

Vascular Disruption and the Role of Angiogenic Proteins After Spinal Cord Injury

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Abstract Spinal cord injuries (SCI) can result in devastating paralysis, for which there is currently no robustly efficacious neuroprotective/neuroregenerative treatment. When the spinal cord is subjected to a traumatic injury, the local vasculature is disrupted and the blood–spinal cord barrier is compromised. Subsequent inflammation and ischemia may then contribute to further secondary damage, exacerbating neurological deficits. Therefore, understanding the vascular response to SCI and the molecular elements that regulate angiogenesis has considerable relevance from a therapeutic standpoint. In this paper, we review the nature of vascular damage after traumatic SCI and what is known about the role that angiogenic proteins—angiopoietin 1 (Ang1), angiopoietin 2 (Ang2) and angiogenin—may play in the subsequent response. To this, we add recent work that we have conducted in measuring these proteins in the cerebrospinal fluid (CSF) and serum after acute SCI in human patients. Intrathecal catheters were installed in 15 acute SCI patients within 48 h of injury. CSF and serum samples were collected over the following 3–5 days and analysed for Ang1, Ang2 and angiogenin protein levels using a standard ELISA technique. This represents the first description of the endogenous expression of these proteins in an acute human SCI setting.

Keywords Spinal cord injury · Angiogenesis · Blood–spinal cord barrier · Angiopoietin · Angiogenin

Introduction

Each year, over 10,000 North Americans suffer acute and permanent paralysis after sustaining a traumatic spinal cord injury (SCI) [46]. Not only is an SCI one of the most physically disabling and psychologically devastating traumas that an individual can survive, the socioeconomic burden is enormous. Estimates of the annual medical and rehabilitative expenses are in the \$1 million range for individuals with high cervical cord paralysis [176]. Whilst historically this has been an injury of the youth, an ageing population prone to suffering cervical cord injuries after falls has altered the demographics of SCI, with a second peak of traumatic SCI appearing in the elderly population aged 65 and above [191].

The poor neurologic prognosis for SCI has prompted the development of a plethora of therapeutic strategies, many of which have shown promise in the laboratory setting [122, 123, 185]. A handful of these have been evaluated in human clinical trials [78], but unfortunately, to date, none has proven to be convincingly efficacious at improving neurologic function for SCI patients [146]. Methylprednisolone remains the most extensively studied pharmacologic agent used for the treatment of acute SCI. However, mounting criticism around the execution and interpretation of data obtained from three landmark clinical trials (NASCIS I/II/III) has prompted many clinicians to abandon this drug, although it remains a ‘treatment option’ for acute SCI.

It has long been understood that when the spinal cord is injured (in, for example, a motor vehicle accident), local mechanical forces disrupt the complex vascular and cellular

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architecture of the cord but rarely transect the cord completely. This mechanical ‘primary injury’ is rapidly followed by an expanding cascade of ‘secondary damage’ mediated by pathophysiological mechanisms including ischemia, excitotoxicity, inflammation and oxidative stress (for review, see [120, 161]). Attenuating these mechanisms to minimize secondary damage and afford ‘neuroprotection’ to regions of the spinal cord that have escaped the primary injury remains a principal therapeutic strategy.

This highlights the fundamental need to understand the pathophysiology of the secondary spinal cord damage that rapidly follows the primary injury. It has become evident from a vast body of literature in rodent models of SCI that the processes that mediate secondary injury are extremely complex. Our focus in this review will be on one aspect of secondary injury after SCI: The damage to local vasculature, its reparative response and the role that angiopoietins 1 (Ang1) and 2 (Ang2) and angiogenin may play in mediating this response.

Vascular Supply of the Spinal Cord

In humans, perfusion of the thoracic spinal cord is largely dependent on the arterial supply from the aorta [135]. Segmental radiculomedullary arteries feed the anterior and posterior spinal arteries, which are the main circumferential arteries outside the cord parenchyma (known as ‘extrinsic’ arteries) [21, 75, 188–190]. These give rise to ‘intrinsic arteries’ which include the central arteries as well as the pial plexus, which arborize into intramedullary arterioles before ending as terminal capillary beds. The intrinsic arteries reside within the spinal cord parenchyma and can be separated into two discrete circuits [21, 75, 188–190]. These circuits flow in opposite directions, creating a watershed region where terminal capillary beds of the two circuits overlap. The ‘centrifugal circuit’ is fed by the anterior spinal artery and supplies the central two thirds of spinal cord capillaries, including the dense capillary networks which support spinal grey matter and the inner regions of white matter [190]. The ‘centripetal circuit’ is fed by the posterior spinal artery as well as the many anastomoses arising from the anterior and posterior spinal arteries [190]. Vessels of this circuit supply much of the posterior (dorsal) spinal cord as well as most of the peripheral white matter and the dorsal grey horns [75, 188–190].

The centrifugal and centripetal vascular circuits meet in a complex network of terminal capillary beds. It is important to note that there is an approximately 5-fold increase in the density of such capillary beds which serve the grey matter as compared to that of the white matter [190]. This is likely attributable to the greater metabolic demands of cell bodies

in the grey matter compared to axons of the white matter [188].

Vascular Injury After Spinal Cord Injury

It has been recognized for many years that trauma to the spinal cord disrupts its local vasculature. As early as 1914, Allen described the development of haemorrhage and oedema within the spinal cord after experimental SCI in dogs [5]. The primary insult causes immediate vascular disruptions at the injury epicentre [5, 26, 64, 89, 204]. This damage primarily affects the microvasculature, resulting in petechial haemorrhage observed at the injury epicentre immediately after injury [26, 42]. This haemorrhage starts near the central canal and is initially confined to the capillaries of the grey matter at 1 h post-injury, then spreads to the white matter by 2 h post-injury [5, 13, 147]. Most of the necrotic damage to endothelial cells occur during the first 24 h post-injury and can largely be attributed to the initial mechanical insult [26]. Further, endothelial cell loss after the first day, manifested as a decrease in intact blood vessel staining by rat endothelial cell antigen-1 [25, 130] and platelet–endothelial cell adhesion molecule (PECAM) [194], is attributed to apoptosis triggered by ischemia [26]. It has also been suggested that there is substantial oncotic endothelial cell death as a result of ionic imbalance due to the sustained activation of Trpm4 channels after SCI [60, 175]. Vessel density continues to decrease during the first 2 days, with little or no observable vessels at the injury epicentre [25, 194].

