Hemorrhagic Changes in Experimental Spinal Cord Injury Models

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ABSTRACT: Early hemorrhagic changes in the spinal cord were compared in three experimental spinal cord injury models in the rat in order to determine the nature and consistency of spinal cord hemorrhage following specific and quantitated forces of injury. The spinal cords were injured by weight-dropping, aneurysm clip and extradural balloon compression techniques. Hemorrhagic changes were assessed quantitatively by the image analyser at 1 and 3 hours after injury. Tissue damage was assessed by determining the percentage of total cross sectional area containing hemorrhage. The extent of hemorrhage at site of injury in the clip and balloon preparations was equal, but several times lower in the weight-drop induced injury. Within each experimental group no appreciable differences were observed at the site of injury between the 1 and 3 hours preparations. The variability of damage within experimental groups was most in the weight-dropping and balloon and least in the clip preparations. Differences were also indicated with respect to the distribution of hemorrhage in grey versus white matter. These findings may be of significance when functional recovery is considered in various experimental acute spinal cord injury models.

RÉSUMÉ: Modifications hémorragiques dans divers modèles expérimentaux de lésions de la moelle Afin de déterminer la nature et la constance de l'hémorragie de la moelle après des forces agressives spécifiques et quantifiées, nous avons comparé trois modèles expérimentaux de lésions: chute de poids, pince à anévrysme et compression extradurale par ballon. Les modifications hémorragiques furent quantifiées par analyse d'image une et trois heures après la lésion. L'atteinte tissulaire fut déterminée en pourcentage de l'aire sectionnaire comprenant des hémorragies. L'étendue de l'hémorragie au site de la lésion était égale pour la méthode ballon et la pince à anévrysme, mais était de beaucoup inférieure avec la chute de poids. Aucune différence ne fut observée au site de la lésion entre une et trois heures après la lésion. La variabilité des résultats était maximale avec les méthodes de chute de poids et du ballon et minimale avec la pince. Il y avait aussi des différences entres les matières blanche et grise. Ces résultats peuvent être d'importance pour l'évaluation du recouvrement fonctionnel après lésion de la moelle dans divers modèles expérimentaux.

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Egyptian physicians described effects of cervical spinal cord injuries about 5000 years ago (Breasted J.H., 1930). One of the first significant experimental histological studies on the injured spinal cords of birds and mammals was reported by Brown-Séquard (1850). For about 100 years thereafter, experimental spinal cord injury was commonly produced by laminectomy and surgical transection of the spinal cord. Allen (1911) was the first to standardize open impact producing injuries on the spinal cords of animals. It took another 25 years before Amako (1936) in Japan, and 42 years before Freeman and Wright (1953) in the United States began to use Allen's method to investigate experimental spinal cord injury. Subsequently several investigators have used the weight dropping technique with minor modifications to produce and study experimental spinal cord injuries in animals.

Tarlov (1957) introduced the extradural balloon compression technique to produce experimental spinal cord injury in dogs. A more recent acute spinal cord injury model in the rat was described by Rivlin and Tator (1978), in which a modified aneurysm clip with a consistent quantifiable force was used to produce a standardized degree of spinal cord injury.

Khan and Griebel (1983) have recently reported on a comparison of experimental spinal cord injury models in the rat using the weight-dropping, aneurysm clip and extradural balloon compression techniques. The present investigation was undertaken in an attempt to assess, compare and determine the consistency of hemorrhagic changes within the spinal cord at 1 and 3 hours following spinal cord injuries in the rat by these techniques.

Materials and Methods

Forty-five Sprague-Dawley adult female rats weighing 250 to 300 grams were used in this study. Each animal was anesthetized with intraperitoneal Pentobarbital 4 mg per 100 gram body

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weight. The spinal cord was injured at the midthoracic level of the spinal canal using the weight-dropping, aneurysm clip and extradural balloon compression techniques as previously described (Khan & Griebel 1983). Ten rats were each subjected to a 50 gram cm force injury (weight-dropping), while a second group of 10 animals had their spinal cords injured by a modified aneurysm clip with a closing force of approximately 50 grams for 10 seconds. A third group of 10 rats had their spinal cords compressed for 5 minutes with a 0.1 cc extradural balloon using a model 12-060-2F Fogarty arterial embolectomy balloon catheter. These forces were chosen on the basis of our previous experience comparing three experimental techniques in acute spinal cord injury in the rat (Khan & Griebel, 1983). In our laboratory, we had noted that when larger forces were used to produce acute spinal cord injuries than those chosen in this study, rather extensive damage was produced, rendering quantitative measurement of hemorrhage inaccurate by our technique. On the other hand, smaller forces produced injuries which were least consistent especially with the weight-dropping and balloon techniques. The 50 gram/cm force was produced using a 5 gram impounder dropped through a vented brass tube from a height of 10 centimeters. The balloon compression injury was produced by inflating the balloon with 0.1cc air and compressing the cord for 5 minutes, whereas the clip compression injuries were produced by placing a modified Kerr-Lougheed aneurysm clip around the cord extradurally for 10 seconds. Animals were sacrificed at 1 and 3 hours respectively following the injury and the entire thoracic spine removed immediately and placed in 10% buffered formalin solution. The thoracic spinal cords were removed 7 days later and sectioned for hematoxlyn and eosin (H & E) staining at the sites of injury, and 0.25 mm and 5 mm above and below this level.

