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Increased Protein Oxidation and Decreased Creatine Kinase BB Expression and Activity after Spinal Cord Contusion Injury

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ABSTRACT

Traumatic injury to the spinal cord triggers several secondary effects, including oxidative stress and compromised energy metabolism, which play a major role in biochemical and pathological changes in spinal cord tissue. Free radical generation and lipid peroxidation have been shown to be early events subsequent to spinal cord injury. In the present study, we demonstrated that protein oxidation increases in rat spinal cord tissue after experimental injury. As early as 1 h after injury, the level of protein carbonyls at the injury epicenter was significantly higher than in control (169%, $p < 0.05$) and increased gradually over the next 4 weeks to 1260% of control level. Both caudal and rostral parts of the injured spinal cord demonstrated a mild increase of protein carbonyls by 4 weeks postinjury (135–138%, $p < 0.05$). Immunocytochemical analysis of protein carbonyls in the spinal cord cross-sections showed increased protein carbonyl immunoreactivity in the epicenter section compared to rostral and caudal sections of the same animal or control laminectomy animals. Increased protein carbonyl formation in damaged spinal cord tissue was associated with changes in activity and expression of an oxidative sensitive enzyme, creatine kinase BB, which plays an important role in the maintenance of ATP level in the CNS tissue. Damage to CK function in the CNS may severely aggravate the impairment of energy metabolism. The results of our study indicate that events associated with oxidative damage are triggered immediately after spinal cord trauma but continue to occur over the subsequent 4 weeks. These results suggest that antioxidant therapeutic strategies may be beneficial to lessen the consequences of the injury and potentially improve the restoration of neurological function.

Key words: creatine kinase; oxidative stress; protein oxidation; spinal cord injury

INTRODUCTION

THERE ARE TWO MECHANISMS of damage to the spinal cord after acute spinal cord injury: the primary mechanical injury and a secondary injury due to many additional damaging processes, including vascular, biochemical, and ionic changes (Anderson and Hall, 1993; Tator and Fehlings, 1991; Young, 1993). Much of the

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damage that follows acute trauma is due to secondary effects such as ischemia, edema, glutamate excitotoxicity, Ca^{2+} overload, compromised energy metabolism, and oxidative stress (Hall and Braughler, 1989; Juurlink and Paterson, 1998; Siesjo et al., 1989). The injured spinal cord provides a fertile environment for the generation of oxygen radicals and lipid peroxidation reactions thought to contribute to neuronal dysfunction and cell loss following traumatic damage to the CNS (Awasthi et al., 1997; Springer et al., 1997; Hall and Braughler, 1986; Hall, 1989). A number of studies indicate that activation of glutamate receptors and the influx of Ca^{2+} lead to the impaired function of the mitochondrial electron transport system and ROS formation (Happel et al., 1981; Moriya et al., 1994). Increased ROS production that overwhelm endogenous scavenging mechanisms contributes to the damage of critical cellular components including nucleic acids, proteins, and phospholipids. Oxygen free radicals also may trigger inflammation (for review, see Dirnagl et al., 1999), which is considered one of the leading secondary events in spinal cord injury.

During the past decade, free radical generation was established to be an early biochemical event subsequent to spinal cord injury. Most reports concentrated on free-radical-induced lipid peroxidation and membrane damage, although it is acknowledged that free radicals damage proteins and nucleic acids as well (Anderson and Hall, 1994; Barut et al., 1993; Braughler et al., 1985; Butterfield and Stadtman, 1997; Butterfield et al., 2001; Floyd and Carney, 1992). The important role of free radicals in tissue damage associated with spinal cord injury is underscored by the fact that treatment with free radical scavengers can be effective. Iwasa et al. (1989) demonstrated the protective effect of vitamin E on experimental compression injury of the rat spinal cord by inhibiting damage induced by lipid peroxidation. Protective effects of vitamin E, methylprednisolone, and tirilazad mesylate against spinal cord injury was demonstrated by Koc et al. (1999). Cyclosporin-A and methylprednisolone were effective upon inhibition of lipid peroxidation after spinal cord injury as was demonstrated by Diaz-Ruiz et al. (2000). Melatonin has been shown to be very effective in protecting the injured spinal cord from lipid peroxidation (Kaptanoglu et al., 2000). Promoting glutathione synthesis after spinal cord trauma was shown to decrease oxidative stress and to allow tissue preservation in spinal cord after compression injury (Kamencic et al., 2001), possibly by blocking damaging effects of lipid peroxidation products (Subramaniam et al., 1997; Pocernich et al., 2001). It has also been shown that the generation of ROS, impaired mitochondrial function, and lipid peroxidation occur very early following spinal cord injury (Kamencic et al., 2001; Azbill et al., 1997; Hall, 1989). In the present study, we examined the oxidation of cytoplasmic spinal cord proteins and the localization of protein oxidation following spinal cord injury in rats up to 4 weeks postinjury.

