Evaluation of time-dependent spread of tissue damage in experimental spinal cord injury by killed-end evoked potential: effect of high-dose methylprednisolone

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Object. Histopathological studies on spinal cord injury (SCI) have demonstrated time-dependent spread of tissue damage during the initial several hours postinjury. When the long tract within the spinal cord is stimulated, a large monophasic positivity occurs at the injury site. This type of potential, termed the killed-end evoked potential (KEEP), indicates that a nerve impulse approaches but does not pass beyond the injury site. The authors tested the hypothesis that the damage spread can be evaluated as a progressive shift of the KEEP on a real-time basis. The effect of high-dose methylprednisolone sodium succinate (MPSS) on the spread of tissue damage was also examined by this methodology.

Methods. The KEEP was recorded using an electrode array placed on the spinal cord at the T-10 level in cats. This electrode array consisted of multiple 0.2-mm-diameter electrodes, each separated by 0.5 mm. Spinal cord injury was induced using a vascular clip (65 g pinching pressure for 30 seconds). The midline posterior surface of the spinal cord was stimulated bipolarly at the C-7 level by applying a single pulse at supramaximal intensity. During the initial period of 6 hours postinjury, the localization of the largest KEEP shifted progressively up to 2.5 mm rostral from the injury site. The amplitude of the KEEP recorded at the injury site decreased to 55 to 70% and became slightly shortened in latency as the localization of the largest KEEP shifted rostrally. These findings imply that the injury site KEEP represents the volume-conducted potential of the largest KEEP at the site of the conduction block. It moved away from the injury site in association with the damage spread, and this was confirmed histopathologically. A decrease in amplitude of KEEP at the injury site appeared to be the most sensitive measure of the damage spread, because the amplitude of the volume-conducted KEEP is inversely proportional to the square of the distance between the recording site and site of conduction block. Administered immediately after SCI, MPSS clearly inhibited these events, especially within 30 minutes postinjury.

Conclusions. The KEEP enables sequential evaluation to be made of the time-dependent spread of tissue damage in SCI in the same animal. It is, therefore, useful for detecting the effect of therapeutic interventions and for determining the therapeutic time window. The efficiency of MPSS to inhibit the spread of damaged tissue appeared to be maximized when it was administered within the initial 30-minute period postinjury.

Key Words • killed-end evoked potential • methylprednisolone • spinal cord injury • cat

The results of histopathological studies of SCI in experimental animals have indicated that tissue damage spreads progressively during the initial several hours postinjury. Various neurochemical, metabolic, and vascular mechanisms have been proposed to underlie such time-dependent damage spread.

Progressive or delayed tissue damage has been demonstrated by various functional measures including the local blood flow and the electrophysiological method. Biological evidence derived from experimental studies in animals indicates that therapeutic intervention including early decompressive surgery may improve neurological recovery after SCI, although the window for effective intervention remains unclear. To date, sequential evaluation of the spread of damage in the same animal has not been readily possible. If the time-dependent damage spread were to be defined quantitatively, the effect of therapeutic intervention to minimize the extent of tissue damage could be effectively evaluated.

When the long tract fibers within the spinal cord are stimulated, a large monophasic positivity is recorded at the injury site. This type of potential, termed the KEEP, indicates that a nerve impulse approaches but does not pass beyond the recording site. The KEEP can be readily recorded in humans, as well as experimental animals, in the spinal epidural spaces and is useful for localizing the injury site. In the present study, we tested the hypothesis that the spread of damage evident in tissue can be demonstrated as a progressive shift in the

Abbreviations used in this paper: ABP = arterial blood pressure; ANOVA = analysis of variance; KEEP = killed-end evoked potential; MPSS = methylprednisolone sodium succinate; SCI = spinal cord injury; SD = standard deviation.
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KEEP. Such documentation would provide detailed information regarding the time course of damage spread following SCI. We also examined whether the effect of high-dose MPSS on the damage spread could be detected in this way, because MPSS has been regarded as having a therapeutic effect in SCI.3–5,7,16,17,21