Angiogenesis is the process of blood vessel growth from existing vessels. This is distinct from the process of vasculogenesis, which is the de novo formation of blood vessels from mesenchymal tissue. After SCI, angiogenesis, presumably sprouting from vessels that are spared from the primary insult, starts 3 [42] to 4 days post-injury [25] and is observed for up to 1 week post-injury [25]. Revascularization to an extent that is comparable to control values [194] or even up 540% [42] in vessel density has been reported at 7 days post-injury [42, 194]. However, these neovessels, which grow longitudinally through the injury epicentre [25], are not associated with neurons, astrocytes [25] or pericytes [61]. The restoration of Glut-1 transporters, which are responsible for transporting a constant supply of glucose across the blood–brain barrier (BBB) to metabolically fragile CNS neurons, has not been observed until the 2-week time point [194]. This suggests that although there is significant angiogenic growth during the early post-injury phases of SCI, these neovessels may not be fully functional, given the important role that astrocytes and pericytes have on vascular function within the nervous system. Perhaps as a consequence of the lack of integration of these neovessels

into a functional neurovascular unit, there is subsequent pruning of these vessels at 2 weeks post-injury [25]. There is a more prolonged phase of angiogenesis from 4 weeks to 2 months post-injury, along with significant deposition of new basal lamina [130]. This further indicates that maturation and organization of neovessels occur after the first angiogenic phase and that much of the endothelial cell sprouts are pruned away based on the metabolic demand of the local area, with only a portion remaining to become stable, functionally integrated blood vessels.

The disruption of local microvasculature reduces perfusion of the remaining cord parenchyma [17, 63, 170]. Neurons have high metabolic requirements and are thus extremely vulnerable to reductions in perfusion and resultant periods of ischemia. This may be compounded by the loss of auto-regulatory mechanisms [65, 66, 170] and systemic hypotension, which is common in acute SCI patients, as the result of hypovolemic and/or neurogenic shock [22, 66, 170, 171, 183]. At rest, neurons require more than three times the energy usage (in terms of ATP) than glial cells [10]. This difference increases to more than five times per second in a neuron that is generating action potentials [10]. This highlights the importance that ischemia holds in the neurological deterioration of SCI patients after the initial insult.

The Blood–CNS Barrier

It is important to realize that the consequences of the aforementioned microvascular damage are not limited to endothelial cell death and the loss of perfusion but also involves the breakdown of the blood–spinal cord barrier (BSCB). Anatomically, the blood–CNS barrier is manifested by several components of the capillary wall including endothelial cells, pericytes, astrocytes and the extracellular matrix. Endothelial cells in the CNS are overlapping, with tight junctions sealing paracellular spaces [23], no fenestrations [23, 47, 158] and minimal pinocytosis [169]. They also have an increased mitochondrial content, presumably to support transporters such as Glut-1 for glucose transport across this barrier [149]. Molecularly, the junctional complexes between adjacent endothelial cells are made of a combination of adherens and tight junctions. Adherens junctions are found in all vessel walls. They mediate the adherence of endothelial cells to one another by linking the actin cytoskeletons of adjacent cells [166]. Tight junctions are only found in blood–CNS interfaces. They are comprised of a complex of transmembrane proteins including junctional adhesion molecules [134], occludins [54] and claudins [56], which span the entire intercellular cleft, and intracellular accessory proteins including the zonula occludens [177], which

link the transmembrane components to the cytoskeleton [45, 55].

Pericytes surround the endothelial cells and have a significant role in regulating BBB properties [9], as well as influencing endothelial cell proliferation, migration and differentiation [9, 156]. Pericytes also mediate proper orientation and positioning of astrocytic foot processes around vessel walls [9]. The basal lamina, made of proteoglycan and laminin components, wraps around the layer of endothelial cells and pericytes, providing physical support for the vessel wall through interaction with other extracellular matrix components [97]. The basal lamina can also stimulate the expression of tight junction-related proteins to help maintain BBB function [163]. Astrocytic foot processes are juxtaposed against the basal lamina-covered capillaries. They have a critical role in the formation and maintenance of the BBB [1, 79, 85, 100, 137] and can modulate endothelial permeability via the secretion of chemical substances. Together, these components constitute a functional neurovascular unit—another example of how the vascular and nervous systems are physiologically linked.

Whilst the BSCB has slight structural and physiological differences from the BBB (some of which are highlighted in [14]), functionally the two play similar roles in protecting the CNS environment from the systemic circulation. There are a number of important aspects to this function, which include controlling ionic balance, regulating nutrient transport and restricting the passage of neurotoxic molecules and inflammatory cells.

The perception that the CNS is an immune privileged zone was first hypothesized in 1925 by Billingham and Boswell, who reported the lack of leukocyte infiltration in the brain [20]. Absolute immune privilege of the CNS has since been disputed with increasing evidence that the brain is indeed subjected to immunological surveillance (for review, see [32, 58]). However, there is clearly a relative difference in immune privilege between the CNS and peripheral tissues. The inflammatory response in the CNS under pathological conditions propagates with a different mechanism and in a different timeframe than that in peripheral tissues [8]. Furthermore, the number of immunological cells in the CNS is still greatly lower (if at all present) compared to the periphery (T-lymphocytes [82, 83, 196], B-lymphocytes [76, 77, 116] and monocytes [7, 8]).

The time course of BBB and BSCB breakdown in multiple sclerosis [104], after traumatic brain injury [4, 87] or SCI [41], has been reported to closely parallel the progression of neuroinflammation. The accumulation of immunological cells in perivascular spaces where basement membrane and astrocytic foot processes were displaced after SCI [182] suggests that BSCB breakdown may be

involved in the expansion of the post-injury inflammatory response after SCI.

Breakdown of the BSCB After SCI

The conceptual integration of the nervous and vascular system is essential in understanding the pathophysiology of SCI and provides a basis for potential intervention strategies aimed at both nervous and vascular systems. In addition to the death of endothelial cells, there is extensive breakdown of the BSCB after SCI [17, 99, 102, 136, 147, 154, 159]. The lack of such a barrier protecting the spinal cord allows for the indiscriminate passage of cellular toxic molecules such as calcium [89, 164], excitatory amino acids [127, 198], free radicals [131], erythrocytes [5, 64, 89, 99, 145, 147, 184] and inflammatory mediators [41] into the injury penumbra, all of which may contribute to secondary injury after SCI. Although BSCB dysfunction has been reported largely from 2 [17, 99, 147, 154] to 4 weeks post-injury [136], chronic abnormalities have been observed at 8 weeks [182] and even 7 months post-injury [159].

In animal studies, BSCB breakdown after SCI is demonstrated by the extravasation of vascular tracers into the spinal cord parenchyma. This has been reported as early as 1 h post-injury and remains elevated for at least 24 h [64, 102, 147, 194]. This early peak in vascular leakage coincides with the acute inflammatory response [41], implicating the role of vascular permeability in the propagation of the inflammatory response after SCI by allowing inflammatory cells access into the injury penumbra. Increased BSCB permeability has also been observed between 3 and 7 days post-injury [17, 99, 154, 182, 194, 204], correlating with the initiation of angiogenesis and revascularization at the injury epicentre [25, 26, 42, 130, 194]. The destabilization of existing vessels by an increase in vascular permeability is necessary for angiogenic remodelling to occur and is evident as a breach of tight junctions, displacement of astrocytic foot processes and separation of the basement membrane [99, 147, 154]. In the chronic phase of injury, up to 5.5 months, overlapping endothelial cell junctions are reformed [99], although the perivascular space continues to expand, with mis-aligned extracellular matrix, collagen layers and displaced astrocytic foot processes [99]. This suggests that despite endogenous reparative efforts, there are chronic morphological abnormalities in the BSCB after SCI.

