented (Table **3**). Worldwide there may be several more of completed/ongoing clinical trials for neuroprotective agents that might render functionality in post CNS trauma, but, certainly, the statistics on the low availability of selective neuroprotective agents for CNS injury corroborates the pressing need for greater experimental and preclinical research, and further clinical trials.

QUEST FOR NEUROPROTECTIVE AGENTS FOR TRAUMATIC SCI

Earlier attempts in our laboratory were focused on the calpain and its inhibitors as therapeutic strategy which has been extensively reviewed [10]. Currently, two endogenous molecules – estrogen and melatonin are being investigated. The review recapitulates the research on their efficacy on experimental spinal cord injury.

ESTROGEN AS A POTENTIAL NEUROPROTECTIVE AGENT FOLLOWING SCI

Neuroprotective efficacy of estrogen is based on diverse biological effects produced by estrogenic steroids [11]. Classically, estrogen signals through a nuclear receptor, which targets transcription of mRNA and cognate protein expression [12]. Estrogen can also act *via* activation of its receptors ER-α and ER-ß and downstream intracellular signaling through kinases [13]. It can act as an anti-inflammatory agent [14] and promote growth of micro vessels [15]. Estrogen can act directly at neurotransmitter receptor complexes or at ion channels resulting in altered neuronal current conductance or trans-cellular ion flux [16]. Thus, the hormone initiates generalized signaling pathways to the nuclear and membrane-localized effectors with ample crosstalk between nuclear activation and membrane-associated events. Estrogen also has important non-cell type-specific actions, such as anti-oxidation and conservation of endogenous free radical scavenging agents [17]. Overall, diverse mechanisms of estrogen mediated neuroprotection includes genomic, receptor-dependent transcriptional

Table 1. Burden of Traumatic CNS Injury on the Society World-Wide

Annual Incidence	Estimated Cost that Burdens the Society	Resources
In US	TBI $56 billion approximately. Stroke over $40 billion. SCI $10 billion approximately.	**NINDS** (National Institute of Neurological Disorder and Stroke) **The Dana Foundation**
In developed countries Western Europe (UK) Australia	 $500 million approximately. $1 billion approximately.	**ICCP** (International Campaign for Cures of Spinal Cord Injury Paralysis)
In developing countries Reports reflecting the exact scenario are not available in most of the cases		

regulation, and non-genomic rapid effects which may or may not be receptor mediated but involves regulation by kinases as well as other effects such as anti-inflammatory, anti-oxidative, and anti-apoptotic, making it a versatile neuroprotectant. Estrogen certainly is a potential candidate with multimodal efficacy against neurotrauma including SCI. Furthermore, it is important to note that both types of estrogen receptors (α and β) are distributed widely in the body in both genders. Estrogen receptor-α predominates in the uterus and mammary gland, whereas estrogen receptor-β has significant roles in the central nervous, cardiovascular, immune systems and several others [18]. Such wide-scale distribution of the estrogen receptors infers greater access of low dose of estrogen to the receptors and underscores the possibility of beneficial effects of estrogen.

ESTROGEN EFFICACY IN EXPERIMENTAL SCI

Applying the *in vitro* findings on estrogen efficacy in rescuing different neural cells from diverse injury and/or stress [19-21], to experimental SCI in rats, we observed protection of the injured cord in acute phase [22, 23]. Supraphysiological levels of estrogen attenuated inflammation, reduced or restricted the lesion volume, prevented axonal degeneration, and preserved myelin in acute experimental SCI. Moreover, the profound proteolytic events of the Ca^{2+}-activated proteinase calpain were reduced which prevented the apoptosis of neurons largely present in caudal penumbra by estrogen treatment when administered immediately after SCI in rats [22, 23]. In addition, similar high dose estrogen could mitigate the damage and restore functionality in chronic SCI in rats [24]. Such neuro-protective studies led to further investigations on the clinical relevance on the efficacy of lower physiological doses of estrogen, applied immediately and at different times in the acute SCI paradigm. Subsequently, results from these studies helped to explore low dose estrogen efficacy in chronic SCI in rats. Indeed, estrogen mediated neuroprotection in SCI in rats was attained at much lower doses in subsequent studies in our laboratory.

