Send Orders for Reprints to reprints@benthamscience.net

SYSTEMATIC REVIEW ARTICLE

The Role of Astrogliosis in Formation of the Syrinx in Spinal Cord Injury

Jacek M. Kwiecien[1,*], Wojciech Dąbrowski[2], Jordan R Yaron[3], Liqiang Zhang[3], Kathleen H. Delaney[1] and Alexandra R. Lucas[3]

[1]*Department of Pathology and Molecular Medicine, Faculty of Health Sciences, McMaster University, Hamilton, ON, Canada;* [2]*Department of Anaesthesiology and Intensive Therapy, Medical University of Lublin, ul. Jaczewskiego 8, Lublin 20-090 Poland;* [3]*Center for Personalized Diagnostics and Center for Immunotherapy, Vaccines and Virotherapy, Biodesign Institute, Arizona State University, Tempe, AZ, U.S.A*

ARTICLE HISTORY

Received: May 11, 2020
Revised: June 09, 2020
Accepted: July 16, 2020

DOI: 10.2174/1570159X18666200720225222

Abstract: A massive localized trauma to the spinal cord results in complex pathologic events driven by necrosis and vascular damage which in turn leads to hemorrhage and edema. Severe, destructive and very protracted inflammatory response is characterized by infiltration by phagocytic macrophages of a site of injury which is converted into a cavity of injury (COI) surrounded by astroglial reaction mounted by the spinal cord. The tissue response to the spinal cord injury (SCI) has been poorly understood but the final outcome appears to be a mature syrinx filled with the cerebrospinal fluid with related neural tissue loss and permanent neurologic deficits. This paper reviews known pathologic mechanisms involved in the formation of the COI after SCI and discusses the integrative role of reactive astrogliosis in mechanisms involved in the removal of edema after the injury. A large proportion of edema fluid originating from the trauma and then from vasogenic edema related to persistent severe inflammation, may be moved into the COI in an active process involving astrogliosis and specifically over-expressed aquaporins.

Keywords: Spinal cord injury, inflammation, cavity of injury, astrogliosis, aquaporins, edema, syrinx.

1. INTRODUCTION

Massive injury to the white matter of the spinal cord, as with motor vehicle accidents and falls in the elderly, frequently results in the formation of the syrinx, an elongated, permanent, fluid-filled cavity [1] but the mechanisms involved in syrinx formation remain poorly understood. The other mechanisms of the CNS response, such as the removal of excess edema fluid post-injury also remain poorly understood. Further, the previously used treatment with methylprednisolone succinate [2] has proven to be ineffective and is associated with serious toxic side effects [3, 4]. Recent systematic studies have demonstrated that the injury to the white matter causes a severe and destructive inflammation [5-8] that in the spinal cord injury (SCI) remains active for >16 weeks post-injury with associated peri-lesional edema [6] before it is eliminated. The rat injured spinal cord reacts by forming a cavity of injury (COI) within the first week post-SCI [6-11] which encompasses the site of hemorrhage and necrosis, and a severe inflammatory infiltration mainly represented by active, phagocytic, CD68+/CD163-, pro-inflammatory macrophages [6, 11]. Since the severe inflammation progresses in the aqueous environment in the COI, it apparently serves as a reservoir of the edema fluid from the surrouding CNS tissue with the concurrent decrease in the tissue water content as determined in MRI studies involving SCI [6, 9, 10]. The formation of the COI is unique to the CNS and does not occur in other organs after trauma. The pumping of the edema fluid out of the injured CNS is an active process [12] that involves remarkable astrocytic hypertrophy and increased expression of water channels, aquaporins (AQPs), particularly AQP4 expressed mainly on astrocytes [13-22]. It appears that the removal of the edema fluid serves two fundamental mechanisms leading to the restoration of homeostasis in the viable tissue after the CNS injury; (1) it limits the volume of the tissue irreversibly destroyed in the acute phase of the injury to forming COI, (2) it allows for the creation of the COI to which necrosis and supervening severe inflammation is sequestered [6, 23]. The elimination of excess edema fluid involving aquaporins expressed on astrocytes occurs *via* 3 routes: (i) *via* the glia limitans externa to the subarachnoid space, (ii) *via* the glia limitans interna to the cerebral ventricles or to the central canal, and (iii) *via* the blood-brain barrier (BBB) or blood-spinal cord barrier (B-SCB) to the blood vessels [24]. The existing treatment of edema uses pharmacological enhancement of the osmolarity of blood such as intravenous administration of 20% mannitol or hyperosmotic NaCl, a >100 years old therapy of limited clinical success. Mechanisms involved in the elimination of edema fluid *via* the COI with involvement of astrogliosis forming around it have been proposed [18, 25] but not yet studied. Importantly, in the rat model of SCI, the confinement of the severe inflammatory process to the COI with its aqueous environment and its direct connection to the