Given the implications of vascular disruption on secondary injury, the mechanisms by which angiogenesis occurs and the BSCB restored are particularly relevant to the topic of how neuroprotection can be achieved in acute SCI. In the next section, we will discuss the role of the

three angiogenic proteins—specifically angiopoietin 1, angiopoietin 2 and angiogenin—in the regulation of angiogenesis and the restoration of the BSCB after SCI.

Introduction to the Angiogenic Proteins

The angiopoietins are a family of growth factors that promote angiogenesis (for review, see [11, 203]). Ang1 and Ang2 are the best-characterized members of the angiopoietin family and are essential for the induction, maturation and maintenance of blood vessels. Angiogenin is a potent endothelial mitogen and belongs to the RNase A superfamily of ribonucleolytic proteins. It is also implicated in the pathogenesis of amyotrophic lateral sclerosis (ALS).

Angiopoietin 1

Ang1 was isolated by Davis et al. in 1996 [39] as a ligand for tyrosine kinase with Ig and endothelial growth factor homology domains (Tie) 2 receptors, which are predominantly expressed on endothelial cells [43, 44]. Ang1 is a 498-amino acid glycoprotein with a dimeric weight of approximately 70 kDa [39, 186]. The Ang1 protein consists of three distinct domains: a short amino terminus which forms a ring-like structure to super-cluster Ang1 homodimers together [40], a coiled-coil domain which mediates the formation of Ang1 homodimers via a disulphide bond at Cys245 [40, 155] and the carboxyl terminus which is homologous to the carboxyl terminus of fibrinogen, hence it being named the fibrinogen-like domain [40, 155]. This domain is responsible for ligand activity [155] and contains the binding site to Tie2 [40, 49].

Ang1 is constitutively expressed at a low basal level in quiescent adult vasculature [39, 107, 132, 195, 197] by perivascular mural cells such as pericytes [181] and smooth muscle [107, 133]. Upon activation of its receptor Tie2, Ang1 induces pro-survival/anti-apoptotic effects on endothelial cells [28, 119, 150, 151]. Ang1 binding induces auto-phosphorylation of Tie2, which activates downstream phosphatidylinositol 3-kinase (PI3K) and Akt [107, 151]. This has numerous downstream pro-survival effects, including the upregulation of survivin [35, 37, 70, 151], mammalian target of rapamycin [2] and the inhibition of caspases 3, 7, 9, BCL2 antagonist of cell death, mitochondrial second mitochondria-derived activator of caspase [27, 70, 151] and Forkhead box protein O1 [37]. Ang1 has been shown to interact with integrin to promote survival through a similar activation of Akt as well as various mitogenic protein kinases [35, 36]. Ang1 also modulates other survival signals such as extracellular signal-regulated kinase 1/2 (ERK1/2) [2, 71], stress-activated protein kinase (SAPK) and c-Jun NH2-terminal kinase (JNK) [71].

In addition to the role that it plays in promoting endothelial cell survival, Ang1 is crucial in maintaining vessel quiescence by limiting vascular permeability and controlling BSCB integrity [197]. Ang1 strengthens paracellular interactions and reduces the number and size of endothelial gaps [12] by inducing the expression of adhesive PECAM-1 [59] and tight junction proteins occludin [88] and ZO-2 [126]. Ang1 further reinforces vessel integrity by inhibiting the transcription of genes associated with vessel destabilization and remodelling [37]. By securing paracellular junctions, Ang1 effectively limits the progression the inflammatory response by restricting the passage of inflammatory cells from the bloodstream to CNS tissue [57]. Moreover, Ang1 activation of PI3K and Akt [110, 111] inhibits the expression of inflammatory cytokine nuclear factor- κ B (NF κ B) [96] and adhesion molecules intercellular adhesion molecule 1, vascular cell adhesion molecule 1 and E-selectin [59, 110], which are required for the migration of inflammatory cells.

Although Ang1 has little to no proliferative effects on endothelial cells [52, 117, 195], it is essential in the later stages of angiogenesis, including the migration and organization of vessel components into mature, stable vessels [197]. Ang1 promotes the migration of vascular components to sites of angiogenesis [2, 24, 52, 53, 72, 106, 162, 195] and the organization of these components into tubule-like structures.

It is hypothesized that the ability of Ang1 signalling to both maintain the vasculature in a quiescent state as well as mediate angiogenesis may be attributed to differential ligand–receptor complexes that Ang1 and Tie2 receptors form in mobile versus confluent cells [53, 162, 205]. In mobile endothelial cells, Ang1 associates and binds to the extracellular matrix [200] via β 1-integrin [125] and releases adhesion molecules which promote cell motility and migration of vessel components [53, 162]. In contrast, when associated with confluent, mature endothelial cells, Ang1 mediates the formation of trans-associated Tie2 homotypic paracellular complexes to reinforce vascular integrity [53, 162, 205].

Angiopoietin 2

Ang2 was first characterized in 1997 by Maisonpierre as the natural antagonist of Ang1 [132]. Whilst Ang1 is expressed at low basal levels constitutively, Ang2 expression is more actively regulated to modify and counteract Ang1 signalling. Ang1 and Ang2 share approximately 60% homology in their amino acid sequence, as well as a common protein structure consisting of an amino terminal that modulates super-clustering, a coiled-coil domain for the formation of homodimers and a fibrinogen-like domain with ligand activity [16, 40, 132]. Ang1 and Ang2 bind to

the same domain on Tie2 with similar affinities [15, 16, 132] and conformation [49]. However, they have opposite effects on receptor phosphorylation and activation.

Whilst binding of Ang1 to Tie2 induces receptor auto-phosphorylation and triggers downstream intracellular signalling pathways, the binding of Ang2 does not [155]. The differential effects that Ang1 and Ang2 have on receptor activation have been hypothesized to be due to their different abilities to form homotypic oligomers. Native Ang1 is largely found in superclusters of tetramers or higher order oligomers, whereas Ang2 is predominantly reported as homodimers [113, 155]. Receptor tyrosine kinases such as Tie2 have been reported to require multimerization for receptor activation [113, 155]. Thus, it is conceivable that although Ang2 dimers are able to bind to Tie2 receptors, they may not be sufficient to elicit receptor auto-phosphorylation, effectively acting as an antagonist for Tie2. Interestingly, Ang2 is able to elicit agonistic effects when driven outside of its natural physiological state, such as at high concentrations, after prolonged exposure, as engineered high-order oligomers or in non-endothelial cells transfected with Tie2 [38, 40, 69, 108], supporting the hypothesis that the antagonistic role of Ang2 in its physiological state is at least in part mediated by its natural tendency to form lower order oligomers.