Fifteen animals were used as controls. Five animals were subjected to two-levels middorsal laminectomies and used as controls for the weight-dropping injured cords. Another group of 5 animals had similar laminectomies with placement of the aneurysm clip around the cord without compression for 10 seconds, while a third group of 5 rats had partial midthoracic laminectomy and introduction of the balloon catheter for 1 cm up the extradural space but without inflation of the balloon for 5 minutes. These 3 groups of control animals were all sacrificed at 3 hours after surgery with immediate removal of the thoracic spine which was placed in 10% formalin for 7 days. The thoracic spinal cords were then removed and sectioned for H & E staining.

The extent of hemorrhage in cord tissue was quantitated with an image analyser. Images of slides were projected onto a screen and hemorrhagic areas were outlined with a cursor. A computer attached to the screen was used to assess the cross-sectional area in the delineated hemorrhagic zones. These zones were identified by the presence of red blood cells in a blind study. The total cross-sectional area of all hemorrhagic areas was determined for both grey and white matter and expressed as a percentage of total cord cross-sectional area.

Results

Changes at site of injury

Figure 1A shows hemorrhagic tissue in spinal cord at the site of surgical intervention.

No statistically significant differences were detectable with respect to total percentage of area occupied by hemorrhagic tissue at 1 vs 3 hours postoperatively in any of the experimental groups (Fig 2). The extent of damage in clip lesioned rats was significantly greater than in weight-drop treated animals at 3 hours after injury (paired t test, p less than 0.05) (Fig 2). The

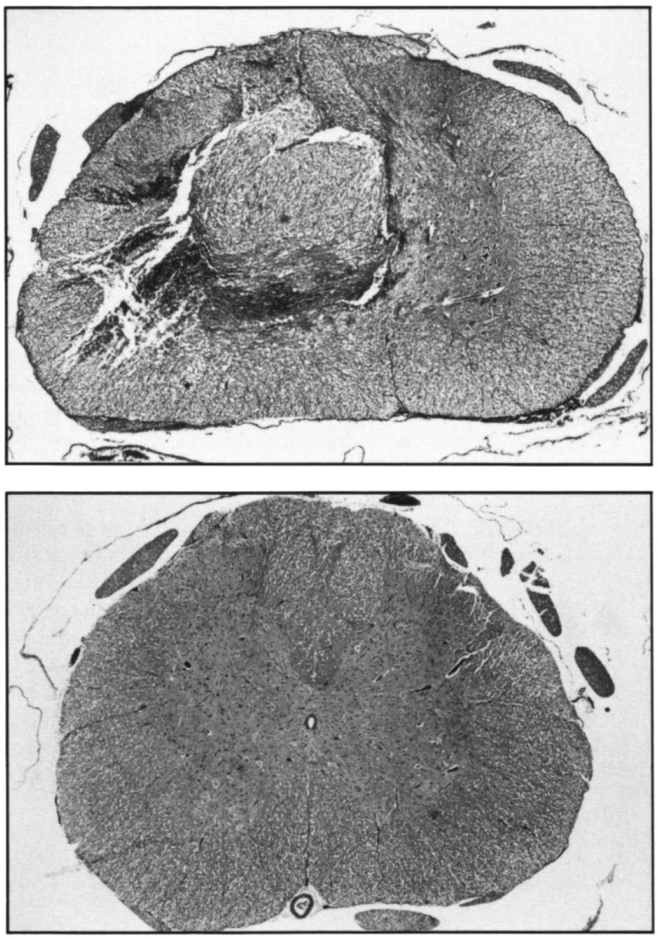

Figure 1 — Light micrography showing clip lesion at 3 hours postoperatively at site of injury (A) and in uncrushed control preparations (B).