* Address correspondence to this author at the Department of Pathology and Molecular Medicine, faculty of Health Sciences, McMaster University, Room HSC 1U22D, 1280 Main Street West, Hamilton, ON, Canada L4S 4K1; Tel: 905-525-9240, Ext: 22827, Fax: 905-522-3580;
E-mail: kwiecien@mcmaster.ca

1570-159X/21 $65.00+.00

subarachnoid space allowed for effective inhibition of macrophage infiltration by sustained subdural infusion of dexamethasone [7, 8] and of Serp-1 and M-T7, Myxoma virus-derived immunomodulatory proteins [11]. This novel model of drug administration allowed to circumvent the BSCB and will serve to test other anti-inflammatory compounds for their neuroprotective activity. The pathologic relationship between severe inflammation in the COI and potentially leaky blood vessels in the surrounding spinal cord leading to chronic edema [6] and reactive astrogliosis around the COI appears obvious but has not been addressed in experimental research.

The enhancement of astrogliosis to accelerate removal of edema fluid *via* the COI is postulated here. Recently studied anti-inflammatory treatments of the SCI resulted in inhibition of macrophage infiltration in the COI and may also inhibit inflammatory vascular damage and consequently, vasogenic edema as a potentially effective, novel therapeutic strategy to treat the SCI, traumatic brain injury (TBI), and stroke.

1.1. Formation of the Syrinx

1.1.1. Inflammatory Response to the SCI

The response of the white matter of the spinal cord to massive traumatic injury differs strongly from a post-traumatic reaction in other non-neural tissues. It involves a severe, destructive, macrophage-rich, and long-lasting inflammation and leads to the formation of a fluid-filled COI limited by an astroglial reaction [6]. Formation of the syrinx is reported to occur in <1-7% in patients with neurological deficits [25-27] and the incidence of radiological diagnosis of cavitations in SCI patients is >50% [28-30] indicating a considerable discrepancy, the reason for which is not clear. Post-traumatic formation of the COI is a complex and insufficiently studied process involving the transformation of a poorly defined area of hemorrhage and cellular necrosis surrounded by tissue edema [6, 9, 10] into a defined cystic structure, filled with fluid containing necrotic debris, red blood cells, and large numbers of phagocytic macrophages [6-8, 11] with apparently simultaneous inflow of edema fluid from the surrounding spinal cord [9, 10, 31]. The COI is well defined 7 days post-SCI and it is delineated by a layer of progressively severe astrogliosis in the surrounding spinal cord [6-8, 11]. Thus it appears that the formation of the COI post-trauma involves; (a) confinement of cellular inflammatory response infiltrating the necrotic debris to a localized area separated from the rest of the spinal cord, (b) transfer of edema fluid from a much larger area in the spinal cord surrounding the site of necrosis into the forming COI, and (c) formation of a layer of astrogliosis surrounding the cavity to separate its pro-inflammatory content from the rest of the spinal cord in an attempt at its preservation and restoration of homeostasis [23, 32]. Reactive astrocytes have been observed in the spinal cord surrounding the cavities following SCI in humans and experimental animals [33-35] and in syrinxes forming without the involvement of mechanical trauma [36]. Severe inflammation plays a key role in this process [6-8, 11]. The inflammatory infiltrate is composed mostly of CD68+/CD163- pro-inflammatory macrophages persistent in the cavity beyond 16 weeks post-injury [11]. The presence of myelin granules in invasive, phagocytic macrophages is indicative of immunogenic character of damaged myelin and after 2 weeks post-SCI [6], also of continuous destruction of myelin sheaths in the white matter of the spinal cord surrounding the COI leading to its expansion, an observation indicated previously [18, 24, 37]. Associated with histological macrophage-rich severity of inflammation is marked increase in the spinal cord of levels of pro-inflamatory cytokines including IL-1β, IFN-γ and IL-6, and chemokines during the first week post-SCI [6].