Ang2 is expressed by endothelial cells [195] and perivascular smooth muscle [133] at sites of active angiogenesis during the vessel destabilization process [38, 132, 178]. Its expression is induced by hypoxia [132, 133, 153], hypoxic factor HIF1 α [202] and vascular endothelial growth factor (VEGF) [148]. Ang2 is stored in intracellular Weibel–Palade bodies in endothelial cells along with von Willebrand factor, which is involved in haemostasis [50]. Stored Ang2 has a half-life of 18 h but can be secreted within minutes of stimulation [50]. In adults, Ang2 secretion is induced by various cytokines such as VEGF and basic fibroblast growth factor [133], inflammatory mediators tumour necrosis factor α and NF κ B [109] or vasoactive molecule thrombin [50]. As the antagonist of Ang1, Ang2 induces the destabilization of vessel integrity [129, 148, 165], thus increasing BSCB permeability, but also thereby allowing vessels to undergo remodelling. Destabilization of existing vessels increases endothelial plasticity and is a primary prerequisite to vascular remodelling.

However, the result of these permeability changes also depends on the local cytokine milieu. In the presence of VEGF, Ang2 induces angiogenic sprouting, whilst in the absence of VEGF, Ang2 destabilization leads to vessel regression [18, 19, 86, 129]. Finally, Ang2 mediates the escalation of the inflammation response by increasing BSCB permeability to prime the endothelium and allow the passage of inflammatory cells through the vessel [19,

51]. By loosening endothelial cell junctions, Ang2 facilitates the migration of inflammatory cells from the bloodstream into peripheral tissue [51, 109].

Angiogenin

Angiogenin is a 123-amino acid, 14-kDa protein that potently induces angiogenesis and neovascularisation [48, 115, 142, 115, 187]. It was the first reported tumour-derived angiogenic protein, characterized by Fett et al. in 1985 [48, 179]. Angiogenin shares 65% homology to bovine pancreatic ribonuclease A [118, 179]. And although it has surprisingly low ribonucleolytic activity [3], the ribonucleolytic site on angiogenin appears to be essential for its angiogenic actions [33, 34, 93, 142, 172, 173].

Angiogenin is predominately expressed and released by endothelial cells, but it is also widely expressed by a variety of anchorage-dependent proliferating cells including aortic smooth muscle cells, fibroblasts and various tumour cells [139]. Angiogenin expression is induced by HIF1 α under cellular stress or hypoxic conditions [74, 144, 168]. A 170-kDa receptor has been identified as the angiogenin receptor [94]. Upon binding, angiogenin is translocated to the nucleus where it regulates genes controlling the proliferation of endothelial cells by activation of ERK [128], Akt [114] and the SAPK/JNK pathways [201]. Angiogenin has also been reported to interact with 42-kDa smooth muscle type α -actin [91, 92]. This induces the formation of angiogenin–actin complexes, which drive the degradation the extracellular matrix and basement membrane of blood vessels in order to promote the migration of vascular components [90, 93].

Aside from its prominent role in promoting tumour angiogenesis [48, 84, 115, 138, 139], angiogenin is well-characterized in the pathogenesis of ALS. Genetic mutations in angiogenin have been linked to both familial and sporadic ALS [30, 62, 105]. These ALS-associated mutants appear to have reduced or abolished survival-promoting activity, leading to the degeneration of motor neurons, an apparent symptom in the pathogenesis of ALS [168, 180, 199].

Angiogenic Proteins as Treatment after SCI

Vascular disruption, ischemia and BSCB permeability all contribute to secondary injury after SCI. Therefore, understanding angiogenesis and manipulating it therapeutically may potentially restore perfusion, reduce ischemic insults and reconstitute the BSCB to ultimately limiting secondary injury. Indeed, Ang1 as a treatment resulted in improved functional and histological outcomes following experimental SCI [67, 81]. Ang1 with the integrin-binding

peptide C16 was shown to rescue vasculature at the injury epicentre [67]. There was also an increased amount of spared white matter at both 7 and 42 days post-injury [67], although no further improvement was observed at 42 than 7 days. Decreased inflammation was observed as early as 24 h post-injury, suggesting that targeting early mechanisms such as vascular dysfunction can indeed result in long-term functional improvements by rescuing vasculature and reducing some aspects of inflammation very soon after SCI. This study is of particular clinical importance, as treatment did not begin until 4 h post-injury, which is a clinically realistic timeframe for a neuroprotective intervention in human SCI [67].

In another study, the combination of Ang1 and VEGF treatment by AAV viral transfection immediately after SCI decreased lesion volume and promoted vascular stability [81]. The treatment decreased oedema, demyelination and BSCB permeability, resulting in improved open-field locomotor function at 56 days post-injury [81]. Interestingly, the combination of Ang1 and other angiogenic molecules such as C16 or VEGF both resulted in synergistic functional outcome compared to each individual treatment, suggesting that in addition to the promotion of angiogenesis, the roles of Ang1 in the formation of stable vessel and regulation of BSCB permeability are crucial in limiting secondary injury after SCI.

Whilst there has been extensive investigation of angiogenic proteins as treatment after SCI, the endogenous expression of these proteins remains largely elusive. One recent report examined changes in several angiogenic growth factors in a rodent SCI model and reported both prolonged (VEGF) and transient (Ang1, PDGF, PIGF) decreases in gene expression up to 4 weeks post-injury [160]. However, the role that these endogenous changes have in the angiogenic response to SCI remains elusive.

Changes in Angiogenic Proteins After Acute Human SCI

The discussion to this point has revolved around what has been studied and reported with respect to Ang1, Ang2 and angiogenin in animal models of SCI. The vast majority of our scientific understanding of the pathophysiology of secondary injury is derived from such animal studies. However, therapies that have been shown to be effective in rodent models of SCI have historically been unsuccessful at demonstrating efficacy when translated to human clinical trials. Whilst there are many potential reasons for this, one is that important biological differences may exist between the pathophysiology of such animal models and that of the human condition.

Studying the biology of human SCI is obviously considerably more difficult than in animal models given that cord specimens can only be obtained postmortem. Our studies have therefore utilized what might be considered ‘the next best thing’—cerebrospinal fluid (CSF). We recognize that this is an indirect measure of what is happening within the spinal cord itself and that it would be challenging to interpret data from human CSF in relation to when the proteins are actually being released or extruded from the injury site. However, animal studies that have compared parenchymal and CSF levels of specific molecules have shown that they do correspond, albeit with much lower levels in the CSF than the cord parenchyma [73, 193]. In this present study, we investigated the temporal changes in three angiogenic proteins: Ang1, Ang2 and angiogenin following acute human SCI. We evaluated the protein levels in both CSF and serum samples of SCI patients and non-SCI controls. We sought to characterize the patterns of expression of these proteins and how their levels might be influenced by injury severity and/or hold implications for neurological recovery after SCI.

Materials and Methods

Patients from a single level 1 regional trauma institution were enrolled in a clinical trial in which lumbar intrathecal catheters were inserted to measure CSF pressure and obtain CSF samples [121]. The clinical trial protocol was approved by the university human ethics committee and was registered on ClinicalTrials.gov (ID: NCT00135278). Inclusion criteria for enrolment included (1) SCI between C3 and T11 inclusive; (2) ASIA impairment score (AIS) A—motor and sensory complete SCI, B—motor complete, sensory incomplete SCI or C—motor and sensory incomplete SCI upon presentation; (3) presentation within 48 h of injury and (4) ability to provide a valid and reliable baseline neurological exam. Patients with concomitant head injuries, major trauma to the chest, pelvis or extremities that required invasive intervention and those who were too sedated or intoxicated to give a valid neurologic examination were excluded. The patients provided informed consent to participate in the study in which an intrathecal drain was installed and left in situ for 3 to 5 days. To obtain control CSF from the ‘non-SCI’ condition, a direct lumbar dural puncture was performed in individuals undergoing lumbar spine surgery.