Materials and Methods

Animal Preparation

Twenty-five adult cats weighing 2.8 to 4.2 kg were used. A 10-mg intramuscular dose of ketamine was initially used to induce anesthesia in all animals. The femoral artery and vein were cannulated to allow recording the ABP, as well as drug administration and fluid infusion, respectively. Anesthesia was maintained subsequently by periodic intravenous administration of pentobarbital sodium (20 mg/kg). Electroencephalographic data, recorded from stainless-steel screws placed on the skull, were monitored to ensure that the cats remained adequately anesthetized throughout the experiment. Muscle paralysis was induced using intravenous succinylcholine chloride (1 mg/kg), and mechanical ventilation was provided using a mixture of room air, 95% O₂, and 5% CO₂. The PO₂, PCO₂, and systemic ABP in all cats were monitored and maintained by adjusting the respiratory volume and/or flow rate of the gas mixture, at 100 to 150 mm Hg, 25 to 40 mm Hg, and 90 to 150 mm Hg, respectively. Data obtained in cats that indicated changes in physiological parameters beyond these ranges, except the initial increase in systemic ABP at the time of SCI, were discarded. The rectal temperature was also monitored and maintained at 37 to 39°C by using a water-heating pad.

After C-7 and T-10 laminectomies were performed, SCI was induced using a vascular clip with 65 g of pinching pressure at T-10. Clip-induced compression was maintained for 30 seconds, and the clip was then released. The protocol for these procedures was approved by the University Committee on the Use and Care of Experimental Animals.

Recording Procedures

Electrical stimulation was conducted using a pair of silver ball electrodes separated by 2.5 mm, placed epidurally at the midline posterior surface of C-7. The stimulation was applied with monophasic square wave pulses of 0.5-msec duration and supramaximal intensities (1–3 mA) at a 2-Hz frequency. The KEEP was recorded with an electrode array placed parallel to the spinal cord at the midline posterior surface of the T-9 to T-11 levels. The electrode array consisted of multiple 0.2-mm-diameter electrodes, each separated by 0.5 mm. These were positioned so that one of the electrodes was situated at a site where the rostral edge of the vascular clip was applied, and at least 10 electrodes were situated rostral to the injury site. A reference electrode was placed on the spine. Signals from the electrodes were fed into an amplifier with a band-pass range of 5 Hz to 3 kHz and were averaged for 30 sweeps by using a signal processor. A total of 20 cats in which electrophysiological monitoring was performed were killed by pentobarbital using a signal processor. A total of 20 cats in which electrophysiological monitoring was performed were killed by pentobarbital

Data Sampling and Analysis

The spinal cord evoked potential recorded obtained from the spinal cord following spinal cord stimulation was typically composed of triphasic waves: a small positive wave (P1) followed by two negative waves (N1 and N2). The N1 was always larger than N2 in amplitude. The conduction velocities of P1 and N1, which were calculated from latencies observed at different locations, were the same in each cat (range 110–130 m/second).

Immediately after the clip was released, the spinal cord evoked potential demonstrated at the injury site was found to have already changed to a large monophasic positivity, which was consistent in nature with the KEEP.

As the recording site moved rostrally, P1 decreased in amplitude, and N1 and N2 became detectable. It was noted that the latencies of P1 and N1 underwent a progressive shortening as the recording site moved more rostrally (Fig. 2).
This indicated that impulses represented as triphasic waves observed at positions rostral to the injury site were conducted along the spinal cord. In contrast, only a positivity was observed caudal to the injury site (Fig. 1). The peak latency of the positive wave was located between the peak latencies of P1 and N1, recorded at the recording site immediately rostral to the injury site. These findings indicated that the monophasic positivity recorded at the injury site represented the KEEP of P1 and N1.

Furthermore, the amplitude decreased rapidly as the recording site moved more caudally (Fig. 1). The latency of such monophasic positivity was identical in all electrodes placed on the side caudal to the injury site (Fig. 2). In both of these time courses, such a time-dependent shift of the greatest positivity was clearly inhibited by high-dose MPSS (open circles) compared with that observed in the controls (solid squares [p < 0.01, ANOVA]).