1.2. Anti-inflammatory Activity in the Injured Spinal Cord

Formation of the COI in the injured spinal cord devoid of myelin such as in the Long Evans Shaker (LES) rat mutant [38-42] involves infiltration by macrophages phagocytizing necrotic debris and red blood cells at 3 days after injury [5]. The inflammatory process however, is completely eliminated from the COI by day 7 in the LES rat indicating a powerful anti-inflammatory activity in the surrounding spinal cord which is water soluble [5] and plays a critical role in elimination of the macrophage infiltration. The anti-inflammatory effect in the LES rat SCI causes an extraordinarily accelerated elimination of inflammation in the COI in comparison to SCI in the normally myelinated spinal cord where it takes more than 16 weeks to eliminate CD68+/CD163- phagocytizing macrophages [6]. The presence of diffuse astrogliosis in the CNS of the LES rat [39, 40] is related to a severe dysmyelination with the lack of formation of the nodes of Ranvier [42] and not to an inflammatory process suggesting that astrogliosis represents a CNS reaction designed to restore homeostasis which may involve a multitude of functions including anti-inflammatory activity [5, 6]. Gradual decline in numbers of consistently pro-inflammatory, CD68+/CD163- macrophages in the COI after 4 weeks supports the notion of growing anti-inflammatory activity and is supported by reduction of levels of pro-inflammatory cytokines and chemokines in the spinal cord [6]. Since these molecular changes coincide with growing severity of peri-lesional, anisomorphic astroglial reaction, this neuroplastic change needs to be considered in anti-inflammatory tissue reaction after SCI.

1.3. Subdural Infusion; Novel Strategies for Anti-inflammatory Treatment in the Cavity of Injury

The aqueous environment of the COI, connects directly with the subdural space [7, 43], allowing for a novel intrathecal route of administration of anti-inflammatory treatments. Short, 24-48 hr intravenous infusion of high dose of methylprednisolone succinate has been proposed earlier [2, 44-46] but is no longer recommended [47] due to the increased risk of adverse side effects and no improved neurologic deficits [4, 11, 44, 48, 49]. However, much of the literature and work to date has examined the use of steroids as potent

acute inhibitors of inflammation. In rat studies, the subdural infusion of dexamethasone sustained for a period of 1-2 weeks [7, 8] allowed for an effective inhibition of macrophage infiltration in the COI interpreted as neuroprotective effect, but of a too short a duration to eliminate the myelin-rich debris and inflammation from the COI [8]. Since the dose of dexamethasone required to achieve inhibition was unduly toxic to rats [7, 11] and a much more protracted subdural infusion was required than 2 weeks [8], novel, powerful anti-inflammatory drugs of low toxicity are required for the inhibition and elimination of the destructive inflammation in the COI. Given the above considerations, controversial current treatment, recommended by the North American Spinal Cord Injury Study (NASCIS) for the SCI patients; an intravenous bolus of 30 mg/kg body weight methylprednisolone succinate over 1 hour followed by the intravenous dose of 5.4 mg/kg b.w./hour for 23-47 hours [2] does not address the pathogenesis of the SCI [6] and is considered not effective.

Histologic observation of the limiting effect of infused dexamethasone on the astrocytic hypertrophy around the COI [7] may suggest that beneficial effects integrated by astrocytes including their potential anti-inflammatory and anti-edema activities may negatively be affected by this powerful synthetic steroid and point to the need of better, anti-inflammatory compounds not inhibitory to astrogliosis.

