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Research Report

The role of hemorrhage following spinal-cord injury ☆

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ABSTRACT

Spinal-cord injury is characterized by primary damage as a direct consequence of mechanical insult, and secondary damage that is partly due to the acute inflammatory response. The extent of any hemorrhage within the injured cord is also known to be associated with the formation of intraparenchymal cavities and has been anecdotally linked to secondary damage. This study was designed to examine the contribution of blood components to the outcome of spinal-cord injury. We stereotaxically microinjected collagenase, which causes localized bleeding, into the spinal cord to model the hemorrhage associated with spinal cord injury in the absence of significant mechanical trauma. Tissue damage was observed at the collagenase injection site over time, and was associated with localized disruption of the blood-spinal-cord barrier, neuronal cell death, and the recruitment of leukocytes. The magnitude of the bleed was related to neutrophil mobilization. Interestingly, the collagenase-induced injury also provoked extended axonal damage. With this model, the down-stream effects of hemorrhage are easily discernible, and the impact of treatment strategies for spinal-cord injury on hemorrhage-related injury can be evaluated.

1. Introduction

Traumatic spinal-cord injury (SCI) causes irreversible axonal damage and neuronal death, resulting in permanent disability. In addition to the initial mechanical injury, a cascade of events takes place that precipitates further axonal damage and neuronal death long after the primary insult. This cascade of events is collectively termed secondary injury (Sekhon and Fehlings, 2001), and was first postulated as early as 1914 by Allen who suggested that noxious agents present in the

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hemorrhagic material might cause further damage to the spinal cord (Allen, 1914). The mechanisms of secondary injury include hemorrhage, tissue ischemia, blood-spinal-cord-barrier (BSCB) breakdown, inflammation, and glutamate toxicity, as well as demyelination and apoptotic signaling (Fleming et al., 2006).

SCI is associated with mechanical insult to the microvasculature, leading to a primary hemorrhage into the spinal cord. Subsequent to the initial bleeding, there is a formation of secondary petechial hemorrhages in the tissue surrounding the primary lesion that have been shown to be related to a dramatic increase in the extent of the lesion (Gerzanich et al., 2009). Blood infiltrating the central nervous system (CNS) tissue has been shown to be toxic by itself (Asano, 1980), and such internal bleeding leads to the following toxic events: elevated thrombin formation, increased extracellular glutamate level, red blood cell lysis, and iron toxicity (Hua et al., 2006; Wagner et al., 2006). Following damage to the microvasculature, there is a local reduction of blood flow resulting in different degrees of ischemia (Senter and Venes, 1978). In addition, the disruption of the BSCB and the resulting inflammatory response disturb the micro-environment and expose the adjacent tissue to molecules that can be noxious to non-injured tissue (Schlosshauer, 1993).

The importance and a description of hemorrhage following SCI has been described previously (Mautes et al., 2000; Sinescu et al., 2010; Tator and Fehlings, 1991; Tator and Koyanagi, 1997). Notably, Tator and Koyanagi (1997) examined the distribution of hemorrhage following spinal cord injury in human. Their findings support the description that vascular damage is principally located in the intramedullary system, while the major arteria on the surface are usually spared. Thus hemorrhage after SCI is mostly distributed within the gray matter, which has proved difficult to model given that most injury models rely on the application of mechanical damage to the dorsal surface.

Hitherto most accepted knowledge about the effect of hemorrhage in the spinal cord has been extrapolated from data from intracerebral hemorrhage (ICH) studies (Mautes et al., 2000), but such studies on the spinal cord have not been carried out. While the spinal cord is part of the CNS and shares many common characteristics with the brain, major immunological and anatomical differences do exist. An important example is the distinctive inflammatory response in the spinal cord compared to the brain following traumatic injury (Schnell et al., 1999). To assess the contribution of hemorrhage in traumatic spinal-cord injury, current mechanical models of spinal-cord injury using compression, contusion and surgical section are of limited value. A model is required which restricts the mechanical component of injury to the vessels.

In the present study, microinjections of bacterial collagenase into the rat spinal cord were used in order to investigate the effect of intraspinal hemorrhage in the absence of severe mechanical injury. Bacterial collagenase digests type VI collagen in the basal lamina of blood vessels and has been used in established animal models of ICH (MacLellan et al., 2008). The following issues have been addressed: whether the degree of hemorrhage and blood-spinal-cord-barrier breakdown induced by collagenase injection is reproducible, whether the degree of inflammatory response following hemorrhage in the spinal cord correlates with the level of hemorrhage, and whether there is evidence of neuronal and axonal damage. To observe the temporal sequence of events, three different time-points after the microinjection were chosen for the tissue collection: 6 h, 1 day and 7 days. We envisage that an improved understanding of the effect of hemorrhage on the spinal cord will aid clinical decisions in the acute setting, and may contribute to development of novel therapeutic interventions in the management of traumatic spinal cord injury.

2. Results

2.1. *Collagenase disrupts the BSCB*

Collagenase (0.12 U) or vehicle (saline) was microinjected into the spinal cord after laminectomy at T8. In order to examine the integrity of the blood-spinal-cord-barrier breakdown, we injected HRP intravenously 30 min before taking out the cords for immunohistochemistry. After processing, Hanker-Yates staining allowed us to quantify the extent, if any, of the damage done to the BSCB (Fig. 1a–c). In the animals injected with collagenase, we observed BSCB breakdown at 6 h ($P<0.001$) and 1 day after the microinjection ($P<0.01$). 7 Days after collagenase injection, a small amount of BSCB breakdown was still visible in some animals, but the difference compared to the vehicle-microinjected animals did not reach statistical significance ($P=0.074$). Little or no damage to the BSCB was observed in animals microinjected with vehicle at any time point. Any low-level damage found is probably attributable to the mechanical insertion of the microcapillary used for the injection.

2.2. *Collagenase leads to intraspinal hemorrhage*

Following the loss of BSCB integrity due to the collagenase injection, we could observe an intraspinal hemorrhage, which was visible under the microscope as a brown-pigmented area (Fig. 1d–f). The pattern of the intraspinal hemorrhage measured was similar to that of the loss of BSCB integrity. Evident hemorrhage was observed 6 h ($P<0.001$) and 1 day ($P<0.05$) after the microinjection of collagenase, but none was detected after 7 days. In the vehicle-injected animals, little, if any, hemorrhage was observed at any time-point.

2.3. *Neutrophil profile in response to hemorrhage*

There is no resident population of neutrophils in the spinal cord, and we were interested to discover whether hemorrhage caused by the microinjection of collagenase into the cord was sufficient to locally recruit neutrophils. Significant numbers of neutrophils were observed at 6 h after the injection of collagenase (Fig. 2a–c, $P<0.001$), and their magnitude remained high at 1 day ($P<0.05$). After 7 days, the number of neutrophils was still elevated in all the animals injected with collagenase compared to those injected with saline, but the numbers were low and the result did not reach significance. Some of the neutrophils observed in the spinal cord could be