Related to the ROS-mediated oxidative damage are changes in energy metabolism after acute spinal cord trauma (Anderson et al., 1980a; Anderson and Hall, 1993; Tator and Fehlings, 1991; Zhang et al., 1993). The phosphocreatine/creatine kinase (PCr/CK) system plays a key role in buffering of ATP levels in the cell under stress conditions (Bessman and Carpenter, 1985; Hemmer and Wallimann, 1993), and creatine kinase isoenzymes are very sensitive to oxidative modifications (Hensley et al., 1994; Konorev et al., 1998; Thomas et al., 1994; Aksenov et al., 2000). In the present study, we investigated temporal changes in protein oxidation and CK activity and expression in rat spinal cord tissue following spinal cord contusion injury.

MATERIALS AND METHODS

Spinal Cord Injury

Adult female Long Evans rats weighting 200–220 g were used in this study. Spinal cord contusion injuries were performed using the New York University impactor device, which provides an accurate and reproductible method for producing contusion injury to the rat spinal cord (Constantini and Young, 1994) and is utilized by the Multicenter Animal Spinal Cord Injury Study (Basso et al., 1996a). Prior to receiving injury, animals were anesthetized with sodium pentobarbital (50 mg/kg, ip), and a dorsal laminectomy was performed to expose the spinal cord at a thoracic (T) level T10 with the dura intact. Clamping at vertebrae T6 and T11 stabilized the vertebral column. The 10-g impact rod was dropped from a height of 25 mm, inflicting a contusion injury of moderate severity (Basso et al., 1996b). Control animals received a dorsal laminectomy. Postinjury care included administration of the analgetic buprenorphine hydrochloride (0.1 mg/kg, i.m., 2× daily, for 2–3 days), the antibiotic Polyflex (3 mg/kg, s.c., once), and 0.9% saline solution (10 mL, s.c., daily, for 5–7 days) to prevent dehydration. If blood was detected in urine, the rats were treated with Polyflex until the urine became clear. The animals were weighed once a day until they regained their pre-operative weight (usually 10–14 days) and twice weekly thereafter. The rats were euthanatized with an overdose of sodium pentobarbital (120 mg/kg, i.p.) 1 h, 6 h, 24 h, 48 h, 1 week 2 weeks, and 4 weeks after contusion injury. A minimum of four animals were examined at each postinjury timepoint.

Tissue Preparation

For immunoblots, the animals were euthanatized with an overdose of pentobarbital and decapitated, the spinal cords were removed quickly, frozen in powdered dry ice, and stored at −70°C. For some experiments rats were euthanatized as described above, and the spinal cords were perfused with saline and removed. From each spinal cord three 10-mm segments were taken, including the impact site and rostral and caudal segments. Tissue samples were homogenized in Tris-saline (50 mM, pH 7.5) containing protease inhibitors (2.5 mg/mL leupeptin, 500 mM EDTA, 1 mg/mL pepstatin, and 20 mM AEBSF). The homogenates were centrifuged at 100,000 *g* for 30 min, the supernatants were collected and stored at −70°C. Protein content was determined using the BSA protein assay (Pierce).

Tissue fixation and immunostaining of spinal cord sections for protein carbonyls were performed as described by Smith et al. (1998) with some modifications (Aksenov et al., 2001). The animals were euthanatized with an overdose of pentobarbital and decapitated, spinal cords removed quickly, and fixed in Methacarn at 4°C overnight (Methacarn does not interfere with measurement of protein carbonyls). A 30-mm spinal cord segment containing the impact site was cut into 10 blocks of 3 mm each, the sections were placed in an embedding cassette and immersed in the same fixative overnight. After dehydration with graded alcohol, the tissue blocks were cleared in xylene and embedded in paraffin wax. Eight-μm-thick cross sections of the spinal cord were cut with a microtome and mounted onto Fisher Superfrost Plus microscope slides. Paraffin was removed and rehydrated sections were covered with 0.1% solution of 2,4-dinitrophenylhydrazine (DNPH) in 2N HCl and incubated in a humid chamber for 1 h to derivatize protein carbonyls. Because derivatization of protein carbonyls was performed in the presence of 2N HCl, inactivation of endogenous alkaline phosphatase activity in sections was also achieved by this treatment. After incubation, sections were washed three times with Tris-buffered saline (TBS) to remove the unbound DNPH and blocked for 30 min with 10% normal goat serum in TBS (NGS/TBS). After blocking, sections were incubated overnight at 4°C with 1:100 dilution of rabbit polyclonal anti-DNP antibody (Oxyblot, Intergen Company, Purchase, NY) in 1% NGS/TBS. Sections were washed three times with TBS and incubated 40 min with secondary anti-rabbit IgG alkaline phosphatase-conjugated antibody (Sigma, St. Louis, MO) diluted 1:1000 in 1% NGS/TBS. After incubation, the secondary antibody solution was removed and sections were again washed three times with TBS. Alkaline phosphatase activity was localized by the development for 15 min with nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (NBT/BCIP) substrate solution (Sigma). Primary antibody was omitted to control the specificity of protein carbonyl immunoreactivity localization. Protein carbonyl immunoreactivity was analyzed with 2× objective of a Nikon TMS microscope. Images were taken by a computer-controlled CCD video camera attached to the microscope.

