

● INVITED REVIEW

# Impact of vasculature damage on the outcome of spinal cord injury: a novel collagenase-induced model may give new insights into the mechanisms involved

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## Abstract

The deleterious effect of vasculature damage on the outcome of spinal cord injury has long been recognized, and numerous clinical studies have shown that the presence of hemorrhage into the spinal cord is directly associated with a poorer neurological outcome. Vascular damage leads to decreased blood flow to the cord and the release of potentially toxic blood-borne components. Here we consider the mechanisms that may be contributing to hemorrhage-induced damage and discuss the utility of a new model of spinal cord hemorrhage, which was urgently required as most of our current understanding has been extrapolated from intracerebral hemorrhage studies.

**Key Words:** spinal cord injury; vasculature; hemorrhage; animal model; collagenase; stereotaxic microinjection

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The central nervous system (CNS) does not have the plasticity of the peripheral nervous system (PNS), and traumatic spinal cord injury (SCI) causes irreversible damage to the cord. In addition to the initial mechanical injury, a long lasting cascade of events, defined as secondary injury, occurs and gives rise to further axonal damage and neuronal death (Sekhon and Fehlings, 2001). The mechanisms of secondary injury include hemorrhage, tissue ischaemia, blood-spinal-cord-barrier breakdown, inflammation, and glutamate toxicity, as well as demyelination and apoptotic signaling (Fleming et al., 2006). However, the relative contribution of each of these components remains unclear. As long as 100 years ago, Allen (1914) suggested that deleterious agents present in the hemorrhage could cause further damage to the spinal cord. In this review, after a brief description of the spinal cord blood supply, we focus on the importance of vascular damage to the outcome of SCI. We will also discuss a new animal model, recently developed in our laboratory, which we hope will help to lead to a better understanding of these events (Losey et al., 2014a).

## Blood supply of the spinal cord

For the investigation of hemorrhage after SCI the rat can be considered to be a good model as the anatomy of arterial supply is almost an exact match to the human. In both rats and humans, the blood supply to the spinal cord is provided by three vessels that run along the spinal cord, namely the

ventral (anterior in a human) spinal artery and the paired dorsal (posterior in a human) spinal arteries [for an extended review, see for example (Bosmia et al., 2013)]. The ventral spinal artery arises from the confluence of two distal branches of the vertebral arteries close to the medulla oblongata and runs caudally along the midline of the ventral aspect of the spinal cord. This artery supplies about 75% of the blood needed by the spinal cord (Wan et al., 2001). The dorsal spinal arteries emerge from either the vertebral arteries or the dorsal inferior cerebellar arteries and run caudally on the dorsolateral side of the spinal cord adjacent to the entrance of the dorsal roots. In the rat, there are sometimes additional longitudinal arteries, namely a median dorsal spinal artery located at or near the dorsal septum, and two lateral spinal arteries located at equidistance between the entrance of the dorsal and ventral roots. The presence of these additional arteries is only frequent in the cervical part, and less in the more caudal parts of the spinal cord (Paxinos, 2004). Below the cervical level, the arterial supply in human and rats is strengthened by the radicular arteries, which location can vary, derived from segmental arteries that split into ventral and dorsal branches.

The microcirculation into the spinal cord is either centrifugal (directed outward from the center) or centripetal (directed toward the center). The centrifugal system is formed by branches of the ventral spinal artery called the sulcal arteries. These branches enter the ventral median fissure of

the cord and provide blood to the greater part of the grey matter and the inside half of the white matter. The centripetal microcirculation is formed by the combination of other branches of the ventral spinal artery, named the pial arterial network, with branches of the dorsal arteries. This centrifugal system supplies the dorsal horns, most of the posterior white matter, and the outer portion of the ventrolateral white matter. It is important to note that the capillary network is much denser in the central grey matter than in the white matter of the spinal cord. Thus in terms of gross spinal anatomy, the cord is the opposite of the brain in this respect where the cortex is highly vascularized. This is likely to have a bearing on outcome after injury to the brain or spinal cord and may contribute to cavitation, which is often observed in the cord after injury, but not in the brain. Venous drainage is also very different in the cord compared to the brain. Veins in the central sulcus, dorsal to the ventral spinal artery run the entire length of the spinal cord and damage to this vessel may result in damage that extends further rostrally and caudally in the cord.