Upon presentation, patients were evaluated by a clinical research nurse, and a neurological examination was performed to assign a baseline AIS and ASIA motor score. Long-term outcome was measured at 6 months and 1 year post-injury with parameters used in the initial baseline

neurological testing including AIS, ASIA motor score and the last normal sensory level.

Intrathecal catheters (PERIFIX® Custom Epidural Anaesthesia Kit; B. Braun Medical Inc., Bethlehem, PA, USA) were inserted at L2/3 or L3/4, and 3 to 4 ml of CSF was collected every 6 to 8 h for 3 to 5 days using a strict aseptic technique. For non-SCI controls undergoing lumbar spine surgery, a sample of CSF was obtained via needle puncture of the dura at the end of their surgery. Samples were immediately centrifuged at 1,000 rcf for 10 min. The supernatant was aliquoted, snap-frozen in an ethanol and dry ice bath and stored at -80°C until analysis. Blood samples were drawn in both SCI patients and non-SCI controls at the same times that CSF samples were collected. The blood samples were left to clot at room temperature for 15 min and then centrifuged at 10,000 rcf for 5 min. The serum was aliquoted, frozen and stored at -80°C until analysis.

The CSF and serum samples were analysed using standard quantitative sandwich ELISA kits for Ang1 (Quantikine® Human Angiopoietin-1) and Ang2 (Quantikine® Human Angiopoietin-2) and a microparticle-based multiplex ELISA kit for Angiogenin (Fluorokine® MAP Human Angiogenesis Base Kit A and Angiogenin bead set). All kits were manufactured by R&D Systems Inc., Minneapolis, MN, USA. For SCI patients, up to 15 CSF and blood samples taken between 8 and 120 h post-injury were analysed. A single baseline sample was analysed for non-SCI controls. All samples were run in duplicate.

Statistical analysis was performed using SPSS Statistics 17.0 software. Normal distribution in the data was tested using the Shapiro–Wilk test, and equality of variances between groups was tested using the Levene test. Kruskal–Wallis H test was used to compare protein levels with baseline AIS. The Friedman test was used to examine temporal changes in individual patients. The Mann–Whitney U test was used to compare values between SCI patients and non-SCI controls at each time point. Spearman’s correlation was used to investigate relationships between protein levels and outcome parameters at 6 months or 1 year post-injury.

Results

For this study, we analysed 15 SCI patients equally divided amongst injury severity, with five AIS A, five AIS B and five AIS C at baseline (Table 1). Twelve individuals suffered cervical SCI, whilst three suffered thoracic SCI. The average age was 41.7 years, with 13 males and two females. Five patients were injured by motor vehicle accidents, five during sporting activities, four from falls and one resulting from a direct blow to the

Table 1 Demographics of SCI patients enrolled in the current study

| Subject | Age/ sex | Injury level | Mechanism of injury | Upon presentation | | | 6-month or 1-year follow-up | | |
|---------|-------------|-----------------|-------------------------------|-------------------|---------------------|------------------------------|-----------------------------|---------------------|------------------------------|
| | | | | AIS | ASIA motor score | Last normal sensory level | AIS | ASIA motor score | Last normal sensory level |
| SCI 1 | 42/M | C6–7 | Transport | B | 19 | C5 | B | 27 | C6 |
| SCI 2 | 64/F | C5–6 | Sports | C | 41 | C5 | D | 98 | C2 |
| SCI 3 | 66/M | C4–5 | Fall | C | 20 | T9 | C ^a | 49 ^a | C4 ^a |
| SCI 4 | 60/M | C4–5 | Fall | B | 6 | C5 | D | 80 | C4 |
| SCI 5 | 46/M | C4–5–6 | Transport | C | 5 | C2 | C ^a | 71 | C5 |
| SCI 6 | 20/M | C6–7 | Transport | B | 30 | C6 | C ^a | 86 ^a | T6 ^a |
| SCI 7 | 39/M | C3–4 | Sports | C | 33 | C3 | D | 83 | C4 ^a |
| SCI 8 | 54/M | C5–6 | Transport | C | 36 | C4 | D ^a | 65 ^a | C5 ^a |
| SCI 9 | 19/F | T10 | Fall | A | 50 | T9 | A | 50 | T7 |
| SCI 10 | 38/M | C5 | Struck on the back of head | B | 17 | C5 | D ^a | 15 ^a | C6 ^a |
| SCI 11 | 22/M | L1 | Transport | A | 60 | C2 | A ^a | 66 ^a | L2 ^a |
| SCI 12 | 25/M | C7 | Sports | A | 43 | C7 | C | 58 | C7 ^a |
| SCI 13 | 51/M | T10 | Fall | A | 50 | T11 | A ^a | 50 ^a | T11 ^a |
| SCI 14 | 55/M | C6–7 | Sports | A | 27 | C6 | UNK | UNK | UNK |
| SCI 15 | 25/M | C5 | Sports | B | 13 | C4 | B ^a | UNK | C4 ^a |

UNK unknown, where follow-up neurological testing was not performed or has not yet been performed at the time this manuscript was prepared

^aSix-month follow-up

back of the head (Table 1). The CSF and serum of these 15 patients were compared against eight ‘non-injured’ control subjects. This population averaged 60.1 years in age and included four males and four females (Table 2).

The concentrations of Ang1, Ang2 and angiogenin were evaluated at 12-h intervals from 24 h to 5 days post-injury (Figs. 1, 2 and 3). The values within groups did not display normal distribution (Shapiro–Wilk test), and variances between groups were unequal (Levene test); hence, non-parametric statistical tests were used for all comparisons. No significant differences in Ang1, Ang2 or angiogenin concentrations were found between AIS A, B or C patients at any time point post-injury (including baseline). Thus, the data from all injury

severities were pooled to compare the SCI condition (AIS A, B and C) against non-SCI controls. For the non-SCI controls, only a single CSF and serum sample was obtained, and we made the assumption that the concentrations would remain largely unchanged over time in these individuals.