Electrophoresis and Western Blot Preparation

SDS-PAGE (12%) was performed in mini-slabs according to the method of Laemmli (1970). The gels were either stained with Coomassie Blue for the verification of the amount of protein applied, or were transferred to nitrocellulose membrane for immunoblot analysis. After the transfer, membranes were blocked in 3% bovine serum albumin in phosphate-buffered saline with 0.01% sodium azide and 0.2% Tween 20 for 1 h at room temperature.

Western Blot Analysis for Protein Carbonyls (Oxyblot)

Protein carbonyl levels are an index of protein oxidation (Butterfield and Stadtman, 1997). To determine the level of protein carbonyls, an Oxidized Protein Detection Kit (Oxyblot, Intergen Company, Purchase, NY) was used. Briefly, the samples were treated with DNPH and derivatization-control solution according to the protocol supplied with the kit before the electrophoresis. After derivatization and neutralization with 2M Tris/30% glycerol/19% 2-mercaptoethanol, samples were loaded onto the gel. Rabbit anti-DNP polyclonal antibody from the Oxyblot kit was used as a primary antibody. Membranes were incubated for 1 h with primary antibody, washed in washing buffer (phosphate-buffered saline with 0.01% sodium azide and 0.2% Tween 20), treated with alkaline phosphatase-conjugated secondary antibody (Sigma, diluted 1:30,000), and developed with BCIP/NBT solution (SigmaFast tablets, Sigma).

Western Blot Analysis for Immunoreactive CK BB

Primary polyclonal antiCK BB antibody (Burbaeva et al., 1992) was diluted 1:1,000 in blocking solution and incubated with the membrane for 1 h at 37°C. Membranes were washed and developed as described above.

CK Activity Assay

Total CK activity in the spinal cord tissue samples was determined colorimetrically by using a commercial kit (Sigma). One unit (U) of total CK activity was determined as the amount of the enzyme that converts 1 μmol of creatine to CrP/min at 37°C, pH 9.0.

Imaging Analysis

Western blots were digitized and quantified by computer-assisted imaging using MCID/M4 software supplied by Imaging Research Inc. (St. Catherine's, Ontario, Canada). Quantitative data from Oxyblots were obtained by digitizing and integration of all anti-DNP-positive immunobands. Final results are expressed as % of control values (mean ± SEM).