Temporal Pattern of Changes in KEEP Localization

The monophasic positivity was initially largest at the rostral edge of the injury site where the vascular clip had been placed. This implied that a conduction block occurred in proximity to the injury site. During the initial period of 2 hours postinjury, the electrode demonstrating the largest
monophasic positivity shifted progressively to the more rostral portion of the electrode array (Fig. 2 center [solid squares]). This tendency continued slowly throughout the observation period. At 6 hours postinjury, the site associated with the largest monophasic positivity had shifted to a level 2.5 mm rostral to the injury site (Fig. 2 lower [solid squares]). The amplitude of the largest positivity did not change significantly throughout the experimental period in either the or control group (Table 2).

As the MPSS group site associated with the largest monophasic positivity moved rostrally, the positivity recorded from the caudal portion of the electrode array decreased in amplitude (Fig. 2, center and lower [solid squares]). In all caudal electrodes the latency of the monophasic positivity was always identical to that of the largest monophasic wave. This indicated that the monophasic positivity occurring at the injury site changed from the KEEP that was generated in proximity to the injury site to the volume-conducted potential, reflecting the KEEP generated by conduction block at more rostral sites.

The amplitude of the injury site–recorded monophasic positivity decreased rapidly during the initial 30-minute period postinjury and slowly thereafter (Fig. 3 solid squares). At 6 hours postinjury, the amplitude of the monophasic positivity recorded at the injury site was reduced to 50 to 70% of the original level (Fig. 3 solid squares).

Histopathological examination showed hemorrhagic necrosis in the central gray matter at the injury site where the vascular clip was applied (Fig. 4 lower). The rostrocaudal extent of white matter damage at 6 hours postinjury was clearly greater than that in cats killed at 2 hours postinjury, although precise quantification was not readily possible. The recording site at which the largest monophasic positivity was noted at 6 hours postinjury (vertical lines in Fig. 4) was always located slightly more rostral to the area of central necrosis and corresponded roughly to the rostral border of white matter damage in each animal.

**Effects of High-Dose MPSS**

The aforementioned time-dependent shift in distribution of the monophasic positivity was clearly inhibited by high-dose MPSS. The rostral shift of the greatest monophasic positivity reached 1.5 mm at 6 hours postinjury, and this was significantly less than that observed in the controls (Fig. 2) \(p < 0.01\). The amplitude of the largest positivity was not significantly different in these groups at any time point (Table 2).

During the initial 30 minutes postinjury, the amplitude of the monophasic positivity recorded at the injury site decreased more slowly than that in the controls, which was rapid. In five of the 10 cats, even a slight positivity–related increase in amplitude was noted within the initial 30-minute period postinjury. The amplitude of the monophasic positivity recorded at the injury site then decreased similarly as in the controls. At 6 hours postinjury, the monophasic positivity–related amplitude recorded at the injury site was reduced to 75 to 85% of its original level. This time course was clearly different from that observed in the controls (Fig. 3) \(p < 0.01\), and this difference could be attributed mostly to the slow decrease in amplitude in MPSS–treated cats during the initial 30 minutes postinjury.

The rostrocaudal extent of white matter damage, as demonstrated histopathologically, in the MPSS–treated cats was clearly limited both compared with and that observed in controls (Fig. 4), although precise quantification was again not readily possible. Similarly as in the controls, the site where the largest monophasic positivity was recorded at 6 hours postinjury (vertical lines in Fig. 4) was always located slightly more rostral to the area of central necrosis and corresponded roughly to the rostral border of white matter damage in each animal.

**Discussion**

**Origin of the KEEP in Response to Stimulation**

We have previously demonstrated that P1 and N1 of the spinal cord evoked potential in response to stimulation of the posterior surface of the spinal cord disappear after transection of the posterolateral funiculus in cats. The conduction velocities of P1 and N1 were the same in each cat and faster than that reported for the corticospinal tract. This
indicates that P1 and N1 represent a nerve impulse that is mediated by fibers of large diameter, which approaches and surpasses the recording site. Such large-diameter fibers within the posterolateral funiculus have been reported for the spino cerebellar tract. In the present study the KEEP recorded appears therefore to represent a nerve impulse mediated by the spino cerebellar tract, which approaches but never surpasses the injury site. We recorded the KEEP with the depth electrode placed in the posterolateral funiculus in some cats (data not shown) and confirmed that the latencies of the KEEPs recorded by the depth and surface electrodes were approximately the same.