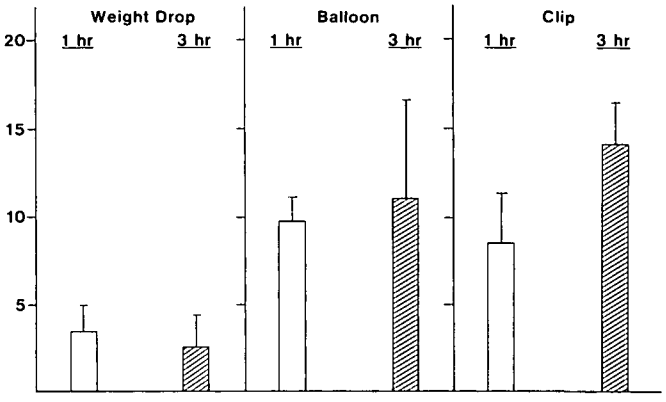

Figure 2 — Histogram showing percent cross-sectional area of spinal cord occupied by hemorrhagic tissue at 1 (unshaded) and 3 (shaded) hours postoperatively at site of lesion for weight drop, balloon compression and clip induced trauma. Shown are means and S.E.M.

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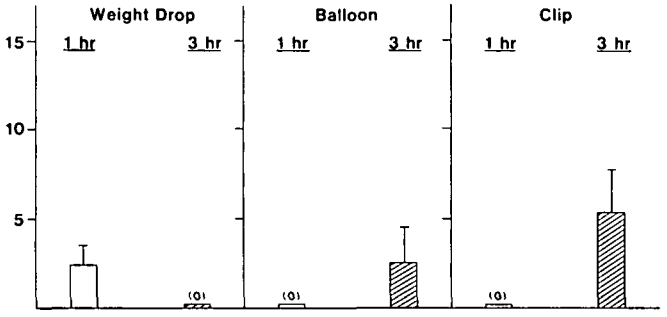

Figure 3 — Histogram showing percent cross-sectional area occupied by hemorrhagic tissue in white matter at site of injury.

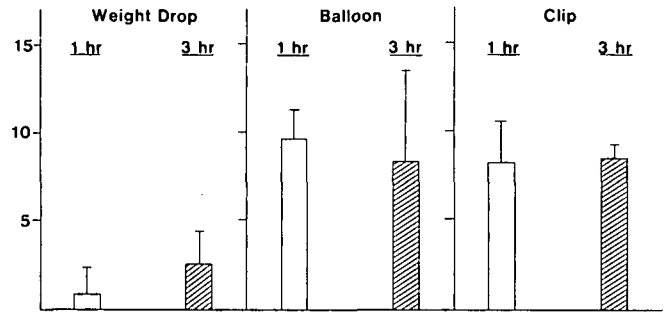

Figure 4 — Histogram showing percent cross-sectional area occupied by hemorrhagic tissue in grey matter at site of injury.

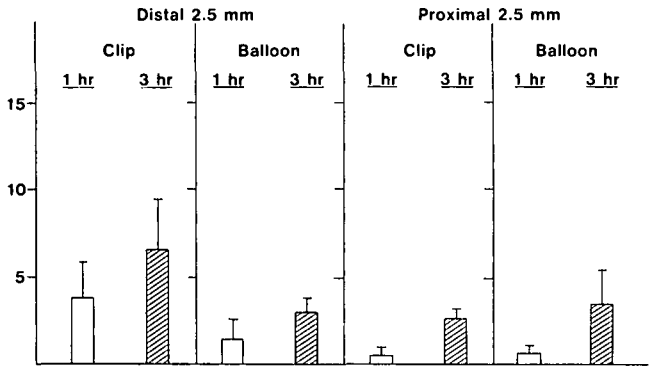

Figure 5 — Histogram showing percent cross-sectional area occupied by hemorrhagic tissue proximal and distal to site of surgically induced lesions.

high degree of variability within the balloon preparation group did not allow statistical comparison with clip or weight-dropping lesioned animals.

Animal to animal variations within groups at 3 hours post-operatively was lowest after clip lesion, and marked following the weight-drop and balloon induced injuries. Differences were indicated with respect to the distribution of damage in grey vs white matter (Fig 3 and 4). In weight drop preparations, more white than grey matter involvement was evident at 1 hour postoperatively whereas more grey than white matter change appeared to take place by 3 hours after surgery. By contrast, results obtained with the clip injury indicated little or no white matter damage at 1 hour post-injury (values *not* being significantly greater than zero) but significant white matter involvement 2 hours later.

The basis for these pathological changes are at this point uncertain. However, variations in distribution of hemorrhagic tissue suggest different mechanisms of injury and, possibly subsequent degenerative-reactive changes as a function of the means used to induce trauma.

Changes distal and proximal to site of injury

Significant spread of damage was evident at 2.5 mm proximal and distal to site of lesion but not at 5.0 mm from the lesion site. Hemorrhagic tissue was observed after balloon and clip injury at the 3 hour point involving both grey and white matter (Fig 5).