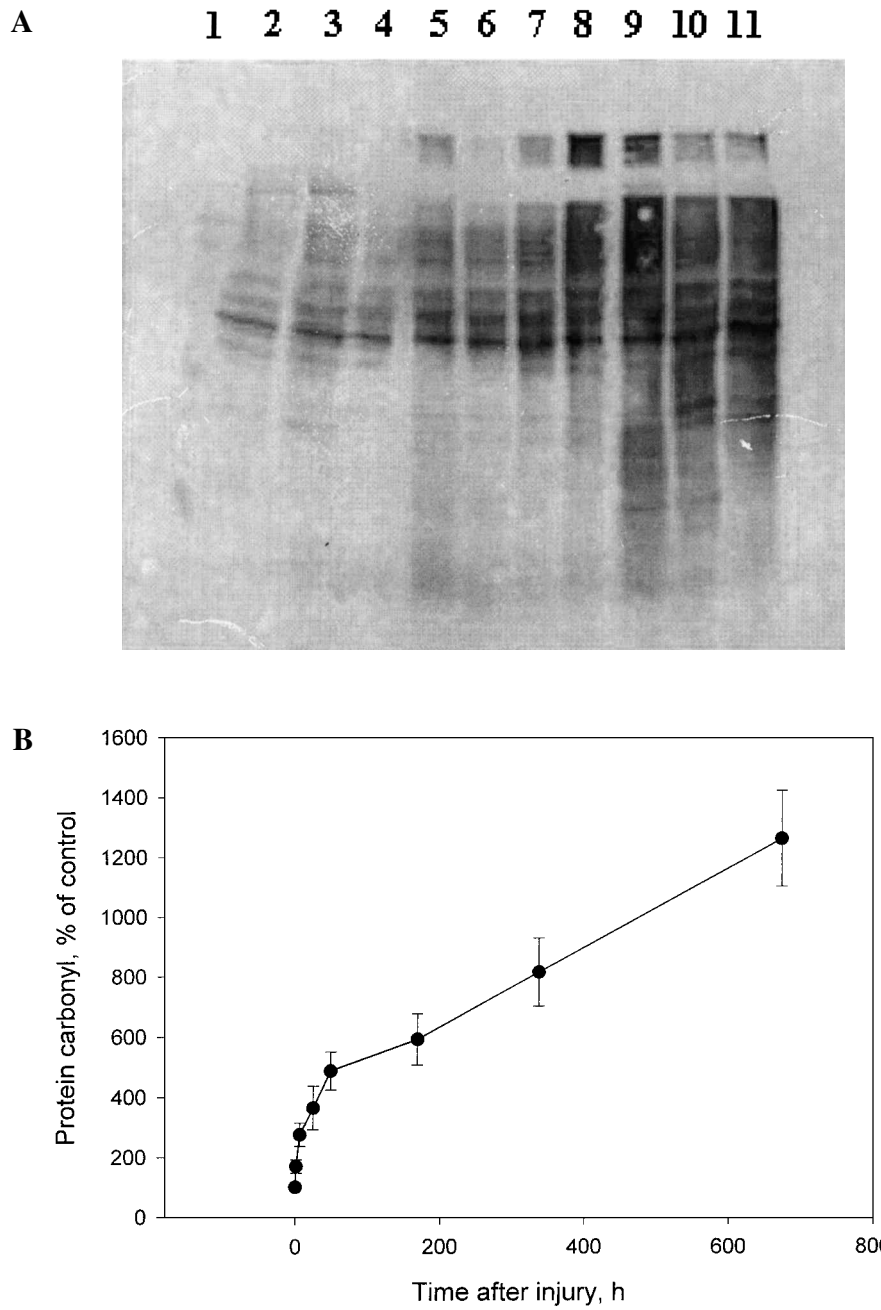


FIG. 1. (**A**) Image of a typical Oxyblot which represents the temporal increase of protein carbonyls in spinal cord tissue at the epicenter area following contusion injury. Lane 2, control laminectomy; lanes 3–4, 6 h postinjury; lanes 5–6, 24 h; lane 7, 48 h; lanes 8–9, 1 week, lanes 10–11, 2 weeks postinjury. (**B**) Protein carbonyls in the spinal cord tissue after SCI. Quantification of the oxyblots demonstrates that following spinal cord injury, the protein carbonyls increase is rapid and extensive. Each point is the mean of at least four animals. Error bars represent SEM values. Significance at $p < 0.001$ at all timepoints.

Statistical Analysis

Statistical comparisons were made using ANOVA followed by Dunnett's test for multiple comparisons. Significant differences were set at $p < 0.05$.

RESULTS

Protein Carbonyl Levels in Spinal Cord Tissue

As early as one hour after injury and at all subsequent time points examined, there was a dramatic increase in the amount of protein carbonyls in the spinal cord tissue at the injury epicenter (Fig. 1). One hour after injury, protein carbonyl level was significantly higher than in control (169 ± 23%) and increased gradually following injury over the 4 weeks period reaching the highest level at 4 weeks (1,260 ± 160%).

In contrast with the rapid increase of the protein carbonyls level at the area of damage, both caudal and rostral parts of the injured spinal cord did not demonstrate oxidative damage to the proteins for up to 2 weeks postinjury (Fig. 2A,B). The amount of protein carbonyls