Electrophysiological Evaluation of Damage Spread

The rostral shift of the largest monophasic positivity, which corresponded to the KEEP, indicated that the location of conduction block moves rostrally during the initial 6-hour period postinjury. Because the KEEP is a functional consequence of white matter damage, white matter damage therefore appears to spread rostrally from the injury site during this period. The shift of the KEEP can thus be regarded as an indicator of the time-dependent spread of white matter damage.

The decrease in injury site–related monophasic positivity during the initial 6-hour period postinjury is consistent with the idea that it is a volume-conducted potential and its source moves progressively away from the injury site. In some cats, we confirmed that the latency of the KEEP, which was recorded using a depth electrode placed in the injury site of the posterolateral funiculus, demonstrated a decrease in amplitude with a similar time course (data not shown). Although the amplitude of KEEP is influenced by many variables, most are related to events occurring at a point of conduction block, such as the injury current. Because there was no significant change in amplitude of the largest monophasic positivity during the experimental period, its observed decrease at the injury site does not appear to be attributable to changes in the KEEP as the source of volume-conducted potential. This implies that the major variable of the observed decrease in positivity at the injury site is the distance of volume conduction.

Decreases in amplitude as well as latency of the injury site positivity can be used as indicators of damage spread.
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The latency of the positivity is proportional to the distance between the stimulation site and the point of conduction block. Because the spread of tissue damage is not large compared with the entire distance between the stimulation and recording sites, a latency change is not practically useful for detecting the damage spread. In contrast, the amplitude of the volume-conducted potential is inversely proportional to the square of the distance between the recording site and its source. The decrease in injury site amplitude is therefore a more sensitive indicator of damage spread than the shortening in latency caused by the shift of the site associated with the actual KEEP. Based on our study results, we find that such a technique is far more sensitive for detecting the damage spread than conventional histopathological examination. Histopathological changes are obviously a more definite injury endpoint. We emphasize, however, that the KEEP, but not histopathological changes, permits sequential evaluations of the damage spread in the same animal.

Therapeutic Time Window for MPSS

The slow but steady decrease in injury site positivity even at 6 hours postinjury indicates that the spread of tissue damage can continue for more than 6 hours postinjury. To minimize neurological deficits in patients with SCI, inhibition of damage spread during the initial several hours is extremely important. Various forms of therapeutic intervention have proven useful for inhibiting the damage spread in experimental animals. Most investigators who reported such effects, however, evaluated the histopathological, biochemical, electrophysiological, or neurological outcome at a certain time point postinjury. Little is known, therefore, about the effects of therapeutic intervention on the temporal pattern of damage spread. Because the KEEP enables sequential evaluations of the damage spread in the same animal, this modality would provide information for determining the time window of therapeutic intervention.

It has been shown that administration of high-dose steroid agents improve spinal cord blood flow and microvascular perfusion following SCI in experimental animals. In various models of SCI, the effects of high-dose MPSS on tissue damage have been studied in detail experimentally. The effects against the free radical–mediated lipid peroxidation have been suggested to represent a major mechanism by which MPSS can attenuate tissue damage following SCI in experimental animals. Investigators for the North American Spinal Cord Injury Study in the United States confirmed that high-dose MPSS administered within the first 8 hours postinjury reduces neurological deficits. Consistent with the results of these studies, our findings confirm that high-dose MPSS inhibits the extent of the spread of tissue damage within 6 hours postinjury. Such an MPSS-related effect was not attributed to improved physiological parameters, because they did not differ significantly between the MPSS-treated and the control group.

In our experiments, an MPSS bolus was administered immediately after injury. Because this is not feasible clinically, the observation in the present study does not have any direct clinical significance. Nevertheless, it is interesting that the effect of MPSS most occurred during the initial 30-minute period postinjury. Assuming that the biological half-life of the MPSS bolus is in the range of 2 to 3 hours, our data appear to indicate that the clinical effect of MPSS could be maximized if it were administered within 30 minutes postinjury.

Conclusions

In summary, the KEEP-related evaluation of damage spread activity is sensitive and quantitative, as well as being more efficient than assessment based on histopathological examinations. Because of these features, the KEEP is useful for assessing the efficacy of therapeutic intervention and for determining the therapeutic time window. Such observations offer hope that this modality may help us to refine therapeutic interventions for SCI.

References

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