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Post-injury treatment with a new antioxidant compound H-290/51 attenuates spinal cord trauma-induced c-*fos* expression, motor dysfunction, edema formation, and cell injury in the rat

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Summary

The neuroprotective efficacy of post-injury treatment with the antioxidant compound H-290/51 (10, 30, and 60 minutes after trauma) on immediate early gene expression (c-*fos*), blood-spinal cord barrier (BSCB) permeability, edema formation, and motor dysfunction was examined in a rat model of spinal cord injury (SCI). SCI was produced by a longitudinal incision into the right dorsal horn of the T10–11 segment under Equithesin anesthesia. Focal SCI in control rats resulted in profound up-regulation of c-*fos* expression, BSCB dysfunction, edema formation, and cell damage in the adjacent T9 and T12 segments at 5 hours. Pronounced motor dysfunction was present at this time as assessed using the Tarlov scale and the inclined plane test. Treatment with H-290/51 (50 mg/kg, p.o.) 10 and 30 minutes after SCI (but not after 60 minutes) markedly attenuated c-*fos* expression and motor dysfunction. In these groups, BSCB permeability, edema formation, and cell injuries were mildly but significantly reduced. These observations suggest that (i) antioxidants are capable of attenuating cellular and molecular events following trauma, and (ii) have the capacity to induce neuroprotection and improve motor function if administered during the early phase of SCI, a novel finding.

Keywords: Spinal cord injury; gene expression; c-fos; blood-spinal cord barrier; edema; oxidative stress; antioxidants; H-290/51.

Introduction

Oxidative stress appears to play an important role in inflammatory damage to myelin sheaths and axons following spinal cord injury (SCI), multiple sclerosis, and in pathogenesis of several other neurodegenerative diseases, e.g., hypertension, stroke, Alzheimer's, and Parkinson's disease [3, 4, 6–9]. Micro-hemorrhages and extravasation of blood components into the central nervous system compartment is associated with oxidative stress and generation of free radicals causing cell injury [11]. In addition, alterations in the balance between cellular oxidants and antioxidants in chronic diseases are also responsible for neurodegenerative changes [6]. Altered expression of several antioxidant enzymes, such as superoxide dismutase, glutathione peroxidase, gamma-glutamylcysteine synthase, catalases, glutathione S-transferase, and quinone reductase in central nervous system injuries and in neurodegenerative diseases are in line with this hypothesis [6–10, 13–15].

Oxidative stress induces breakdown of the blood-spinal cord barrier (BSCB) and up-regulates heat shock protein (HSP 72) response [16, 25, 26]. Release of cytokines and immunoglobulins following oxidative and/or cellular stress contributes to cell injury or cell death through mechanisms involving apoptosis and/or necrosis [27, 31, 36]. Furthermore, oxidants are capable of enhancing expression and/or DNA binding of several immediate early genes (IEG) and transcription factors that are involved in inflammation and DNA damage including *fos*, *jun*, *myc*, *erg*-1, heat shock factor, and nuclear factor kappa-B [5–7, 10]. One of the IEG, *cellular*-fos (c-*fos*), is a primary response gene that can be detected within 20 to 90 minutes after neuronal excitation [3, 4, 6, 16, 23]. Prolonged expression

of c-*fos* precedes programmed cell death *in vitro* [23, 27, 34, 36]. Thus, it is likely that c-*fos* up-regulation following trauma may represent a neuronal marker for cell injury [23]. However, the detailed involvement of oxidative stress-induced IEG expression and cell death in SCI is unclear.

There are reasons to believe that antioxidants and lipid peroxidation inhibitors play important roles in attenuating spinal cord cell and tissue injury following SCI [12, 16, 18, 20–22, 24–26]. A significant reduction in BSCB permeability, edema formation, and cell damage in SCI in animals pretreated with a potent chain-breaking new antioxidant compound, H-290/51 (Astra-Zeneca, Mölndal, Sweden), further supports this hypothesis [24, 26]. However, it is unclear whether the compound is still effective in reducing trauma-induced IEG expression, motor dysfunction, and spinal cord pathology, if given at various time intervals after SCI.