Other protective effects of estrogen administration following SCI thus far include prevention of astrogliosis and microgliosis, reduction of proteolytic and apoptotic markers, and preservation of the axon-myelin structural unit. The estrogen-mediated attenuation of all these parameters is essential and important for recovery of neurological function following SCI. Functional recovery may be enhanced further by promoting microvessel growth and restoring blood supply, needed for cell survival as cells may die due to ischemia caused by disruption of blood vessels following the primary injury to SC. Since, estrogen is known to promote angiogenesis and microvessel growth; its administration may help protect cells from ischemic damage following SCI. One of our goals is also to explore the angiogenic mechanism in both acute and chronic experimental SCI.

A few contemporary studies from other laboratories have also suggested the estrogen efficacy in SCI. An increase in expression of the estrogen receptors ER-α and ER-β mRNA in lumbar SC motoneurons is reported after axomotomy following sciatic nerve crush injury in bilaterally ovariectomized mice where exogenously supplied estrogen capsules (24 μg) rendered a sustained supraphysiological level of serum estrogen for the first three weeks [25]. Estrogen treatment induced gene expression resulting in acceleration of the growth and maturation of the axons. Furthermore, estrogen receptors were transported from the perikaryon into regenerating neurites, where they promoted local regeneration through the non-genomic ERK-activated signaling pathway [25]. Such protective effects of estrogen on motoneurons reflected well in experimental SCI. Pretreatment with estrogen (3-300 μg) improved functional recovery in the injured rat, in part, by reducing apoptotic cell death [26]. Furthermore, in a post-treatment paradigm in the same study, male rats were given a single injection of estrogen (100 μg/kg) immediately post-injury, which also showed significant recovery in locomotor activity coupled with decreased morphologic outcome. Subsequently adopting a 1 h post-SCI treatment regimen, the same group confirmed the steroid's neuroprotective mechanism being partly mediated by induction of Bcl-2 through PI3K/Akt-dependent CREB activation [27]. Estrogen also reduced the severity of autonomic dysfunction in SCI in male mice with administration of physiological dosage of estrogen in mice, wherein involvement of non-central/non-spinal mechanisms has been suggested [28]. Protection by estrogen was further confirmed in SCI induced by complete crush injury. Estrogen effects in such severe SCI were assessed by comparing non-ovariectomized, ovariectomized control, and ovariectomized with low physiological level estrogen supplementation (corresponding to 20 pg/ml in blood) in premenopausal and postmenopausal female rats. The study reported improved BBB scores, white matter sparing, and lower motor neuron survival by 21-day post injury [29]. Besides, another group reported that pretreatment with estrogen reduced the development of inflammation, tissue injury, neutrophil infiltration, expression of iNOS, COX-2 activity and several apoptotic markers associated

Table 2. International Conference on Neuroprotective Agents (ICNA)