For Ang1, both the CSF and serum levels of Ang1 at the earliest time point were analysed, around 24 h post-injury ($p < 0.05$, Mann–Whitney U test) (Fig. 1). The median Ang1 value in CSF in SCI patients at this time was 50.03 pg/ml [interquartile range (IQR) 43.70–84.19], whereas the median value for non-SCI controls was 39.72 pg/ml [IQR 35.54–48.31]. In the serum, the median value for SCI patients was 4,661.70 pg/ml [IQR 3,959.77–

Table 2 Demographics of non-SCI controls enrolled in the current study

| Subject | Age/sex | Diagnosis |
|---------|---------|--|
| CTRL 1 | 49/M | L4–5 recurrent disc herniation |
| CTRL 2 | 85/F | L2–3, 3–4, 4–5, 5–S1 stenosis |
| CTRL 3 | 52/F | Right S1 radiculopathy |
| CTRL 4 | 68/F | Degenerative L3–4 spondylolisthesis |
| CTRL 5 | 45/M | L5–S1 disc herniation |
| CTRL 6 | 61/F | L5–6 disc herniation |
| CTRL 7 | 62/M | L5–S1 degenerative disc disease with neural foraminal stenosis |
| CTRL 8 | 59/M | L3–4 spinal stenosis; L4–5 degenerative spondylolisthesis |

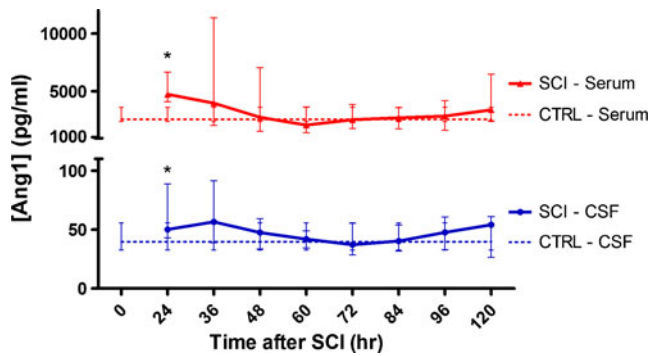


Fig. 1 Median Ang1 protein levels in CSF and serum after acute human SCI. SCI patients showed significantly higher Ang1 values than non-SCI controls in both CSF and serum at 24 h post-injury. Median CSF value for SCI patients at 24 h post-injury was 50.03 pg/ml whereas median value for non-SCI controls was 39.72 pg/ml. Median serum value at 24 h post-injury for SCI patients was 4,661.70 pg/ml, and median value for non-SCI controls was 2,542.25 pg/ml. Blue lines (circles) represent CSF values and red lines (triangles) represent serum values. SCI patients are represented with solid lines, and non-SCI controls are represented with dotted lines. Data presented as median±IQR. * $p < 0.05$, Mann–Whitney U test

6,226.92] and 2,542.25 pg/ml [IQR 2,426.67–3,306.99] in non-SCI controls. These elevations in Ang1 diminished over the subsequent 12 h, and by 36 h post-injury, SCI and control levels were no longer significantly different in CSF or serum. There were also no statistically significant correlations between Ang1 levels and the 6- and 12-month neurologic outcomes.

In contrast to the early peak in Ang1, a delayed and prolonged increase in CSF Ang2 expression was observed

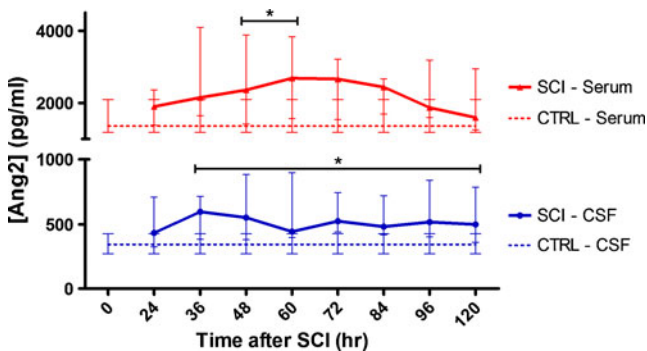


Fig. 2 Median Ang2 protein levels in CSF and serum after acute human SCI. SCI patients showed significantly higher Ang2 values from 36 h post-injury until the end of the study period at 120 h post-injury in the CSF. Serum samples from SCI patients were also significantly higher than non-SCI controls between 48 and 60 h post-injury. The peak in CSF at 36 h post-injury in SCI was 599.16 pg/ml compared to 344.80 pg/ml in non-SCI controls. The peak in serum is observed at 60 h post-injury, with SCI value at 2685.61 pg/ml and non-SCI control value at 1,358.77 pg/ml. Blue lines (circles) represent CSF values, and red lines (triangles) represent serum values. SCI patients are represented with solid lines, and non-SCI controls are represented with dotted lines. Data presented as median±IQR. * $p < 0.05$, Mann–Whitney U test

(Fig. 2). Increased Ang2 levels were observed in SCI patient CSF from 36 h post-injury and stay upregulated until the end of the study period. The maximal difference in CSF between SCI patients and non-SCI controls is observed at 36 h post-injury, with SCI patient median at 599.16 pg/ml [IQR 394.19–688.38] and non-SCI control median at 344.80 pg/ml [IQR 280.05–392.72]. The maximal difference in serum levels is observed at 60 h post-injury, with SCI patient median at 2,685.61 pg/ml [IQR 1,588.76–3,823.93] and non-SCI control median at 1,358.77 pg/ml [IQR 1,207.85–2,080.15]. The differences between SCI and non-SCI values were statistically significant in CSF from 36 h until the end of the study and in serum between 48 and 60 h post-injury ($p < 0.05$, Mann–Whitney U test). Again, no significant correlations were found between Ang2 values and the neurologic outcome at 6 months or 1 year post-injury.

For angiogenin within the CSF, the concentration in SCI patients appeared to decrease starting around 36 h post-injury, and between 72 and 84 h post-injury, levels were significantly lower than in non-SCI controls ($p < 0.05$, Mann–Whitney U test) (Fig. 3). Maximal difference is observed at 72 h post-injury with median value for SCI at 6.73 ng/ml [IQR 5.27–7.46] and median value for non-SCI controls at 8.81 ng/ml [IQR 8.45–10.32]. In the serum, there appear to be no changes until 60 h post-injury, with an increasing trend from 60 to 120 h post-injury. Median serum value for SCI at 120 h post-injury was 465.17 ng/ml [IQR 402.77–631.82], and non-SCI median was 232.18 ng/ml [IQR 204.11–316.50]. However, these values were not significantly different between SCI and non-SCI controls ($p < 0.05$, Mann–Whitney U test).

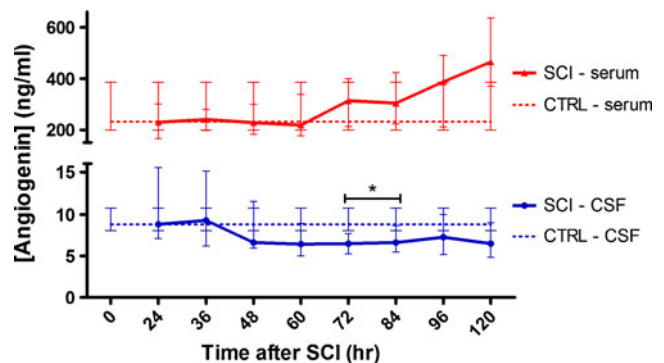


Fig. 3 Median angiogenin protein levels in CSF and serum after acute human SCI. SCI patients showed significantly lower angiogenin values in CSF between 72 and 84 h post-injury. No significant differences were observed between SCI patients and non-SCI controls in serum samples. Median CSF value in SCI patients at 72 h post-injury was 6.73 ng/ml, and median for non-SCI controls was 8.81 ng/ml. Blue lines (circles) represent CSF values, and red lines (triangles) represent serum values. SCI patients are represented with solid lines, and non-SCI controls are represented with dotted lines. Data presented as median±IQR. * $p < 0.05$, Mann–Whitney U test

