Spinal cord contusion injury: experimental dissociation of hemorrhagic necrosis and subacute loss of axonal conduction

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Previously reported experimental models for spinal cord contusion injury do not allow the independent control of compression and contact velocity required for interpretation of experimental data relating kinematics of vertebral injury to spinal cord injury. Therefore, controlled dynamic compression of the spinal cord was used to study compression and contact velocity as independent variables. Cord conduction was assessed using the latency of somatosensory evoked potentials in response to hindlimb stimulation. The latency increase at 4 hours after contusion differed significantly between control and 50% compression results, and between 25% and 50% compression results. A small nonsignificant increase in latency was observed with increase in contact velocity. The extent of hemorrhagic necrosis correlated with contact velocity rather than with the amount of compression. This study demonstrates, for the first time, a dissociation between hemorrhagic necrosis and loss of neuronal conduction in the subacute phase. Although long-term effects of hemorrhagic necrosis on cord structure and conduction remain to be evaluated, the data suggest that delayed loss of neuronal conduction seen clinically may result from moderate levels of cord compression at high contact velocity. Such an injury is not reproducible by weight-drop techniques for cord injury.

KEY WORDS: spinal cord injury • spinal cord conduction • contact velocity • somatosensory evoked potentials • hemorrhagic necrosis • neural conduction

OVER the past 75 years, various animal models have been developed to study the mechanisms of spinal cord neural and vascular injury in an effort to reduce the pathology and dysfunction resulting from cord injury in man. The weight-drop technique first introduced by Allen in 1911 has been the most widely used, with some modifications. Extrudural balloon compression of the cord as introduced by Tarlov has also been widely used. Each of these models has limitations, however. The balloon compression model is limited to slow rates of compression, representing tumor growth rather than traumatic cord compression. The weight-drop model provides dynamic compression, but exhibits high variability with slight variations in the experimental parameters, and does not permit independent control of the amount of compression and the contact velocity.

When slow compression of the spinal cord is compared to dynamic compression of an equal amount, the extent of spinal cord dysfunction is determined by the contact velocity of the compression. Slow cord compression by balloon inflation blocks neural conduction through the site of compression only when it is sufficient to block blood flow in the area. Clinical observations further support the conclusion that spinal cord conduction is resistant to slow compression, since neurological deficits from protracted gradual-onset cord compression are reduced by cord decompression.

Dynamic compression, whether by weight-drop or by rapid balloon inflation, has different results. Levels of compression which have no effect when applied slowly cause an immediate loss of conduction through the injured site after a dynamic compression. The permanent dysfunction occurs even though changes in local blood flow parallel alterations seen following slow compression.

Mechanical damage to nerve fibers in the region of compression may be a critical factor in determining permanent loss of neuronal conduction, with local ischemia and hypoxia playing secondary roles. The present project tests the hypothesis that neuronal membrane damage, and hence functional impairment, is a function of the amount of applied compression.
depend on the speed of applied compression. Our pneumatic contusion technique gives accurate control of the amount of compression and contact velocity independently. Thus, the interaction of contact velocity and amount of compression in defining injury severity can be determined over a range of velocities which have relevance to clinical injuries.

Materials and Methods

A total of 67 adult male ferrets (*Mustela putorius furo*), each weighing 1.0 to 1.5 kg, were obtained from a long-established closed colony. Anesthesia was induced and maintained using halothane (0.5% to 1.0%), nitrous oxide, and oxygen (1:1) by inhalation. A midline dorsal incision was made and the muscular tissue was retracted to expose the dorsal surface of the cervical spine. The interspinous and posterior spinal ligaments were removed at the C5–6 intervertebral space to expose the dura, which remained intact. No laminectomy is required for sufficient exposure of the ferret cervical spinal cord. Temperature, blood pressure, electrocardiographic data, and expiratory CO₂ were monitored and maintained within normal limits. Animals were mechanically ventilated.

Somatosensory evoked potentials (SEP's) were recorded from bipolar silver ball electrodes inserted in the cisterna magna with rostrocaudal electrode separation of 1 cm. Stimuli were delivered via bipolar needle electrodes in the forepaw and hindpaw footpads. Current intensity was adjusted to four times the threshold level necessary for paw twitch. Responses to both hindpaw and forepaw stimulation were recorded, with forepaw response used as a control for systemic effects on evoked response. Normally the response to forepaw stimulation is unaffected by cord injury at the C5–6 level in the ferret.