The first few hours following SCI are crucial for outcome as the early events following the primary insult set the stage for later development of spinal cord cell and tissue injury leading to long-term deficit and disability [16–19, 26]. Suitable therapeutic intervention initiated within the first 3 hours in spinal cord injury victims improves functional recovery, whereas delayed pharmacological treatment beyond 3 hours is largely ineffective [16, 18]. Thus, further studies on the cellular and molecular mechanisms of early events following SCI are necessary to explore new therapeutic strategies to minimize later development of spinal cord cell and tissue injury.

The present study was undertaken to investigate the effects of a potent antioxidant compound H-290/51 [28, 30, 33] on *c-fos* expression, motor dysfunction, and cord pathology when given 10, 30, and 60 minutes after SCI in a rat model.

Materials and methods

Animals

Experiments were carried out on 60 male Sprague Dawley rats (200–250 g) housed at controlled room temperature of $21 \pm 1\,^{\circ}C$ with a 12-hour light, 12-hour dark schedule. Food and tap water were supplied *ad libitum* before the experiment.

Spinal cord injury

SCI was induced by making a longitudinal incision (about 5 mm) over the right dorsal horn of the T10–11 segments under Equithesin anesthesia (0.3 mL/100 g, i.p.). The deepest part of the lesion was mainly located around the Rexed's laminae VIII to X [18, 19, 26]. Experiments were approved by the Ethical Committee of Uppsala University, Uppsala, Sweden, and Banaras Hindu University, Varanasi, India.

H-290/51 treatment

H-290/51 (Astra-Zeneca, Mölndal, Sweden) was dissolved in water and administered 50 mg/kg, p.o. by gastric tube [20, 24–26] in separate groups of rats (n = 5) at 10, 30, or 60 minutes after SCI.

Functional paralysis

Functional paralysis of the hind limb was determined using a semiquantitative analysis during open field walking using the modified Tarlov scale: 0 = total paraplegia; 1 = no spontaneous movement but responds to pinch; 2 = spontaneous movement; 3 = able to support weight but unable to walk; 4 = walking with gross deficits; 5 = walking with mild deficits; 6 = normal walking [19, 26, 29].

Inclined plane test

Motor disturbances in the rat after SCI were determined using the inclined plane test. Each rat was trained on a plane using an angle in such a way that the rats could stay on it for 5 seconds without falling [19, 26].

Perfusion and fixation

Five hours after SCI, rats were perfused through the heart with 0.1 mol phosphate buffer (pH 7.0) followed by 4% buffered paraformaldehyde in 0.1 mol phosphate buffer. Perfusion pressure was maintained at 90 torr throughout the process [17–19].

c-fos immunohistochemistry

Immunohistochemistry for c-*fos* was performed on free-floating vibratome sections obtained from the T9 segment of the cord using monoclonal c-*fos* antiserum (Calbiochem, Boston, MA) according to the manufacturer's protocol [23].

BSCB permeability and edema formation

BSCB permeability was measured using Evans blue (0.3 ml/100 g) and $^{[131]}$Iodine (10 μCi/100 g) as described previously [26]. Spinal cord edema formation was examined by measurement of the spinal cord water content [16, 20, 24, 25].

Spinal cord pathology

Spinal cord pathology was examined by light and electron microscopy. Spinal cord tissue pieces were embedded in paraffin or Epon and examined by light microscopy. Epon-embedded tissue pieces were processed for transmission electron microscopy [16, 20]. The cell changes were graded from 1 (minimum) to 4 (maximum) and analyzed [17].

Statistical evaluation

The quantitative or semiquantitative data obtained were analyzed using ANOVA followed by Dunnet's test for multiple group comparison from one control group. A p-value less than 0.05 was considered significant.