Discussion of Angiogenic Changes After Human SCI

The expression of angiogenic and BSCB-integrity-promoting proteins in the acute phase post-SCI could limit the spread of secondary damage by tightening the interactions between endothelial cells and mural cells to close the paracellular junctions in vessel walls, therefore preserving the BSCB. This could also eliminate the extra-vascular space which has been reported to become a conduit for inflammatory cells invading the site of injury [182]. Furthermore, by preserving distal circulation in the overlapping vascular networks of the spinal cord, adequate perfusion to the site of injury could be maintained. In an attempt to investigate the role of angiogenesis in the acute phase after SCI, we have previously measured the levels of another potent angiogenic factor, VEGF, using a similar biochemical assay but were not able to detect it at any measurable levels in the CSF of the current series of acute SCI patients [124].

A decrease in the expression of Ang1 mRNA has been reported after acute SCI in rats from 6 h to 2 weeks post-injury [160]. Ang1 activity early after SCI could help preserve the integrity of the BSCB, which when compromised promotes the invasion of inflammatory cells and other toxic blood products into the injury penumbra [147, 154, 194]. Although a transient increase in Ang1 expression is observed at 24 h, this is likely due to the release of Ang1 from damaged endothelial cells into the CSF as a result of the initial mechanical impact. The absence of a sustained increase in Ang1 levels within the CSF in the current study supports the contention of investigators such as Han et al. [67] and Herrera et al. [81] who administered Ang1 in the hopes of increasing angiogenesis and restoring the integrity of the BSCB after acute SCI. These authors showed that Ang1 alone or in combination with another angiogenic factor resulted in functional improvements after experimental SCI [67, 81].

Conversely, the prolonged upregulation of Ang2 expression from 36 h post-injury observed in the current study could exacerbate secondary injury by destabilizing endothelial junctions to increase BSCB permeability. A delayed angiogenic response after SCI has been reported in several studies, ranging from 3 to 7 days [17, 99, 154, 182, 194, 204]. No further neurological changes were correlated to the new angiogenic status in these studies, adding evidence to support that although endogenous reparative response of the damaged tissue and vasculature is observed after acute SCI, these neovessels fail to integrate into a functional neurovascular unit. Furthermore, it is likely that the increase in BSCB permeability during the early stages of injury, in addition to the lack of continued robust Ang1 response, contributes to the exacerbation of secondary injury. Angiogenesis and the maintenance of the BSCB

after acute traumatic SCI is not only a neuroprotective strategy—to preserve remaining neurons and glia and prevent further cell death by ischemia—but blood vessels also provide trophic support and a scaffold for both endogenous and potential regeneration strategies [143].

However, along with stimulating angiogenesis, this prominent increase in Ang2 after SCI may allow the passage of deleterious inflammatory cells and cytokines, as well as cytotoxic molecules into the injury penumbra, exacerbating secondary injury. A marked inflammatory response has been established from 3 to 7 days post-injury [41, 154, 194], which coincides with revascularization of the injury epicentre [25, 42, 130, 194]. The time course of BSCB repair also closely parallels that of the appearance of the glial scar [130]. It has recently been reported that a subpopulation of pericytes, perhaps those which proliferate during the first angiogenic stage but are not integrated into a functional neurovascular unit, leave the vessel wall, trans-differentiate to express fibroblast markers, migrate into the lesion epicentre and are responsible for a majority of the extracellular deposition of the glial scar sealing the injury epicentre [61].

There is a significant decrease in the CSF expression of angiogenin between SCI and non-SCI control groups from 72 to 84 h post-injury, substantial inter-individual variation and statistical noise may mask any potential differences between SCI patients and non-SCI controls. However, the decreased expression of angiogenin after SCI suggests that the angiogenic changes that occur in the acute phase post-injury are not driven by the same mechanism as tumorigenic angiogenesis that has been reported to be associated with angiogenin [48, 84, 138].

The temporal changes examined in this study represent the changes of all sample SCI patients compared to all non-SCI controls. However, it has been reported that there is a substantial variation in inter-individual protein expression levels in CSF even in healthy individuals [167]. We too observed considerable variation in our SCI patients and uninjured controls. Certain patterns of change present after SCI in our sample population could potentially have been masked by this great inter-individual variation, as it would be logistically impossible to acquire a non-SCI baseline measure for each SCI patient enrolled in this study to use as a comparison for their post-SCI expression values. However, a majority of the values recorded for our non-SCI controls reflect the values of controls which have previously been reported (Table 3) [6, 29, 31, 68, 80, 84, 95, 98, 101, 103, 112, 138, 140, 141, 152, 157, 174, 192].

In the current study, all protein levels in serum were considerably higher, by orders of magnitude, than in CSF. However, the serum and CSF concentrations did not appear to be changing in parallel, which suggests that the changes seen in the CSF are indeed local CNS changes, and not a

Table 3 Summary of serum or CSF Ang1, Ang2 and angiogenin values reported in the current study and in literature