The spinal cord contusion technique has been described in detail elsewhere. Briefly, it consists of a constrained-stroke pneumatic cylinder mounted on an adjustable crosshead frame. The spine is fixed in relation to the base of the frame, and the amount of compression can be chosen by adjusting the crosshead height relative to the animal. The velocity of compression is determined by the input of air pressure, with a cylinder stroke controlled by a solenoid air valve. The system provides a high degree of mechanical reproducibility, and yields consistent injuries across a spectrum of injury severity.

Animals received 25%, 50%, or 75% compression at a contact velocity of 0.6, 3.0, or 10.0 m/sec (Table 1). The 10 animals receiving the 75% compression level exhibited no recovery of SEP's at any of the contact velocities.

Somatosensory evoked potentials were measured at 30 minutes, 60 minutes, and 4 hours after contusion. Effect of injury on spinal cord conduction was assessed by comparing SEP's at 4 hours with those obtained before compression. Both SEP amplitude and latency were compared for pre- and post-contusion potentials, concentrating on changes in the initial positive-negative peak. Latency to the positivity of this peak has been highly reproducible in control experiments, and will be the experimental parameter reported here. Forelimb evoked potentials were recorded as an internal control for systemic changes which might generally affect SEP's rather than the specific effects on conduction through the injured region of the spinal cord. Since the forelimb fibers enter the spinal cord above the level of the injury in the ferret, forelimb evoked potentials reflect only systemic influences such as anesthetic depth and brain or spinal cord temperature.

At the end of the 4-hour period, animals were deeply anesthetized, and a laminectomy was performed to allow removal of a four-segment portion of the spinal cord spanning the lesion area. Following removal of the spinal cord segment, animals were sacrificed with intravenous T-61* (0.7 ml/kg), followed by a bilateral pneumothorax. Spinal cord samples were immersion-fixed in formalin and examined by light microscopy for the extent of hemorrhagic necrosis.

**Results**

The changes in latency following cord contusion are summarized in Table 2 for the 25% and 50% compression levels. Although evoked potentials were recorded at intermediate time points, only the data from the 4-hour post-contusion recording are shown here. Typical pre- and post-contusion SEP recordings are shown in Fig. 1. In all cases, the recording immediately after injury showed an absence of conduction through the

* Veterinary euthanasia solution obtained from Taylor Pharmacal, Decatur, Illinois.
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When the comparison between contact velocities is made for a constant amount of cord compression, there is no statistical difference between the tested velocities. However, there is a trend toward increased latency at the higher contact velocity, evident for both 25% and 50% compression. At intermediate time points, the recovery of evoked potential latency was slowed for the high-velocity contacts in comparison to the lower velocity.

The amplitude of the positive-negative wave exhibited greater variability than the latency measurements, but a reduced amplitude reflecting impaired neuronal conduction correlated with the amount of compression delivered. Amplitude of the evoked response was only slightly affected by increased contact velocity, and while the reduction in amplitude between the 25% and 50% levels was statistically significant, the difference between the various contact velocities was not.

Typical histology is shown in Figs. 2 and 3, contrasting the amount of hemorrhagic necrosis at 25% and 50% compression delivered at 0.6 and 10.0 m/sec. The extent of hemorrhage and resultant necrosis is increased at the higher velocity contact for a given level of compression. In fact, the hemorrhage resulting from a 25%, 10.0 m/sec compression is more extensive than that from a 50%, 0.6 m/sec compression, although the latency increase was greater in the 50% compression case. The results for a 75% compression were similar, with the extent of hemorrhage and resultant necrosis being greater for the higher velocity contact. However, in the case of 75% compression, recovery of the evoked response did not occur for either the 0.6 or the 10.0 m/sec contacts.

FIG. 1. Typical somatosensory evoked potentials recorded pre- and post-compression, for a 25%, 3.0 m/sec and a 50%, 3.0 m/sec compression.

injury site, with slow recovery of the latency over the 4-hour time period. However, the individual variation was greater at the intermediate time points, preventing comparisons between different levels of compression or contact velocities.

The 3.0 m/sec contact velocity was comparable to previous findings, and to the calculated contact velocity for a weight-drop contusion injury from a height of 20 cm. Essentially no effect was observed for the 25% compression, regardless of contact velocity over the range tested. A 50% compression resulted in a consistent increase in latency, to approximately double the normal values. This increase was statistically significant in comparison to both control values and to values obtained after a 25% compression.