Table 1. *Post-trauma treatment with H-290/51 on the SCI-induced motor dysfunction, c-fos expression, BSCB permeability, spinal cord edema formation, and cell injury in T9 segment in rats*

Parameters measured	n	Control	5 h SCI	H-290/51 treatment in 5 h SCI§		
				+10 min	+30 min	+60 min
Motor dysfunction						
Tarlov scale	5	6 ± 0	2 ± 1**	5 ± 1[a]	4 ± 1[aa]	2 ± 1[ns]
Capacity angle	5	60 ± 0	30 ± 2**	43 ± 4[a]	40 ± 6[aa]	32 ± 6[aa]
BSCB permeability						
Evans blue mg %	6	0.24 ± 0.04	1.65 ± 0.12**	0.68 ± 0.12[aa]	0.79 ± 0.12[aa]	1.58 ± 0.42[ns]
[131]Iodine %	5	0.35 ± 0.06	1.96 ± 0.14**	0.72 ± 0.08[aa]	0.91 ± 0.09[aa]	1.88 ± 0.48[ns]
Edema formation						
Cord width mm	6	3 ± 0.5	5 ± 0.5**	4 ± 0.5[a]	4 ± 1[aa]	5 ± 1[ns]
Water content %	5	66.12 ± 0.18	69.34 ± 0.23**	66.64 ± 0.18[a]	67.38 ± 0.12[a]	69.73 ± 0.28[ns]
Structural changes						
c-*fos* positive cells	6	nil	34 ± 8	8 ± 4#	12 ± 6#	28 ± 8[ns]
Neuronal damage	5	nil	4	2 ± 1#	3 ± 1#	4 ± 1[ns]
Glial cell injury	5	nil	4	1 ± 1#	3 ± 1#	3 ± 1[ns]
Myelin damage	5	nil	4	2 ± 1#	2 ± 1#	4 ± 1[ns]
Endothelial injury	6	nil	4	2 ± 1#	2 ± 2#	4 ± 1[ns]

Cord width was measured in formalin fixed spinal cord specimens before embedding in paraffin [16]. § H-290/51 (50 mg/kg, p.o.) was administered 10 min, 30 min or 60 min after SCI. The cell changes were graded from 1 (minimum) to 4 (maximum) and analyzed in blinded fashion [see 16].
Values are Mean ± SD of 5–6 rats in each group.
BSCB Blood-spinal cord barrier; *SCI* spinal cord injury; * = $p < 0.05$; ** $p < 0.01$ (compared from control); a = $p < 0.05$; aa = $p < 0.01$ (compared with 5-hour SCI), ANOVA followed by Dunnett's test from one control. # = $p < 0.05$, Chi-square test from 5-hour SCI group; ns = not significant (from 5-hour SCI group).

Results

Effect of H-290/51 on motor function

Animals subjected to SCI showed profound motor dysfunction at 5 hours. Administration of H-290/51 to rats either 10 or 30 minutes after SCI significantly improved hind-limb function using the Tarlov scale or the capacity angle derived from the inclined plane test (Table 1). However, when the compound was administered 60 minutes after SCI, no significant improvement in motor function was seen.

Effect of H-290/51 on c-fos immunohistochemistry

Untreated SCI rats exhibited marked up-regulation of c-*fos* expression in neurons of the injured as well as adjacent segments in the edematous region of the spinal cord. Administration of H-290/51 10 or 30 minutes after SCI significantly attenuated c-*fos* expression in the cord. This reduction in c-*fos* expression was most marked in the ventral gray matter on the contralateral side. In contrast, no apparent reduction in c-*fos* expression was seen in rats treated with H-290/51 60 minutes after injury (Fig. 1).

Effect of H-290/51 on BSCB permeability

SCI rats that received H-290/51 either 10 or 30 minutes after injury showed a significant reduction in Evans blue, radioiodine, or lanthanum extravasation across the BSCB. However, treatment received 60 minutes after SCI did not reduce BSCB breakdown to these tracers.

Effect of H-290/51 on spinal cord edema formation

Rats that received H-290/51 either 10 or 30 minutes after injury did not exhibit much swelling and/or increase in spinal cord water content. However, drug administration 60 minutes after SCI failed to reduce spinal cord water content or swelling.