1.4. Arachnoiditis and Formation of the Syrinx

Previous theories indicating the formation of the syrinx as the result of changes in the movement of the CSF in the subdural space caused by arachnoiditis with a localized increase of hydrostatic pressure [43, 50, 51] cannot be discounted at this time. Arachnoiditis is an inflammatory process where macrophages, fibroblasts and capillary blood vessels originate from the subarachnoid space and invade a superficial site of the spinal cord trauma as opposed to deep trauma converted into the COI [6]. Arachnoiditis is essentially granulomatous inflammatory response devoid of glial cells that obliterate the traumatized area with the loss of the spinal cord tissue and expands against the adjoined spinal cord that counteracts by mounting an astroglial reaction that progresses to form a wall of numerous hypertrophied GFAP-positive processes, similar to that surrounding the COI [6]. Ultimately arachnoiditis becomes a collagen-rich scar, not a glial scar, that is excluded from the spinal cord and is walled off from it by astrogliosis. Formation of arachnoiditis may lead to changes in the flow pattern of the CSF in the subdural space and to the creation of localized increase in hydrostatic pressure previously proposed as the mechanisms leading to the formation of the syrinx [55]. The increase in the hydrostatic pressure however, implies mechanical damage to the spinal cord which then may lead to necrosis, hemorrhage and edema in surrounding tissue, not unlike in trauma initiating the formation of the COI deep in the spinal cord or of arachnoiditis on its surface [6].

2. MECHANISMS INVOLVED IN THE REMOVAL OF EDEMA IN THE SPINAL CORD INJURY

2.1. CNS Edema

Edema formation following trauma to the CNS poses an acute and serious medical problem for intensive therapists. A large volume of trauma can result in remarkable swelling of the brain and consequently, to the increase in the intracranial pressure, cerebellar herniation and death [12, 56-59]. Edema develops acutely after the SCI; its severity is dependent on the volume of damaged tissue and is considered directly related to the severity of neurologic deficits [60-62]. Treatment of CNS edema involves the intravenous administration of 20% mannitol, a hyperosmotic solute that has been used to treat the CNS in the last >100 years. A frequent occurrence of hyponatremia, <135 mMol liter^{-1} in a TBI patient's serum [63, 64] requires complex fluid and Na^+ management by intensive therapists involving intravenous administration of hyperosmotic saline (1.8 or 3%) to elevate the Na^+ content in the blood circulation and in the brain. It needs to be pointed out however, that the current treatments of the CNS edema address the flow of solutes and water *via* the BBB and do not address the flow of water *via* the pia terminalis externa to the cerebrospinal fluid (CSF) in the subarachnoid space and *via* pia terminalis interna into the cerebral ventricles and central canal (discussed below). The current understanding of the mechanisms involving AQP4 in astrogliosis potentially leading to a progressively greater outflow of fluid from the edematous CNS needs to be addressed in the development of novel therapies of the CNS edema following a traumatic injury and subsequent inflammation.

A substantial body of recent research on the role of bidirectional water channels, AQPs in the water management in the brain [13, 69, 65] and the spinal cord [70] allows for a better understanding of mechanisms that are involved in the removal of the edema fluid from the CNS [71, 72] and may offer new directions for improved pharmacological management of spinal cord edema [73-75].

2.2. Role of Aquaporins in Removal of CNS Edema

Aquaporins conduct a passive bidirectional movement of water into and out of the cells. Pioneering studies of aquaporins by Agre *et al*. resulted in a Nobel Price in Chemistry in 2003. In the CNS, AQP4 has been the most studied water channel [22, 24, 71, 72, 76-78]. In the intact CNS, it is expressed on end feet of astrocytes particularly at the wall of capillary blood vessels, at the glia limitans externa facing the CSF of the subarachnoid space, and at the glia limitans interna facing the ependyma of the cerebral ventricles and the central canal in the spinal cord [13, 18, 20, 22]. This specific distribution of AQP4 has been interpreted as the mechanism of water movement out of the CNS *via* the 3 main exits with particular concentration of AQP4 channels [24]. A systematic study on mechanisms behind the elimination of the acute cerebral edema in cats revealed that 87% of edema fluid was eliminated into the CSF *via* glia limitans externa