ICNA (1st through 10th, 1991-2010)	Neuroprotective Agents for CNS Trauma	References
1st ICNA In Rockland, Maine, USA, 1991	Organized publication not available	
2nd ICNA In Lake George, NY, USA, 1994 **FOCUS:** Clinical potential for the use of neuroprotective agents	NMDA antagonists, Growth factors (IGF-1, TGF-b), Calcitonin gene-related peptide, Glutamate antagonists	ANNYSc, Vol 765, (1995)
3rd ICNA In Lake Como, Italy, 1996 **FOCUS:** Clinical & Experimental aspects of neuroprotective agents	Endogenous moiety – Adenosine, Calpain inhibitors	ANNYSc, Vol 825, (1997)
4th ICNA In Annapolis, MD, USA, 1998 **FOCUS:** US FDA Strategies for neuroprotective agents	Hypothermia CP-101, 606, Magnesium + Mexiletrine, Calpeptin + Methylprednisolone, Glutamate receptor agents, Benzamide, Free radical scavengers, Neuregulin	ANNYSc, Vol 890, (1999)
5th ICNA In Lake Tahoe, CA, USA, 2000 **FOCUS:** Combinational and time-course application of multiple therapy; complexity of clinical neuroprotection.	Adenosine receptors, Calpain inhibitor (E-64), Nicotinamide	ANNYSc, Vol 939, (2001)
6th ICNA In Hilton Head, SC, USA, 2002 **FOCUS:** Neuroprotection Trek – The next generation or neuromodulation techniques by DBS, VNS, TMS	Topiramate, Estrogen, Melatonin	ANNYSc, Vol 993, (2003)
7th ICNA In Pacific Grove, CA, USA, 2004 **FOCUS:** Developmental stage susceptibility of neurotoxicity; combination therapy	NfkB modulators, Nitric oxide synthase inhibitors, Antiapoptotic agents	ANNYSc, Vol 1053, (2005)
8th ICNA In Mackinac Island, MI, USA, 2006 **FOCUS:** Pathophysiology of CNS assault, neurotropic factors and neuroprotection, nanotechniques and nanoprotection	Neurotrophins, Nanodelivery, Nanomedicines	ANNYSc, Vol 1122, (2007)
9th ICNA In MBL, Woods Hole, MA, USA, 2008 **FOCUS:** Neuroinjury, neuroprotection, neurorepair	Estrogen, Ramelteon, Statins	ANNYSc, Vol 1199, (2010)
10th ICNA Held in Sep 2010, Pacific Grove, CA, USA.		

with SC trauma [30] whereas we observed similar effects in a more preclinical post-treatment approach [22-24]. The estrogen receptor antagonist ICI 182,780 was used to confirm the estrogen-receptor involvement in neuroprotective action of estrogen following SCI [30]. A separate study, which adopted our previously reported estrogen dosing regimen (a higher 4 mg/kg and a lower 100 μg/kg), highlighted a transient neuroprotective window through which estrogen protected SC by stimulating early cytokines release and astroglial responses [31]. Investigators suggested that such stimulations might prevent the spread of lesion and retard inflammatory cells to migrate into the surrounding tissue during the critical first week following SCI. The study further reported improved locomotor-recovery over 4 weeks after injury and inferred them as probably the consequence of the transient hike in astroglial reactivity due to estrogen [31]. As opposed to all the affirmative reports on estrogen efficacy following SCI, a solitary report suggests that gender differences in SCI are not estrogen-dependent and hence estrogen may not provide a viable therapy following SCI [32]. On the contrary, estrogen-related gender differences on the survival of rats following traumatic brain injury has been reported [33]. However, multiple reports on estrogen efficacy in experimental SCI conducted in diverse animal models spanning from acute through chronic models with various dosage regimens and confirming different aspects of neuroprotection certainly are in favor of the multi-active estrogen as a therapeutic agent. The results further validate its use as a promising candidate for the treatment of SCI. A major challenge is the establishment of the minimal effective dose that is beneficial for the SCI individuals irrespective of their