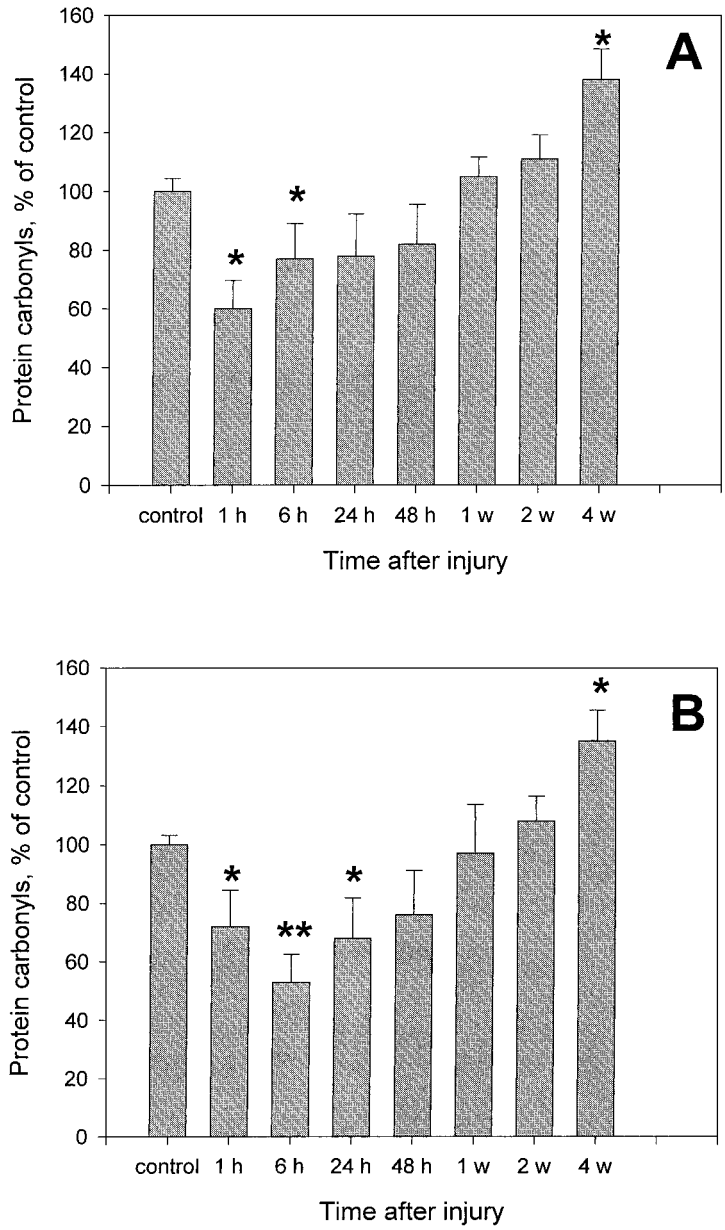


FIG. 2. Temporal changes of protein carbonyls in the rostral (**A**) and caudal (**B**) parts of the spinal cord after SCI at the T10 level. Error bars represent SEM values. Significance at $*p < 0.05$ and $**p < 0.03$.

dropped significantly (50–60% of control level) at 1–6 h after injury, returned back to normal at one week after injury, and then elevated significantly ($p < 0.05$) at 4 weeks following injury by 35–38% in the caudal and rostral injured spinal cord sections. Compared to the dramatic increase of protein carbonyls at the injury epicenter, the elevation of carbonyl level in rostral and caudal parts of the traumatized spinal cord, though significant, was mild (Fig. 3).

Since it is possible that degenerating blood products make significant contribution to the carbonyl content in the injury epicenter, an additional experiment was performed to avoid this potential technical complication. At 1 week following injury, rats were euthanatized as described previously, and the spinal cords were perfused with saline and removed. Protein carbonyl levels were detected at the injury epicenter, and the results obtained for perfused tissue were not different from the results obtained for the non-perfused tissue (data not shown).

Immunocytochemical Analysis of Protein Carbonyls in Spinal Cord Tissue

Anti-DNP immunostaining of the spinal cord cross-sections showed increased protein carbonyl immunoreactivity in the epicenter section compared to rostral and/or caudal spinal cord section of the same animal (Fig. 4A,B), or compared to control laminectomy animals (Fig. 4C). In rostral and caudal sections of the injured animals and control laminectomy animals, faint protein carbonyl immunoreactivity was evenly distributed and no significant differences were found between these samples (Fig. 4A,C). When primary antibody was omitted, no protein carbonyl immunostaining in the spinal cord was detected.

Creatine Kinase in Spinal Cord Tissue

The CK activity per mg of total protein in the spinal cord tissue at the injury epicenter remained relatively stable throughout one-week postinjury, but decreased rapidly after that to approximately 30% of control levels at 2 weeks (Fig. 5A). At 4 weeks following injury, the level of CK activity in the damaged tissue returned back to normal (124% of control, NS $p > 0.05$).

In the CNS, cytosolic CK BB (85-90%) and mitochondrial uMtCK (10–15%) represent total CK activity. Predominantly water-soluble protein extracts used in our study were essentially free of uMtCK isoenzyme, as demonstrated by enzyme activity staining following non-denaturing gel electrophoresis (data not shown). Thus, it is likely, that the observed decline of the total CK activity in the spinal cord tissue extracts can be attributed to the decreased amount of the CK BB isoenzyme. At 6 h following injury, the content of immunoreactive cytoso-