Effect of H-290/51 on cell injury

Treatment of rats with H-290/51 either 10 or 30 min following SCI reduced the gross expansion of the cord

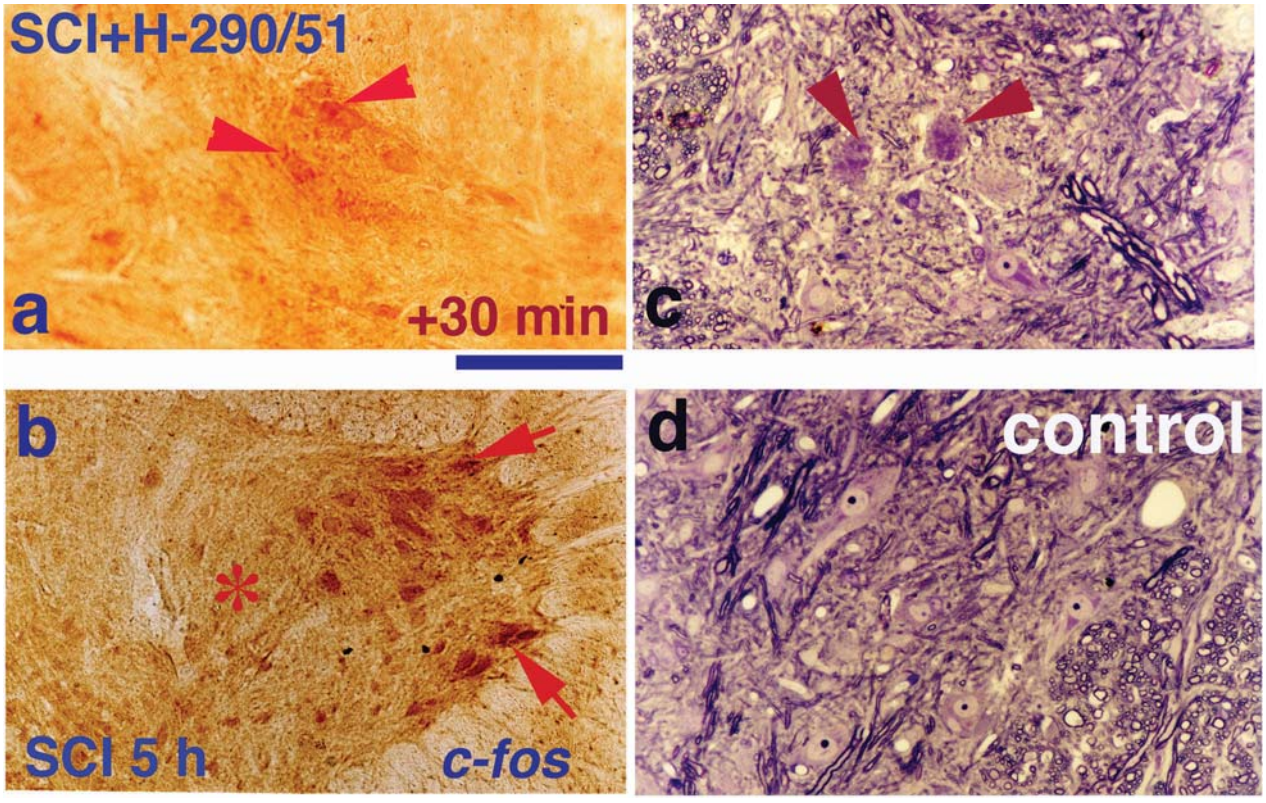

Fig. 1. c-*fos* expression (a,b) and cell changes (c,d) in the spinal cord ventral horn of the T9 segment 5 hours after SCI in control (b,d) H-290/51 treated rats (a,c). H-290/51 was given 30 minutes after SCI. Administration of H-290/51 was neuroprotective. Marked up-regulation of c-*fos* expression (arrows) was seen in the edematous area (*) after 5 hours SCI in control rats (b), but this was attenuated by H-290/51 (arrowheads) (a). H-290/51 also induced neuroprotection (c). Only a few damaged nerve cells can be seen (arrowheads) after drug treatment compared to no treatment (d)

edema, and micro-hemorrhages and damage to neuropil were considerably attenuated. A clear distinction between the gray and white matter was visible in these rats. Several nerve cells with distinct were present and swelling of neurons, astrocytes, and damage to myelin were much less evident in the treatment groups than in controls (Fig. 1, Table 1). At the ultrastructural level, signs of vacuolation, perivascular edema and myelin vesiculation were much less evident. On the other hand, H-290/51 failed to exert any neuroprotective effects on the spinal cord cell injury seen either at the light or electron microscopic level 60 minutes after SCI (results not shown).