Control Group

Minimal subarachnoid hemorrhage along the posterior cord surface was present in all animals subjected to two levels middorsal laminectomies. This was only present at level(s) of the laminectomy(ies) (Fig 1B). In addition, 3 of the 5 aneurysm clip control cords had minimal subarachnoid hemorrhage along the lateral surface of the cord where the clip was introduced. No subarachnoid hemorrhage was noted in the balloon control cords. There was no evidence of hemorrhage within the spinal cords of any control animal.

Discussion

Despite several varieties of methods by which a spinal cord may be injured experimentally, we are not familiar with any report in which a comparison of the hemorrhagic changes in these various models have been documented. In a previous report on experimental spinal cord injury in the rat (Khan and Griebel, 1983), the relationship between different types of injuries and subsequent clinical recovery was studied. The aneurysm clip compression technique resulted in relatively consistent cord injuries with respect to subsequent clinical recovery in comparison to the weight-dropping and balloon compression methods. The results of the present study indicate variability of hemorrhage within the spinal cord was most in the weight-dropping and balloon and least in the clip injuries.

One of the first reports on hemorrhagic changes in experimental spinal cord injuries is that of Allen (1914) who noted that intramedullary hemorrhage and edema followed his impact injury and reached a maximum at about 4 hours. Later, Tarlov (1957) noted extensive hemorrhages and disruption of nerve tissue within the spinal cord of dogs in cases of severe balloon compression.

The light microscopy of experimental spinal cord injuries has been detailed by several authors (Yeo et al, 1957; Goodkin and Campbell, 1969; Wagner et al, 1969; White and Allen, 1970; Ducker et al, 1971; Osterholm, 1974; Wagner and Dohrmann, 1975; Windle, 1980). These studies document central spinal cord injuries which appear to increase with time after trauma.

Nelson et al (1977), postulated damage to the vascular endothelium of the spinal cord, either primary as a result of injury or secondary due to vasospasm, as the major contributing factor in the pathogenesis of central hemorrhagic necrosis after acute cord trauma.

Koenig and Dohrmann (1977) described the histopathological variability in "standardized" spinal cord trauma in cats using the weight-dropping technique. The results of their study indicated considerable variability in the degree of hemorrhage within the cord following impact with 400 g cm force. Several other

investigators have employed this technique to produce experimental spinal cord injuries with major discrepancies in the results of the experiments (Koozekanami, 1976; Albin et al, 1967; Freeman and Wright, 1953; Kajihara et al, 1973). In the present study, similar wide variations in the degree of hemorrhage were noted in the spinal cord of rats injured by the weight-dropping technique.

Ballentine (1978) described early changes in blood vessels and the development of tissue necrosis in the spinal cords of adult Sprague-Dawley rats injured by the weight-dropping technique. He noted that the intramedullary hemorrhages immediately after trauma were mainly petechial and involved in aggregate an average of 11% of the cord at the level of maximal bleeding. This increased in size over the ensuing 8 hours, occupying an average of 27.8% of the cross-sectional area of the cord, but with variation from 10 to 41%. Larger hemorrhages involved both white and grey matter. On our study, no significant increase in hemorrhage was detectable at the 1 versus 3 hour post-injury periods in any of the three experimental groups. Differences were also noted with respect to the distribution of hemorrhage in grey versus white matter.

Rawe et al (1978) studied the effect of systemic blood pressure on histopathological changes of experimental spinal cord trauma and indicated that most hemorrhages probably occur within the first hour. Our present study concurs with this finding. We did not monitor the systemic blood pressure in these animals. Thus, the considerable variation in hemorrhage noted in the balloon compression and weight-dropping injured cords may be secondary to variation in systemic blood pressure, although this degree of variation was not present in animals injured by clip compression.

Rivlin and Tator (1978) described regional spinal cord blood flow in rats after severe cord trauma produced by the aneurysm clip compression technique. Hemorrhages in the spinal cord were noted to be radioactive 15 minutes after injury but were not radioactive in the 2 hour or 24 hour specimens. This was thought to be due to cessation of bleeding between 15 minutes and 2 hours after injury.

The present study examined the extent and distribution of hemorrhagic issue associated with 3 experimental models of spinal cord injury. The extent of total damage appeared to be greatest for clip lesion and lowest with respect to weight-drop preparations. Variations between animals in the same experimental group were least after clip lesion, and marked following weight-drop or balloon induced injury. Marked differences were also noted with respect to the distribution of this damage between the 3 preparations. While the pathological basis for these differences are highly speculative at this time, they suggest different mechanisms by which these surgical interventions induce pathological alterations in the spinal cord within the first 3 hours after injury. These findings are probably of significance when functional recovery is asessed in various experimental acute spinal cord injury models. Further studies will be required to determine if the 3 experimental preparations employed here produce marked differences in terms of subsequent degenerative or regenerative changes in traumatized spinal cord tissue.

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