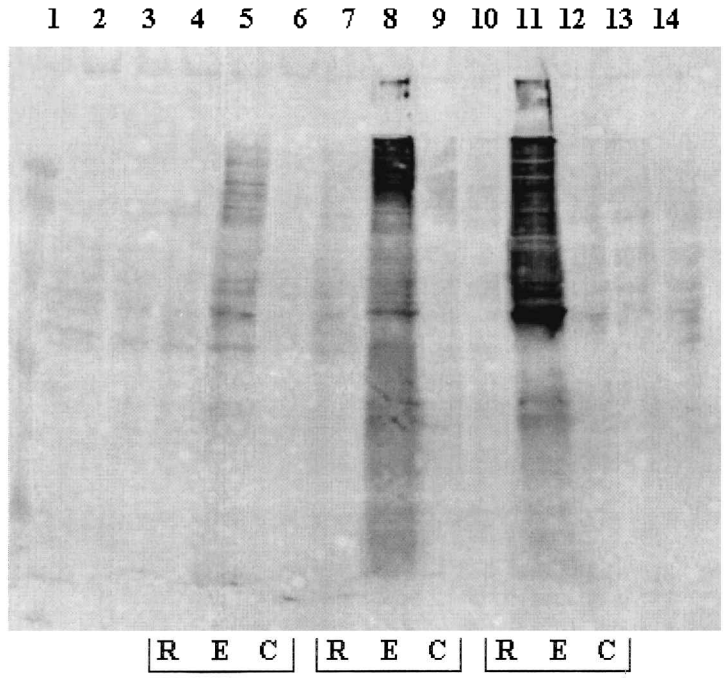


FIG. 3. Typical image of an oxyblot representing protein carbonyls in the spinal cord tissue of the same animal (injury site, caudal part, and rostral part) at different timepoints after SCI. Lanes 2, 3, 13 and 14, control laminectomy animals; lanes 4–6, 6 h postinjury; lanes 7–9, 1 week; lanes 10–12, 2 weeks. R, rostral; E, epicenter; C, caudal.

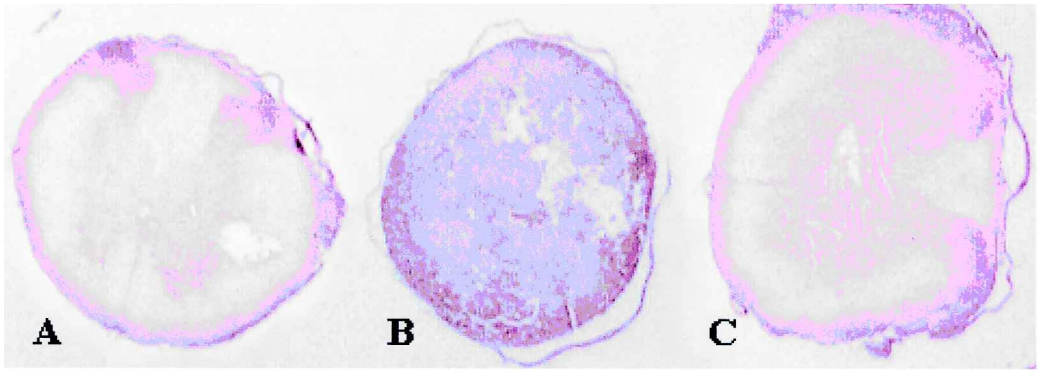


FIG. 4. Representative microscopic images illustrating results of immunohistochemical detection of protein carbonyls in the rat spinal cord sections 24 h after injury. (**A**) Rostral spinal cord section of the injured animal. (**B**) Injury epicenter section. (**C**) Thoracic spinal cord section of a control laminectomy animal. All images were captured at the same lighting conditions and magnification.

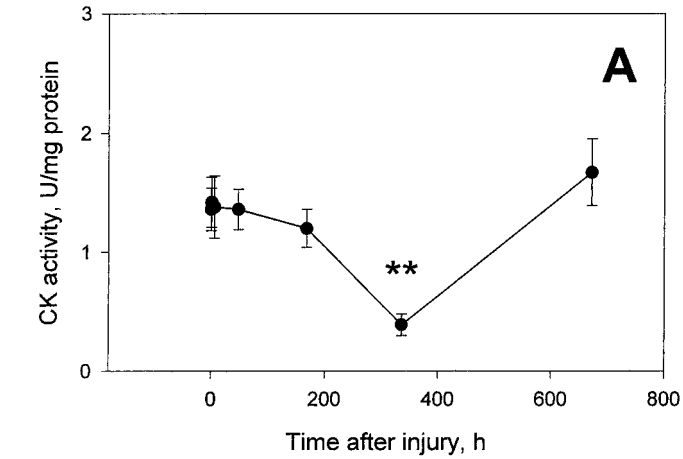
lic CK BB was slightly elevated compared to controls, but the difference was not statistically significant. By 24 and 48 h following injury, the CK BB level was significantly increased ($p < 0.05$) by 113% and 122%, respectively (Fig. 5B). However, at 1 week after injury there was a dramatic decline of the CK BB level (78% of control, $p < 0.01$), which preceded a slow return back to normal level throughout the remainder of the 4-week postinjury period examined in this study. When relative changes in CK activity was normalized to the immunoreactive content of the isoenzyme, this ratio was found to be lower at 2 weeks after injury, indicating the presence of inactive CK BB molecules (Fig. 5C). In rostral and caudal parts of the injured spinal cord total CK activity and the content of immunoreactive CK BB were no different from controls (data not shown).