Discussion

The salient new findings of the present study are that the chain-breaking antioxidant compound H-290/51, if administered within 30 minutes after SCI, attenuates motor dysfunction and spinal cord pathology at 5 hours. These observations suggest that oxidative stress during the early hours of SCI plays an important role in the secondary injury cascade that results in sensory motor dysfunction and spinal cord cell and tissue injury. However, when the antioxidant compound is administered 60 minutes after SCI, no significant neuroprotection or improvement in motor function was noted. This suggests that blockade of lipid peroxidation and/or generation of free radicals within the first hour after trauma is neuroprotective in SCI.

Previous reports from our laboratory indicate that treatment with H-290/51 requires 30 minutes to inhibit lipid peroxidation and, thus, block generation of free radicals [12, 20, 24]. The compound H290/51 in the present study has a short time to onset, reaching protective concentrations in the central nervous system within 30 minutes, with maximal effect lasting more than 6 hours [12]. Thus, administration of H-290/51 10 and 30 minutes after SCI is likely to inhibit further production of free radicals around 40 minutes to 1 hour after trauma. On the other hand, administration of the compound 1 hour after SCI exerts its influence

on free radical generation around 90 minutes after injury, suggesting that blockade of lipid peroxidation and/or generation of free radicals within 1 hour after SCI is beneficial in nature, whereas later blockade of free radical formation is ineffective. The detailed cellular and molecular mechanisms of such time-related neuroprotection with the antioxidant in SCI are not known and require additional investigation.

The antioxidant compound H-290/51 was able to attenuate IEG expression in the cord as seen using c-*fos* immunostaining. Since, the up-regulation of c-*fos* was mainly located within the edematous expansion of the spinal cord [23], a reduction in cell and tissue injury with H-290/51 is probably responsible for the diminished IEG expression in drug-treated spinal cord injured rats. These findings indicate that generation of free radicals and lipid peroxidation are important factors in the *c-fos* up-regulation, a novel finding. The fact that no reduction in c-*fos* expression was found in spinal cord injured animals that received H-290/51 treatment 60 minutes after injury is in line with this hypothesis.

Trauma to the spinal cord results in the release of numerous molecules, free radicals, vasoactive compounds, neurochemicals, growth factors, cytokines and other proteins/factors in a cascade of events leading to cell and tissue injury [16–19, 25, 26]. It is believed that several endogenous compounds/factors released after trauma may have the ability to induce neuroprotection, whereas numerous other endogenous factors/elements increased after injury are likely to have neurodestructive capabilities [16, 18]. Thus, a balance between endogenous neuroprotective and neurodestructive elements is crucial for cell injury and/or survival. It remains to be seen whether an interaction among different compounds/factors will synergistically potentiate or neutralize the neurodestructive and/or neuroprotective capabilities of certain elements *in vivo*. Thus, pharmacological blockade of release and/or synthesis of endogenous neurodestructive elements *before* the injury are likely to achieve neuroprotection [1–3, 7]. However, when the same compound is administered *after* the insult, the neuroprotective effect is either diminished or neutralized, as several other endogenous compounds/factors are likely to influence the outcome [32, 35]. Time-related neuroprotection induced by H-290/51 treatment *after* SCI in our present investigation is consistent with this hypothesis.