Fig. 2. Cross sections of contused spinal cord. H & E, x 20. Left: Cord subjected to 25% compression at 0.6 m/sec contact velocity. Right: Cord subjected to 50% compression at 0.6 m/sec contact velocity.
FIG. 3. Cross sections of contused spinal cord. H & E, × 20. Left: Cord subjected to 25% compression at 10.0 m/sec contact velocity. Right: Cord subjected to 50% compression at 10.0 m/sec contact velocity.

Discussion

The results of this study dissociate the extent of hemorrhagic necrosis from loss of spinal cord conduction following contusion, and suggest that during the subacute postinjury period, impaired conduction correlates with the amount of compression rather than the contact velocity. Correlation between extent of hemorrhagic necrosis and subacute loss of conduction, as reported in the literature, may result from the biomechanics of the injury model used rather than the pathophysiological basis of neuronal dysfunction.

The loss of spinal cord conduction following a standard amount of cord compression is clearly affected by the contact velocity of the compression when velocities are sufficiently distinct. Slow cord compression by balloon inflation blocks neural conduction through the site of compression only when compression is sufficient to block blood flow in the area. Such local ischemia requires compression in excess of 80%. The long-range effects of slow compression are also minor; compression of 75% can be sustained for up to 7 minutes with no permanent effect on conduction. Neurological deficit from long-standing compression of the cord is reduced by cord decompression, provided the compression had slow onset.

Dynamic compression of the spinal cord has a more severe effect on conduction. Levels of compression that have no effect when applied slowly cause an immediate loss of conduction through the injured site after a dynamic compression. The effect of maintained compression is also more severe. When dynamic compression sufficient to block conduction is maintained for more than 1 minute, permanent loss of conduction through the compression site results. The distinction between effects of slow and fast compression is not a result of vascular effects, since changes in local blood flow after dynamic compression parallel alterations seen following slow compression. In both cases, ischemia occurs during compression, and a period of hyperemia follows cord decompression.

The duration of compression in pneumatic spinal cord contusion is less than 5 seconds, yet compression of 75% or more at a contact velocity of 3 m/sec produces a loss of evoked potentials for at least 6 hours. Slow application of the same amount of compression has no permanent effect unless maintained for more than 7 minutes. Dynamic 40% compression results in impaired conduction (increased latency, decreased amplitude) through the injured site when response is assessed over the same period.

Results from weight-drop experiments are difficult to compare directly to dynamic compression data, since the amount of compression and the contact velocity are not often reported for weight-drop contusion of the cord. Indirect measures of the amount of compression have been made in two studies, however, and contact velocity can be estimated from the height of the drop. The data from these two studies demonstrate significant impairment or loss of neuronal conduction following compression of 40% or more, delivered at approximately 2 m/sec. Mechanical damage to nerve fibers in the region of compression may be a critical factor in determining permanent loss of neuronal conduction, with local ischemia and hypoxia playing secondary roles. Neither local ischemia nor hypoxia can fully explain the differences in functional results when comparing fast versus slow compression. Measurements of blood flow in compressed spinal cord have documented comparable levels of ischemia for both fast and slow compression, yet with different functional outcome. When direct vascular manipulations have been at-
tempted, the severity of permanent functional injury correlates with the dynamics of concurrent cord compression rather than with the extent of ischemia or hypoxia.6,7

An important point relative to the current study, however, is that function was not evaluated beyond 4 hours post-contusion. It is possible that delayed loss of function would occur in the less severe compressive injuries that exhibited extensive central hemorrhage. The hemorrhage within central gray matter may induce local vasospasm and promote degradation of neuronal and vascular membranes, thus contributing to loss of neuronal conduction and to impaired microvascular circulation and tissue fluid balance.8 Effects on neuronal conduction would not be expected within the 4-hour post-contusion period evaluated in the current study.

In the clinical setting, SEP's at the time of hospital admission do not always correlate with ultimate functional recovery.16 This is in contrast to observations made using the weight-drop contusion model for experimental spinal cord injury. The discrepancy may reflect delayed pathophysiological changes in the spinal cord, which have not been observable in the experimental situation because of the injury model used, and which do not have acute effects on cord conduction. The availability of an injury model which allows dissociation between contact velocity and compression amount will be useful for a more careful analysis of delayed effects of developing pathophysiology on neuronal conduction.

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References