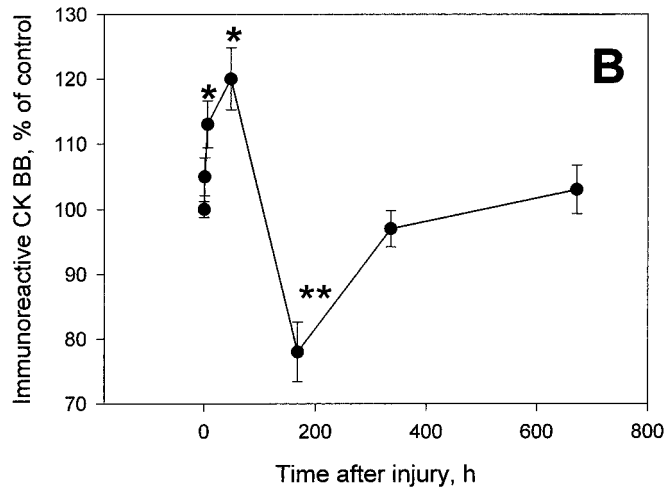
DISCUSSION

The role of ROS in producing irreversible modifications of fundamental macromolecules has been widely studied in different models of CNS injury (Floyd and Carney, 1992; Hall and Braughler, 1989; Hall et al., 1995; Juurlink and Paterson, 1998). Traumatic spinal cord injury triggers a number of secondary events that compromise cellular functions. Mitochondrial dysfunction and energy perturbation are generally believed to be important pathophysiological factors in acute spinal cord trauma. Among the consequences of impaired mitochondrial function are the generation of a ROS cascade and the inability to generate sufficient levels of ATP. Azbill et al. (1997) demonstrated that mitochondrial function is significantly impaired within 1 h following contusion injury to the spinal cord, and remains impaired for up to 24 h. These researchers also demonstrated that ROS levels became elevated after mitochondrial function was impaired for some time following spinal cord injury.

The results of this current study indicate that protein oxidation occurs rapidly (as soon as 1 h) after spinal cord injury. Other authors reported significant increase of protein carbonyls 3 h after injury (Leski et al., 2001), or 24 h after injury (Kamencic et al., 2001). The increase appears to have a long time course; after 4 weeks postinjury, there was no sign of decrease. Immunocytochemical analysis of the spinal cord cross-sections revealed intense protein carbonyl immunoreactivity restricted to the injury epicenter section. Both white matter and gray matter demonstrated high level of protein oxidation in the epicenter sections. Rostral and caudal sections of the same animal demonstrated faint staining, as well as control laminectomy sections.

In this study, we noticed a delayed increase in protein carbonyls levels in both rostral and caudal parts of the injured spinal cord 4 weeks after injury. Oxidative stress damage in spinal cord segments adjacent to the injury epicenter also has been demonstrated recently by the work of Kamencic et al (2001) and by Leski et al. (2001) who found a rapid increase of protein carbonyls in rostral and caudal parts of injured spinal cord, possibly because of much higher impact used by these authors (up to 100 g-cm force of impact by dropping a weight from a height of up to 15 cm). These results provide clear evidence of oxidative damage to proteins taking place in the parts of the spinal cord, which were not in the immediate vicinity of the injury site. Although regulated proteolysis which includes the ubiquitin-proteosome system is one of the main systems designed to degrade and remove damaged proteins (Goldberg et al., 1997), generalized mobilization of antioxidant defenses in response to a local free radical dam-





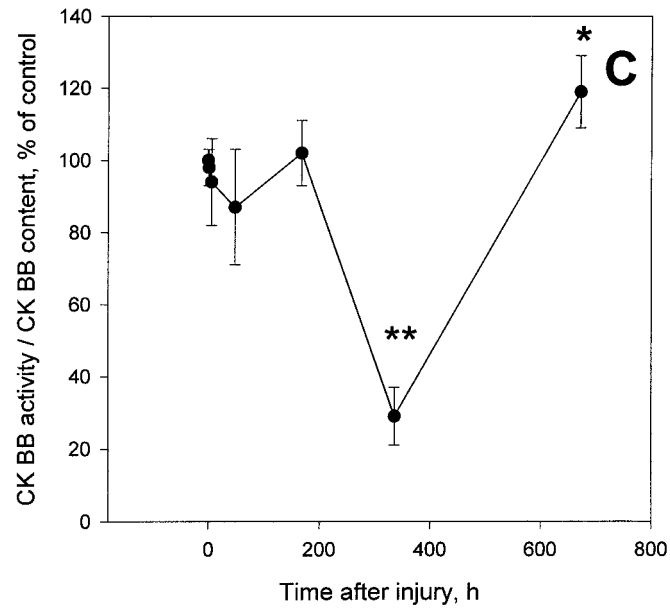


FIG. 5. Total creatine kinase activity (**A**), immunoreactive CK BB content (**B**), and activity normalized for immunoreactive CK BB content (**C**) in spinal cord tissue after SCI (mean ± SEM). Significance at $*p < 0.05$ and $**p < 0.01$.

age would help to decrease protein carbonyl levels in caudal and rostral parts of the injured spinal cord observed in our model at the early timepoints after injury. Since the oxidative stress was relatively mild in those parts compared to the injury site, the antioxidant defense appeared to be successful for up to 1–2 weeks after injury and then failed under the enormous amount of ROS spreading from the injury epicenter.