Our results suggest that generation of free radicals and oxidative stress plays an important role in SCI-induced cell and tissue injury. Recent evidence has shown that oxidative stress, generation of free radicals, and nitric oxide can up-regulate vascular endothelial growth factor (VEGF) expression in the microvascular endothelium [1, 2, 13, 14, 27, 32, 34–36]. An up-regulation of VEGF is associated with breakdown of microvascular permeability [32]. VEGF is a 45-kD glycoprotein secreted in the vascular wall by endothelial and smooth muscle cells [14, 32]. VEGF is a major regulator of angiogenesis and increased microvascular permeability [5, 7, 8]. Thus, VEGF up-regulation by oxidative stress and generation of free radicals could be one of the important factors in BSCB disruption in SCI. The BSCB breakdown following SCI in this investigation and its amelioration with H-290/51 is in line with this idea. However, further studies on expression of VEGF in SCI and its modification with H-290/51 are needed to confirm this hypothesis.

A reduction in BSCB permeability to macromolecules in the spinal cord microenvironment results in either quick resolution of edema or prevention of water accumulation in the spinal cord [21, 24]. Alternatively, in the absence of direct cell membrane damage, accumulation of water in the spinal cord extra- or intracellular compartments is less likely [24]. Obviously, a reduction in the BSCB permeability and edema formation by H-290/51 will induce neuroprotection [1, 2, 20].

Our observations show a close parallelism between improvement in motor function and spinal cord cell and tissue injury, indicating improvements in motor function are related to spinal cord pathology [19, 26]. Improvement in motor function by antioxidants suggests that cell and membrane damage by free radicals is likely to contribute to functional paralysis. The mechanisms by which antioxidants improve sensory and motor functions are not known. However, it appears that antioxidant-induced stimulation of neurotrophins and/or growth factor receptors might play some role.

Oxidative stress is known to stimulate VEGF and other neurotrophins that are involved in necrosis/apoptosis and to down-regulate neuroprotective neurotrophins, such as brain-derived neurotrophic factor, glial-derived neurotrophic factor, and nerve growth factor [3, 5–8, 10]. Exogenous supplement of neurotrophins, e.g., brain-derived neurotrophic factor and glial-derived neurotrophic factor in SCI, improves motor function and cell injury and is consistent with this idea [17]. However, further studies on expression

of neurotrophins and/or their receptors in H-290/51-treated SCI animals are needed to clarify these points.

Conclusion

The results presented in this investigation show for the first time marked neuroprotection and improvement of motor functions when the antioxidant compound H-290/51 is administered 10 to 30 minutes after SCI. These observations suggest that blockade of lipid peroxidation and/or generation of free radicals within the first hours after trauma is crucial for spinal cord function. However, the antioxidant was ineffective when administered 60 minutes after SCI, suggesting that late blockade of lipid peroxidation and/or generation of free radicals are incapable of attenuating spinal cord pathology. Understanding the cellular and molecular mechanisms of time-related neuroprotection with the antioxidant requires additional investigation.