and interna, and only 11% into the blood stream [12] in support of the thesis by Klatzo [79] that most of the cerebral edema fluid is evacuated through the glia limitans into the CSF of the subarachnoid space and of the cerebral ventricles. The hyperosmotic treatments of cerebral edema with intravenous mannitol, hyperosmotic NaCl and diuretics [63, 64] address the removal of edema fluid *via* the blood-brain barrier (BBB) and intravascular blood. However, this offers limited clinical benefits pointing to the need of utilizing other, apparently greater avenues of elimination of edema fluid; *via* the astrocytic end feet forming glia limitans externa and interna.

The classic work by Klatzo [79] introduced 2 main types of cerebral edema; (I) cytotoxic edema, not actually involving a toxic etiology but indicating the lack of damage to BBB and swelling of astrocytic processes in acute water overload [57], and (II) vasogenic edema where BBB is damaged due to a pathologic event such as an actively growing tumor mass, trauma and infection [80]. The critical role of AQP4 in the formation and elimination of cerebral edema was clearly demonstrated in transgenic mice lacking AQP4 [81, 82] who were resistant to the formation of cytotoxic edema [82, 83] but also unable to efficiently eliminate vasogenic edema in comparison to wild type mice [83]. Enhancement of the expression of AQP4 channels has been proposed as a therapeutic strategy to faster eliminate edema fluid following SCI [25, 83-85]. The CNS edema invariably initiates two related events; astrogliosis and remarkable change in expression of AQP4 that appears to be biphasic; the initial decline in AQP4 is followed by remarkable increase of this water channel after 2 weeks [18, 19] when much edema fluid is eliminated as indicated by systematic studies using direct measurement of water content in the tissue [25] or T2-weighed MR imaging in live animal models [25, 62] or patients [60, 63]. Astrogliosis involves migration of astrocytes towards the site of injury, a process mediated by AQP4 and remarkably slowed in the absence of this water channel [86, 87]. Astrogliosis with enhanced expression of AQP4 in the tissue around 1-2 year old syrinxes in the human spinal cord was reported [70] indicating that the processes involved in the formation of the COI and related involvement of astrogliosis and hyperplasia of AQP4 studied in rodents may be relevant to the neuropathology of the human SCI. Studies of the old rat SCI lesions established that the severity of astrogliosis and remarkable hyperplasia of AQP4 persisted for over 11 months indicating that this change is irreversible [25]. Although it is now clear that the formation of the COI following the SCI involves severe inflammation in the cavity and widespread vasogenic edema in the surrounding spinal cord [6] and the subdural infusion of anti-inflammatory compounds inhibited the inflammation [8.9.11], the effect of inhibition of inflammation in the COI on the vasogenic edema remains unknown. It is however, anticipated that the inhibition and elimination of the inflammatory process in the COI would likely translate into inhibition of the vasogenic effect followed by rapid removal of CNS edema by reactive astrocytes.

AQP1 is expressed on the ependymal cells of the choroid plexus and it plays an important role in the secretion of the CSF [17, 19] since mice lacking this aquaporin have diminished production of the CSF by 25% [21]. The role of AQP1 in the CNS tissue reaction to edema has been postulated due to the increase of the expression of either aquaporin in the antibody-stained sections, increase in the expression of the protein on Western blots or the elevation of the specific mRNA [19] implicated in the formation of cavities post-SCI [19], however, the role of AQP1 in the removal of the edema fluid remains unclear.