As one of the most important secondary mechanisms of acute spinal cord trauma, impaired energy metabolism in the spinal cord tissue is well-documented. Decrease of ATP and GTP (Anderson et al., 1980a; Vagnozzi et al., 1999), increase of lactate and decrease of glucose (Anderson et al., 1980b; Lewen and Hillered, 1998), depleted levels of phosphocreatine and increased lactate/pyruvate ratio (Anderson et al., 1980b), and the overall decrease of mitochondrial metabolic activity (Azbill et al., 1997) were reported. Lipid peroxidation and protein oxidation are thought to contribute to a subsequent depression in CNS energy metabolism. Creatine kinase isoenzymes play an important role in the maintenance of ATP level in the CNS tissue (Bessman and Geiger, 1981; Wallimann et al., 1998). Recent reports suggest that the CK and phosphocreatine shuttle may play an important role in protection against free radical damage and ATP depletion (Chung et al., 1998; Klivenyi et al., 1999; Miller et al., 1993). Under the condition of oxidative and bioenergetic stress the PCr/CK system can provide an energy source necessary to support functioning of ATP-dependent enzyme complexes and enable gradual recovery of injured neurons. Our data demonstrate that injury of the spinal cord causes a temporal decrease of total CK activity and content of cytosolic CK BB isoform. CK activity normalized to immunoreactive CK BB content shows the accumulation of inactive CK BB molecules after 2 weeks postinjury. At 4 weeks, the activity and content of CK BB returned to normal levels, which may indicate the early stages of metabolic recovery since animals did not die after spinal cord injury. In the present study we did not investigate the involvement of the uMtCK in the activity decline, which is very possible since the impaired mitochondria are one of the major sources of ROS in spinal cord inquiry.

The loss of CK activity and CK BB protein levels may be related, at least in part, to the degeneration of the dendritic network in the injured part of the spinal cord. The relationship between cytosolic CK alteration and degeneration of neurites was demonstrated by Tomimoto et al. (1993), who investigated CK BB after cerebral ischemia in gerbils by immunoelectron microscopy. These authors observed propagation of ischemic and postischemic damage with disintegration of microtubules in the dendro-somatic direction in neurons, which progressed in parallel with dispersion and loss of microtubule-associated CK BB immunoreactivity in dendroplasm. After reperfusion of the ischemic brain, CK BB was observed in the extracellular space. CK BB leakage from neural cells into serum and/or CSF as a result of brain damage was documented in patients with head trauma (traumatic edema, brain contusion) (Norby and Urdal, 1982; Wang et al., 1994), and hypoxia (hangings, cardiac arrest, carbon monoxide, and drug poisoning; Karkela et al., 1993; Vazquez et al., 1995). Active site of CK isoenzymes contains an essential cysteine residue and tyrosine residues, which could be the targets for oxidative modifications (Koufen and Stark, 2000; Banerjee et al., 1991; Fucci et al., 1983; Konorev et al., 1998; Thomas et al., 1994; Yatin et al., 1999). Therefore, CK is likely to be one of the primary targets for ROS, which is overproduced in the damaged spinal cord tissue. In our previous studies we demonstrated that oxidative modification of CK BB contributes to the decrease of CK activity in the AD brain (Aksenov et al., 2000; Aksenova et al., 1999). Damage to CK in the CNS after acute trauma may severely aggravate the impaired energy metabolism and eventually lead to neuronal death.

The results of our study indicate that events associated with oxidative damage are triggered immediately after induction of spinal cord trauma. Lipid peroxidation and protein oxidation can impair the function of numerous cellular components including creatine kinase, and may significantly delay the recovery of primary biochemical functions and the restoration of neurological function. Since antioxidants and free radical scavengers such as PBN, vitamin E, methylprednisolone, melatonin, dihydrolipoic acid, etc, have been shown to lessen the consequences of lipid peroxidation after acute CNS traumatic injury (Kamencic et al., 2001; Mu et al., 2000; Kaptanoglu et al., 2000; Diaz-Ruiz et al., 2000; Koc et al., 1999; Lewen and Hillered, 1998; Hall et al., 1995; Zhao et al., 1994; Prehn et al., 1994; Iwasa et al., 1989), the same therapeutic strategies may be beneficial in reducing the levels of protein oxidation. Our data demonstrate that trauma is directly responsible for the triggering of the oxidative damage to the proteins, and this damage becomes exacerbated during the postinjury period. This result suggests that antioxidants, even when given after spinal cord injury, may be effective in improving neurological function in patients with acute spinal cord trauma. Such studies are in progress.

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