Table 3. Neuroprotective Agents for CNS Trauma Undergoing Clinical Trials

Drug [Clinical Trial ID#]	Trial objective	Subjects	Progress
A. Neuroprotective Drugs in Brain Injuries			
Melatonin **# NCT00649961**	To find ideal dosage of melatonin to prevent brain injury.	Preterm babies (31 weeks of gestation to 7 days old).	Not yet recruiting.
Modafinil **# NCT00702637**	Prevention of daytime sleepiness, fatigue, cognitive improvement.	18-65 year olds.	Completed.
Modafinil **# NCT00233090**	Preventing post TBI fatigue.	18 yrs and up.	Ongoing.
Modafinil **# NCT00489892**	To test effects on working memory in patients of moderate to severe TBI.	Between the ages 18-45.	Currently recruiting.
Armodafinil **# NCT00893789**	To test efficacy against excessive sleepiness in mild to severe closed TBI patients.	18-65 year olds.	Currently recruiting.
Topiramate and phenytoin **# NCT00598923**	Prevention of acute seizures and development of epilepsy after TBI.	18 years and older.	Ongoing.
Dexanabinol **# NCT00129857**	Prevention of glutamate-induced damage, swelling and brain death.	18-65 years.	Completed.
Rivastigmine **# NCT00219245**	Cognitive deficits after a TBI.	18-50 years.	Completed.
Rivastigmine **# NCT00171795**	Efficacy and safety test on TBI and cognitive impairment.	18-65 yrs old.	Completed.
Xenon gas with hypothermia **# NCT00934700**	Effect on cerebal integrity, metabolism in infants with perinatal asphyxia encephalo-pathy.	Up to 12 hrs after birth.	Ongoing.
Xenon (gas) and hypothermia **# NCT00879892**	Interactive mechanism between neuroprotectant xenon and hypothermia.	18-80 yr olds (cardiac arrests and cerebral ischemia.	Ongoing.
Citicoline **# NCT00545662**	Effects on mild, moderate, severe TBI patients in a 90-day study.	18-70 year olds.	Ongoing.
Recombinant Erythropoietin **# NCT00808704**	Effects on infants with perinatal asphyxia-induced brain injury.	Up to 48 hrs after birth.	Completed.
Recombinant Erythropoietin **# NCT00910234**	Neuroprotective effects in preterm infants to avoid deleterious injuries like periventricular leukomalacia.	Up to 6 hrs after birth.	Ongoing.
Erythropoietin **# NCT00413946**	Prevention of perinatal injury to brain (retina), lung, gut and improvement in neurodevelopment if any by early administration.	Up to 3 hrs after birth.	Ongoing.
B. Neuroprotective Drugs in Spinal Cord Injuries			
Methylprednisolone and tirilazad **# NCT00004759**	Compare MP vs. tirilazad in 24 and 48 h infusions and neurological recovery in 24 vs. 48 h following an acute SCI.	14 years and older.	Completed.
Riluzole **# NCT00876889**	Effects on acute traumatic SCI.	18-70 years old.	Ongoing.
Erythropoietin and methylprednisolone **# NCT00561067**	To compare tolerance and efficacy after spinal shock.	18-65 years old.	Ongoing.
C. Neuroprotective Drugs in Stroke			
Deferoxamine **# NCT00777140**	Safety and tolerance after acute ischemic stroke, brain edema; pharmacokinetic study of bolus administration.	18-80 years old.	Ongoing.

genders. To this end, our studies essentially on clinically relevant post-injury treatment with estrogen, progressively lowering the dose from supraphysiological to physiological level indicates estrogen as a promising agent in the treatment of SCI.

MELATONIN AS A NEUROPROTECTIVE AGENT IN EXPERIMENTAL SCI

Melatonin is another such endogenous candidate with enormous neuroprotective potential. It is a versatile anti-oxidant, anti-nitrosative agent, immunomodulator, oncostatic, and overall a potent neuroprotectant. Melatonin is non-toxic,

safe and has been tested for long-term human usage at both physiological and pharmacological doses. A designated drug status for melatonin in clinics for treatment of CNS trauma is being tried. We investigated the role of melatonin as an intervening agent for ameliorating Ca^{2+}-mediated events, including activation of calpain in moderately severe experimental SCI [34]. Calpain, a Ca^{2+}-dependent neutral protease, is known to be a key player in the pathogenesis of SCI. In an acute SCI regimen, immunofluorescent labeling was used to identify calpain expression in neurons, glia, or macrophages. A combination of TUNEL and double immunofluorescent labeling was used to identify neuronal apoptosis in spinal cord. Furthermore, the effect of melatonin on axonal damage was assessed using an antibody, which was specific for dephosphorylated neurofilament protein. Treatment of SCI animals with melatonin attenuated calpain expression, inflammation, axonal damage, and neuronal death, indicating that melatonin was highly neuroprotective in this situation. Moreover, examination of levels of calpain and caspase-3 expression and activity indicated significant reductions in the proteolytic events in SCI animals after treatment with melatonin. Taken together, our studies strongly suggest that melatonin may be an effective neuroprotective agent for the treatment of SCI [34]. Melatonin efficacy has been confirmed by other researchers in diverse models of experimental traumatic CNS injury as reviewed recently [35].

CONCLUSION

Further experimental and preclinical research on endogenous, non-toxic molecules like estrogen or melatonin as potential neuroprotective agents may help restore functionality in individuals with traumatic CNS injury, as there are no proven neuroprotective agents thus far for them.

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