AQP9 is an aquaglyceroporin permeable to water as well as to small molecules including glycerol, urea and lactate, expressed on astrocytes and on certain populations of neurons in the brain stem [13, 16]. In the intact brain of the mouse and the rat, the AQP9 is expressed in astrocytic processes forming the glia limitans externa and the glia limitans interna [15, 16]. The levels of AQP9 are remarkably increased in hypertrophied astrocytes surrounding infarct lesions [15] suggesting involvement of this aquaporin in the water movement in cerebral edema, a possibility that still requires specific experimental determination in the SCI.

2.3. The Role of Astrogliosis and of the Cavity of Injury in Removal of CNS Edema

Although the direct association of astrogliosis with elevated AQP4 expression has been documented in models of brain and spinal cord edema [18], the role of astrogliosis as the cellular mechanism of the tissue response in neuropathology remains unclear. Astrocytes are the most numerous cells in the CNS and fulfill a very complex and demanding role of maintaining homeostasis. In the intact CNS astrocytes establish local, non-overlapping control units that regulate the function of synapses, maintain physiologic levels of glutamate and K^+ as well as physiologic pH [88-92]. Positioned between neurons and the blood vessels, astrocytes maintain the blood-brain barrier or blood-spinal cord barrier and regulate the blood flow according to the demands of changing activity in a given area of the CNS [91-93]. Astrocytes are interconnected *via* gap junctions rich in connexins, particularly Cx43 [94, 95] effectively involved in formation of regional syncytial networks suitable for the dissipation of locally high concentrations of neurotransmitters and of ions by passing them as well as other small active molecules to adjacent astrocytes thus lowering the interstitial concentration of an active factor [94, 95].

Astrogliosis is a CNS tissue reaction to injury that is designed to lead to the restoration of homeostasis in the surrounding, non-injured tissue [23, 32]. A massive tissue damage in the SCI leads to remarkable glial cell proliferation around the site of injury leading primarily to the generation of astrocytes [6, 96-98] that tend to migrate towards the site of injury [86, 87] where they participate in formation of the wall of the COI [6, 23, 32]. Following the SCI, vasogenic edema is efficiently removed from the interstitial space by the astrocytic syncytium [92] that leads to transiently swollen astrocytic processes [90, 99]. In the CNS edema, astrogliosis with specifically increased expression of AQP4 should therefore be considered as an integrative process serving to move excess water out of the brain and the spinal

cord. This raises a question of whether 3 main paths of water removal; *via* the glia limitans externa to the subarachnoid space, *via* the glial limitans interna to cerebral ventricles and *via* the parenchymal blood vessels are the only routes for the elimination of edema fluid? Or is edema fluid also actively pumped by hypertrophied astrocytes (Fig. **1**) equipped with increased numbers of AQP4 channels into the forming COI such as in the SCI [6-8, 11, 18, 25]? The remarkable intensity of astrocytic hypertrophy that is particularly evident around the COI constantly increases during 16 weeks post-SCI [6], and the elevated expression of AQP4 around the cavity is evident by 2-3 weeks post-SCI with the concurrent decline in the edema in the surrounding spinal cord [9, 10, 18, 25] providing a morphologic support for this concept. Furthermore, a direct connection of the COI with the subdural space of the injured spinal cord [7] may allow the excess fluid to escape from the cavity without a build-up of hydrostatic pressure considered damaging to the surrounding tissue (Fig. **2**). It needs to be noted that the connection with the subdural space could allow for the inflow of the CSF into the COI as demonstrated in subdural infusion of dexamethasone, Serp-1 and M-T7 resulting in an anti-inflammatory effect in the cavity [7, 8, 11]. Although the source of water in the forming COI can be from subarachnoid space, it is the possibility of edema fluid being pumped into the COI (Fig. **2**) that has important medical implications and needs to be studied further. In a large brain, such as in a human patient, a closed, non-communicating cavity resulting from TBI or stroke may often form deep in the tissue with a potential for the hydrostatic pressure to build up. This potential clinicopathological problem will have to be first addressed, confirmed and then, if present, treated perhaps by the creation of an artificial outflow from the cavity to the subarachnoid space or extra-cranially which should have a therapeutic effect on the outcome of cerebral edema. This hypothesis and the understanding of the function and distribution of the water channels in the brain and in the spinal cord may indicate a direction of novel therapeutic management of cerebral and spinal cord edema. Since astrogliosis appears to play central role in the removal of edema in the SCI, experiments leading to the enhancement of astrogliosis [23] should address a novel therapeutic approach; the enhancement of the spinal cord tissue reaction designed to accelerate the removal of edema fluid. A more immediate pharmacological approach to eliminating edema may be the use of anti-inflammatories to inhibit [7, 9, 11] and eliminate [100] inflammation from the COI and thus inhibit vasogenic edema. The breakdown of the BSCB or of the BBB due to severe inflammation following the initial traumatic event [6] is a complex process involving proteolytic cleavage of the endothelial tight junctions and breakdown of the capillary basement membrane [101] leading to an uncontrolled leakage of fluid and solutes to the perivascular CNS parenchyma [102, 103]. Metalloproteinases, particularly MMP-9, activated by thrombin generated during the thrombotic/thrombolytic cascade, have been reported to be active in disrupting the BBB [102, 103]. The related vasogenic edema is further complicated by the activation of microglia and the release of pro-inflammatory cytokines, including IL-1β and TNF-α [6, 102, 103] and infiltration of the perivascular parenchyma by leukocytes [102, 103]. Specific inhibitors of thrombin, tissue plasminogen activator (tPA), urokinasePA and plasmin such as a viral-derived Serp-1 or its active fragment have been able to inhibit these pro-inflammatory vascular events [104, 105], and their anti-inflammatory effects have already been studied in the rat models of SCI [11, 106] but specific vasogenic edema-inhibiting effects have not been yet addressed.