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References

1. Alm P, Sharma HS, Hedlund S, Sjoquist PO, Westman J (1998) Nitric oxide in the pathophysiology of hyperthermic brain injury. Influence of a new anti-oxidant compound H-290/51. A pharmacological study using immunohistochemistry in the rat. Amino Acids 14: 95–103
2. Alm P, Sharma HS, Sjoquist PO, Westman J (2000) A new antioxidant compound H-290/51 attenuates nitric oxide synthase and heme oxygenase expression following hyperthermic brain injury. An experimental study using immunohistochemistry in the rat. Amino Acids 19: 383–394
3. Bazan NG (2005) Lipid signaling in neural plasticity, brain repair, and neuroprotection. Mol Neurobiol 32: 89–104
4. Calabrese V, Scapagnini G, Colombrita C, Ravagna A, Pennisi G, Giuffrida Stella AM, Galli F, Butterfield DA (2003) Redox regulation of heat shock protein expression in aging and neurodegenerative disorders associated with oxidative stress: a nutritional approach. Amino Acids 25: 437–444
5. Caldwell RB, Bartoli M, Behzadian MA, El-Remessy AE, Al-Shabrawey M, Platt DH, Liou GI, Caldwell RW (2005) Vascular endothelial growth factor and diabetic retinopathy: role of oxidative stress. Curr Drug Targets 6: 511–524
6. Chan PH (2005) Mitochondrial dysfunction and oxidative stress as determinants of cell death/survival in stroke. Ann N Y Acad Sci 1042: 203–209
7. Chong ZZ, Li F, Maiese K (2005) Oxidative stress in the brain: novel cellular targets that govern survival during neurodegenerative disease. Prog Neurobiol 75: 207–246
8. Chong ZZ, Li F, Maiese K (2005) Stress in the brain: novel cellular mechanisms of injury linked to Alzheimer's disease. Brain Res Brain Res Rev 49: 1–21
9. Kirby J, Halligan E, Baptista MJ, Allen S, Heath PR, Holden H, Barber SC, Loynes CA, Wood-Allum CA, Lunec J, Shaw PJ (2005) Mutant SOD1 alters the motor neuronal transcriptome: implications for familial ALS. Brain 128: 1686–1706
10. Kowaltowski AJ, Fiskum G (2005) Redox mechanisms of cytoprotection by Bcl-2. Antioxid Redox Signal 7: 508–514
11. Laplace C, Huet O, Vicaut E, Ract C, Martin L, Benhamou D, Duranteau J (2005) Endothelial oxidative stress induced by serum from patients with severe trauma hemorrhage. Intensive Care Med [Epub ahead of print]
12. Mustafa A, Sharma HS, Olsson Y, Gordh T, Thoren P, Sjoquist PO, Roos P, Adem A, Nyberg F (1995) Vascular permeability to growth hormone in the rat central nervous system after focal spinal cord injury. Influence of a new anti-oxidant H 290/51 and age. Neurosci Res 23: 185–194
13. Poulet R, Gentile MT, Vecchione C, Distaso M, Aretini A, Fratta L, Russo G, Echart C, Maffei A, De Simoni MG, Lembo G (2005) Acute hypertension induces oxidative stress in brain tissues. J Cereb Blood Flow Metab [Epub ahead of print]
14. Rodriguez JA, Nespereira B, Perez-Ilzarbe M, Eguinoa E, Paramo JA (2005) Vitamins C and E prevent endothelial VEGF and VEGFR-2 overexpression induced by porcine hypercholesterolemic LDL. Cardiovasc Res 65: 665–673
15. Rogerio F, Teixeira SA, de Rezende AC, de Sa RC, de Souza Queiroz L, De Nucci G, Muscara MN, Langone F (2005) Superoxide dismutase isoforms 1 and 2 in lumbar spinal cord of neonatal rats after sciatic nerve transection and melatonin treatment. Brain Res Dev Brain Res 154: 217–225
16. Sharma HS (2004) Pathophysiology of the blood-spinal cord barrier in traumatic injury. In: Sharma HS, Westman J (eds) Blood-spinal cord and brain barriers in health and disease. Elsevier Academic Press, San Diego, pp 437–518
17. Sharma HS (2005) Post-traumatic application of brain derived neurotrophic factor and glia derived neurotrophic factor in combination over the traumatized rat spinal cord enhances neuroprotection and improves motor functions. Acta Neurochir [Suppl] 96: 359–364
18. Sharma HS (2005) Pathophysiology of blood-spinal cord barrier in traumatic injury and repair. Curr Pharm Des 11: 1353–1389
19. Sharma HS (2005) Neuroprotective effects of neurotrophins and melanocortins in spinal cord injury. An experimental study in the rat using pharmacological and morphological approaches. Ann NY Acad Sci 1053: 407–421
20. Sharma HS, Sjöquist PO (2002) A new antioxidant compound H-290/51 modulates glutamate and GABA immunoreactivity in the rat spinal cord following trauma. Amino Acids 23: 261–272
21. Sharma HS, Winkler T (2002) 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 [Suppl] 11: S83–S91
22. Sharma HS, Westman J, Alm P, Sjoquist PO, Cervos-Navarro J, Nyberg F (1997) Involvement of nitric oxide in the pathophysiology of acute heat stress in the rat. Influence of a new antioxidant compound H-290/51. Ann N Y Acad Sci 813: 581–590
23. Sharma HS, Alm P, Sjoquist PO, Westman J (2000) A new antioxidant compound H-290/51 attenuates upregulation of constitutive isoform of heme oxygenase (HO-2) following trauma to the rat spinal cord. Acta Neurochir [Suppl] 76: 153–157