### Vascular response following SCI

As a spinal trauma occurs, the integrity of the spinal arteries and those adjacent to the spinal column are often compromised. The deeper arteries are, fortunately, rarely affected as such damage leads to devastating neurological deficits. However, the spinal cord microcirculation system is commonly damaged by SCI, giving rise to intraparenchymal or intramedullary hemorrhage. The presence of hemorrhage in the human spinal cord after SCI has been connected with significantly decreased motor function (Flanders et al., 1996). While 21% of the patients in the Flanders study with incomplete motor injuries presented with evidence of hemorrhagic lesions on their initial exams, 85% of the patients with complete motor deficits had evidence of spinal hemorrhage. Numerous other clinical studies confirmed that the presence of hemorrhage in the cord after SCI is associated with poor neurological outcome [for example (Andreoli et al., 2005; Boldin et al., 2006; Parashari et al., 2011)].

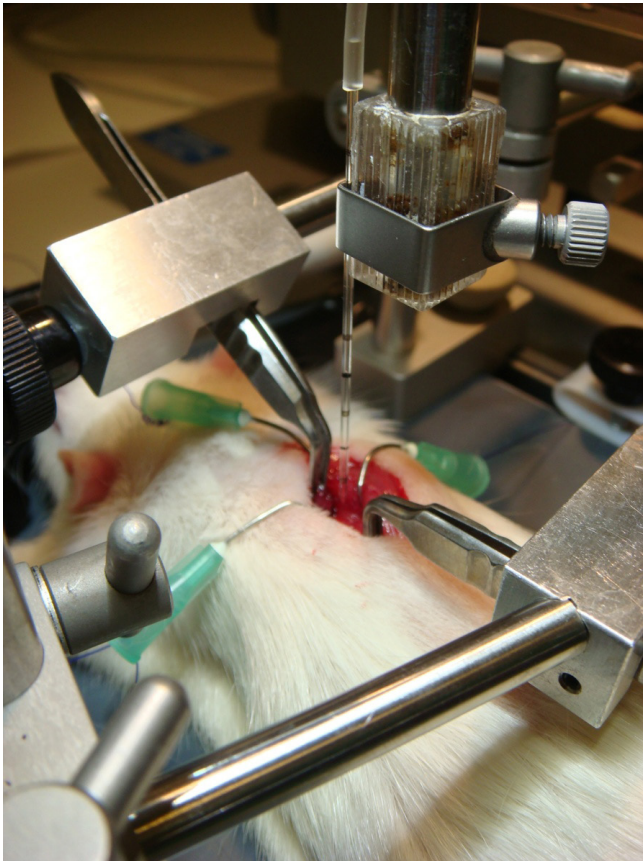
Secondary petechial hemorrhages follow the primary bleed around the primary injury site and are related to an enlargement of the initial lesion (Gerzanich et al., 2009). In the surrounding region of the hemorrhage, blood flow also drops. If we solely consider the hemorrhagic component of SCI, the reduction of flow could be, as suggested by data extracted from intracerebral hemorrhagic studies (Vespa, 2009), a consequence of low rates of oxygen consumption due to the impairment of mitochondrial function in the surrounding tissue, and would not represent per se an ischemic state. This matter is still controversial among intracerebral hemorrhage (ICH) researchers, but it is of interest to note that in a recent animal study, improving the blood flow in a SCI contusion model did not reduce acute microvessel, motor neuron or oligodendrocyte loss and failed to lead to improved functional recovery (Muradov and Hagg, 2013). On the other hand, the same laboratory showed that rescuing the vasculature using angiopoietin-1 and  $\alpha v\beta 3$  integrin

peptide was protective after SCI in mice (Han et al., 2010) and had lasting effects when treated from 4 hours after injury and during a week. Reducing ischemia could be one of the explanations for the improving the outcome, but the prevention of the leakage of blood/plasma into the spinal cord is also likely to play a key role. Blood *per se* has been shown to be toxic to CNS populations (Asano, 1980) and internal bleeding leads to elevated thrombin formation, increased extracellular glutamate level, red blood cell lysis, iron toxicity, inflammation and vasospasm (Hua et al., 2006; Wagner et al., 2006; Sinescu et al., 2010).

The presence of hemorrhage has also been related to edema formation *via* thrombin production and erythrocyte lysis. This is important as after SCI as edema volume is directly proportional to a poorer neurological outcome. In a recent clinical study, it was shown that removing the clots in patients with ICH significantly decreased the perihematomal edema (Mould et al., 2013). In this study, the percentage of the clot removed was also positively correlated to the perihematomal edema reduction. The potential benefit of clot removal after ICH is only possible due to the recent advances and refinement of minimally invasive surgery. In the spinal cord, Zhang et al. (2004) showed that clot removal, approximating the cord and closing the dura can lead to a significant improvement in outcome in a laceration SCI animal model. In order to test the potential beneficial outcome of intraspinal clot removal following SCI, further studies need to be performed using advanced minimally invasive surgery before moving to humans.