| Author | Study | Population | Serum (ng/ml) | CSF (ng/ml) |
|-------------------|-------------------------------|--|---|--|
| Ang1 | | | | |
| Pg | Current study | SCI (<i>n</i> =15) | At 24 h post-injury: 4.66 [3.96–6.23] | At 24 h post-injury: 50.03 [43.70–84.19] |
| | | <i>Ctrl</i> (<i>n</i> =8) | 2.54 [2.43–3.31] | 39.72 [35.54–48.31] |
| Joshi | Clin Biochem. 2011 | Multiple myeloma (<i>n</i> =62) | 36.28 (19.8–44.0) | |
| | | <i>Ctrl</i> (<i>n</i> =50) | 37.05 (35.7–39.2) | |
| Reed | Kidney International. 2011 | Autosomal dominant polycystic kidney disease (<i>n</i> =71) | 35.52±21.03 | |
| Choe | Joint Bone Spine. 2010 | Bencet's disease (<i>n</i> =59) | 284.5±101.2 | |
| | | <i>Ctrl</i> (<i>n</i> =65) | 237.1±76.4 | |
| Han | Hypertens Pregnancy. 2010 | Preeclampsia (<i>n</i> =16) | Plasma 12.65 (1.27–17.5) | |
| | | <i>Ctrl</i> (<i>n</i> =29) | 10.35 (1.43–31.89) | |
| Karapinar | Heart and Vessels. 2010 | Hypertension (<i>n</i> =49) | 26.95±11.63 | |
| | | <i>Ctrl</i> (<i>n</i> =21) | 43.34±9.77 | |
| Anagnostopoulos | Br J Haematol. 2007 | Waldenstrom's macroglobulinemia (<i>n</i> =56) | 18.4 (1.7–107.5) | |
| | | <i>Ctrl</i> (<i>n</i> =30) | 23.2 (0.1–45.9) | |
| Ang2 | | | | |
| Pg | Current study | SCI (<i>n</i> =15) | At 60 h post-injury: 2.69 [1.59–3.82] | At 36 h post-injury: 599.16 [394.19–688.38] |
| | | <i>Ctrl</i> (<i>n</i> =8) | 1.36 [1.21–2.08] | 344.80 [280.05–392.72] |
| Joshi | Clin Biochem. 2011 | Multiple myeloma (<i>n</i> =62) | 4.45 (2.1–13.25) | |
| | | <i>Ctrl</i> (<i>n</i> =50) | 1.67 (0.25–3.45) | |
| Reed | Kidney International. 2011 | Autosomal dominant polycystic kidney disease (<i>n</i> =71) | 2.35±0.96 | |
| Han | Hypertens Pregnancy. 2010 | Preeclampsia (<i>n</i> =16) | Plasma 11.2 (2.3–21.9) | |
| | | <i>Ctrl</i> (<i>n</i> =29) | 3.9 (1.4–14.7) | |
| Helfrich | Clin Cancer Res. 2009 | Melanoma (<i>n</i> =98) | 2.03 [1.71–3.28] | |
| | | <i>Ctrl</i> (<i>n</i> =82) | 1.24 [0.93–1.57] | |
| Moreau | Amyotroph Lateral Scler. 2009 | ALS (<i>n</i> =40) | | 86.75 [67–132] |
| | | <i>Ctrl</i> (<i>n</i> =40) | | 82.5 [30–147] |
| Anagnostopoulos | Br J Haematol. 2007 | Waldenstrom's macroglobulinemia (<i>n</i> =56) | 2.6 (1.0–11.3) | |
| | | <i>Ctrl</i> (<i>n</i> =30) | 1.4 (0.6–5.1) | |
| Angiogenin | | | | |
| Ng | Current study | SCI (<i>n</i> =15) | At 120 h post-injury: 465.17 [402.77–631.82] | At 72 h post-injury: 6.73 [5.27–7.46] |
| | | <i>Ctrl</i> (<i>n</i> =8) | 232.18 [204.11–316.50] | 8.81 [8.45–10.32] |
| Moreau | Amyotroph Lateral Scler. 2009 | ALS (<i>n</i> =40) | | 288.0 [267–307] |
| | | <i>Ctrl</i> (<i>n</i> =40) | | 282.5 [244–326] |
| Ilzecka | Acta Clin Croat. 2008 | ALS (<i>n</i> =20) | | 0.328 (0.208–0.45) |
| | | <i>Ctrl</i> (<i>n</i> =15) | | 0.286 (0.153–0.483) |
| Patel | Ann Med. 2008 | Chronic heart failure (<i>n</i> =109) | 466 [314–739] | |
| | | <i>Ctrl</i> (<i>n</i> =112) | 310 [264–376] | |
| Anagnostopoulos | Br J Haematol. 2007 | Waldenstrom's macroglobulinemia (<i>n</i> =56) | 398.1 (147.4–1,180.6) | |
| | | <i>Ctrl</i> (<i>n</i> =30) | 226.9 (145.8–398.7) | |
| Huang | Eur Neurol. 2007 | Acute cerebral infarction (<i>n</i> =30) | At 48 h: 415.1±76.8 | |

Table 3 (continued)

| Author | Study | Population | Serum (ng/ml) | CSF (ng/ml) |
|------------|-----------------------------|---|---------------------------------|-------------|
| Siebert | Diabetes Care. 2007 | <i>Ctrl (n=20)</i> | <i>334.9±93.9</i> | |
| | | Diabetes mellitus type 2 (n=43) | 319.7±107.04 | |
| Cronin | Neurology. 2006 | <i>Ctrl (n=43)</i> | <i>550.54±187.99</i> | |
| | | ALS (n=79) | 396.7±120.9 | |
| Kim | Leukemia and Lymphoma. 2005 | <i>Ctrl (n=72)</i> | <i>334.6±106</i> | |
| | | Leukaemia (n=43) | 277.6 (145.9–533.7) | |
| Molica | Eur J Haematol. 2004 | <i>Ctrl (n=18)</i> | <i>226 (68–349.8)</i> | |
| | | Leukaemia (n=77) | 295 (74–1,700) | |
| Hisai | Clin Cancer Res. 2003 | <i>Ctrl (n=15)</i> | <i>264 (29–1,835)</i> | |
| | | Hepatocellular carcinoma (n=39) | 362.3±84.1 | |
| Verstovsek | Br J Haematol. 2001 | <i>Ctrl (n=31)</i> | <i>331.9±133.8</i> | |
| | | Leukaemia/myelodysplastic syndrome (n=101) | Plasma 609.7 (127.6–1,054.0) | |
| Miyake | Cancer. 1999 | <i>Ctrl (n=11)</i> | <i>197.1</i> | |
| | | Urothelial carcinoma (n=135) | 434.86±186.02 | |
| | | <i>Ctrl (n=52)</i> | <i>337.5±71.4</i> | |

Data shown as mean±SD and (range) or median and [IQR]. Control values are shown in italics

spillover from serum expression due to systematic injuries. This is evident in the changes seen in Ang2. Whilst Ang2 levels in the serum were much below that of Ang1, they become substantially higher than Ang1 levels in CSF. This is not unexpected, as Ang1 is constitutively expressed in the maintenance of quiescent adult vessels, whilst Ang2 is only found at sites of active angiogenesis. Given the context of BSCB breakdown after SCI and the antagonistic role of Ang2 against Ang1, this suggests that this increase is indeed an active upregulation in the expression and/or secretion of Ang2 locally, which may indicate the destabilization of local vasculature and breakdown of BSCB after SCI.

Much of the current knowledge regarding the mechanisms of secondary injury has been deciphered in animal models of SCI, whilst the direct observation of human injuries is far less common. It is important to keep in mind when examining animal studies that there are considerable differences in both anatomy and physiology between such animal models and human injuries. For example, humans may be more susceptible to spinal cord ischemia compared to rodents because of the increased distance between segmental arteries that are exclusively dependent on diffusion from the capillary supply. Within the thoracic cord, anatomical differences also exist, with a ‘watershed’ region of the human thoracic cord dependent upon the ascending lumbar artery (and thus vulnerable to ischemia) as

compared to the rodent thoracic cord which has sufficient collateral supply. Furthermore, the development of the progressive secondary injury is observably different between species with regards to cavitations and scarring. These differences may dampen the ability to translate a potential therapeutic treatment into the clinical setting. The reverse translation of information gathered from clinical studies back to the controlled environment of the laboratory could be crucial to extract the differences that exist between animal models and the clinical setting and accelerate the tedious process of clinical translation of pharmaceutical interventions for the treatment of SCI.

In conclusion, this article summarizes a portion of the published literature regarding the vascular injury that occurs after SCI and presents novel findings on the expression of three angiogenic proteins: Ang1, Ang2 and angiogenin in CSF and serum after acute human SCI. The intimate relationship of the neurovascular unit and its pathophysiology remains an important focus as a neuro-protective/neuroregenerative strategy for SCI and other CNS disorders alike.

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