24. Sharma HS, Sjöquist PO, Westman J (2001) Pathophysiology of the blood-spinal cord barrier in spinal cord injury. Influence of a new antioxidant compound H-290/51. In: Kobiler D, Lustig S, Shapira S (eds) Blood-brain barrier: drug delivery and brain pathology. Kluwer Academic/Plenum Publishers, New York, pp 401–416
25. Sharma HS, Sjoquist PO, Alm P (2003) A new antioxidant compound H-290/51 attenuates spinal cord injury induced expression of constitutive and inducible isoforms of nitric oxide synthase and edema formation in the rat. Acta Neurochir [Suppl] 86: 415–420
26. Sharma HS, Gordh T, Wiklund L, Mohanty S, Sjoquist PO (2005) Spinal cord injury induced heat shock protein expression is reduced by an antioxidant compound H-290/51. An experimental study using light and electron microscopy in the rat. J Neural Transm [in press]
27. Strosznajder RP, Jesko H, Zambrzycka A (2005) Poly(ADP-ribose) polymerase: the nuclear target in signal transduction and its role in brain ischemia-reperfusion injury. Mol Neurobiol 31: 149–167
28. Svensson L, Borjesson I, Kull B, Sjoquist PO (1993) Automated procedure for measuring TBARS for in vitro comparison of the effect of antioxidants on tissues. Scand J Clin Lab Invest 53: 83–85
29. Tariq M, Morais C, Kishore PN, Biary N, Al Deeb S, Al Moutaery K (1998) Neurological recovery in diabetic rats following spinal cord injury. J Neurotrauma 15: 239–251
30. Thornwall M, Sharma HS, Gordh T, Sjoquist PO, Nyberg F (1997) Substance P endopeptidase activity in the rat spinal cord following injury: influence of the new anti-oxidant compound H 290/51. Acta Neurochir [Suppl] 70: 212–215
31. Tormos C, Javier Chaves F, Garcia MJ, Garrido F, Jover R, O'Connor JE, Iradi A, Oltra A, Oliva MR, Saez GT (2004) Role of glutathione in the induction of apoptosis and c-fos and c-jun mRNAs by oxidative stress in tumor cells. Cancer Lett 208: 103–113
32. Valable S, Montaner J, Bellail A, Berezowski V, Brillault J, Cecchelli R, Divoux D, Mackenzie ET, Bernaudin M, Roussel S, Petit E (2005) VEGF-induced BBB permeability is associated with an MMP-9 activity increase in cerebral ischemia: both effects decreased by Ang-1. J Cereb Blood Flow Metab [Epub ahead of print]
33. Westerlund C, Ostlund-Lindqvist AM, Sainsbury M, Shertzer HG, Sjoquist PO (1996) Characterization of novel indenoindoles. Part I. Structure-activity relationships in different model systems of lipid peroxidation. Biochem Pharmacol 51: 1397–1402
34. Xu W, Chi L, Xu R, Ke Y, Luo C, Cai J, Qiu M, Gozal D, Liu R (2005) Increased production of reactive oxygen species contributes to motor neuron death in a compression mouse model of spinal cord injury. Spinal Cord 43: 204–213
35. Yokoi M, Yamagishi SI, Takeuchi M, Ohgami K, Okamoto T, Saito W, Muramatsu M, Imaizumi T, Ohno S (2005) Elevations of AGE and vascular endothelial growth factor with decreased total antioxidant status in the vitreous fluid of diabetic patients with retinopathy. Br J Ophthalmol 89: 673–675
36. Yu F, Sugawara T, Maier CM, Hsieh LB, Chan PH (2005) Akt/Bad signaling and motor neuron survival after spinal cord injury. Neurobiol Dis (Epub ahead of print)

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