### New model to study the vascular injury

In conventional animal models of SCI it has always been difficult to explore the contribution of hemorrhage to the outcome of SCI in the absence of the trauma. Traumatic lesions invariably generate hemorrhage, but the amount of bleeding is often very variable. To isolate the impact of hemorrhage on the outcome of spinal cord injury, our laboratory has established a new animal model (Losey et al., 2014a). With the microinjection of collagenase into the rat spinal cord (Figure 1), we were able to disrupt the blood-spinal cord barrier and produce an ongoing intra-spinal bleeding in a controlled manner. Collagenase digests type VI collagen of the blood vessels basal lamina and it has become accepted as a useful model of ICH (MacLellan et al., 2008). However, as we have discussed above, the vascular anatomy of the spinal cord is very different from the brain. The principal technical advantage is that the collagenase is injected stereotaxically such that the position of the hemorrhage can be preselected and the amount of hemorrhage is very reproducible, which is very useful for preclinical trials. Classical SCI animal models usually affect the integrity of the dorsal (posterior) arteries, while in human SCI, it is mostly the anterior (ventral) arteries microvasculature integrity that is compromised (Therom et al., 1978). The dose of collagenase (0.12U) was chosen (Losey et al., 2008) to generate hemorrhage in the absence of any immediate and direct neurotoxicity (Matsushita et al., 2000).



**Figure 1** Microinjection technique.

The skin overlying the thoracic spine is shaved and a small incision is made in the midline. The subcutaneous tissues and muscle layers are blunt-dissected and a partial laminectomy is performed at thoracic level 8 ( $T_8$ ) to expose the underlying spinal cord without opening the dura mater. To minimize movement, the animal is suspended in the stereotaxic frame by clamping of  $T_7$  and  $T_9$  spinous processes. The tip of a finely drawn calibrated glass capillary tube is stereotaxically inserted into the grey matter of the spinal cord, 0.4 mm right lateral of midline and at a depth of 1.6 mm. The injection site was selected to ensure penetration into the spinal cord parenchyma and for its proximity to the white matter of the lateral funiculus. 0.5  $\mu\text{L}$  of collagenase (0.24 U/ $\mu\text{L}$ ) (or saline vehicle) is then injected over 2 minutes and the capillary is left in place for a further 3 minutes before being slowly withdrawn. The muscle layer is closed with 4/0 sutures and the skin approximated with wound clips. After surgery of this type, the animals recovered well and we did not observe evidence of overt distress or discomfort during the survival times of up to 7 days.

Using this model, we could show that disrupting the intraspinal vasculature and produced ongoing bleeding that was sufficient to induce extended neuronal damage. Damage to neurons was observed from six hours and neutrophils recruitment was also a feature at this time point, as well as the appearance of ED-1-positive macrophages and activated microglia. While the presence of recruited neutrophils into the spinal cord of collagenase-injected animals strongly diminished over a week, the number of ED-1 positive cells continued to increase over time for at least one week. Importantly, axonal damage, that was observable at six hours after the injection of collagenase continued for seven days. This was at a time-point well beyond the period of blood-spinal cord bar-

rier breakdown. We know that the staining technique used is only sensitive to the presence of relatively newly generated axonal end bulbs (Newman et al., 2001). From a behavioral perspective, the rats exhibited locomotor deficits during the first few days, but recovered then quite well. The plasticity of the rat nervous system is unlikely to explain this rapid recovery (Darian-Smith, 2009), and thus some of the dysfunction we observed is likely to be owing to axonal conduction block caused by pressure induced by the blood occupying space and the edema in the spinal cord following the injection of collagenase. Interestingly, another group recently published a study using a similar model to ours by injecting a different type of collagenase into the spinal cord and they also showed that bleeding affected the integrity of the white matter (Sahinkaya et al., 2014).

In conclusion, the spinal cord-microinjected collagenase model is a simple and very reproducible model that confirmed the important role of hemorrhage after spinal cord injury. We expect that this model will allow a better understanding of the molecules and mechanisms involved and will so have an important impact on the development of new spinal cord injury treatment strategies.

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