Although the build-up of astrogliosis in the spinal cord surrounding the COI (Fig. **1**) is evident [6-8, 11] and apparently associated with potent anti-inflammatory activity [5, 6] its role in potentially counteracting edema related to the inflammation in the cavity of injury has not been addressed (Fig. **2**).

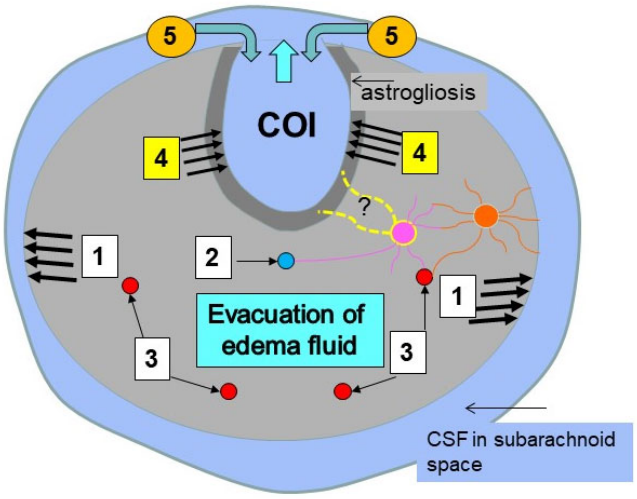

Fig. (1). Conceptual mechanisms of edema elimination from the spinal cord post-injury. *(A higher resolution / colour version of this figure is available in the electronic copy of the article)*.

LEGEND: A massive injury to the spinal cord results in a cavity of injury (COI). It contains necrotic debris, hemorrhages, infiltrating macrophages and fluid, and is surrounded by a wall of astrogliosis (see Fig. **2**) and by edematous spinal cord. As the severity of astrogliosis increases, the edema fluid is moved with participation of aquaporin-4 channels expressed by astrocytes (orange and pink) via; glia limitans externa to the subarachnoid space (1), glia limitans interna, under the ependymal cells lining the central canal (2), and the blood-spinal cord barrier (3). Given the active formation of the COI that accumulates water after the injury, we propose that a large proportion of edema fluid is actively moved into the COI by reactive astrocytes (pink, 4). The blue vertical arrow indicates that there is a direct connection between the COI and the subarachnoid space, therefore the excess edema fluid can be moved from the COI into the subarachnoid space thus alleviating a potential increase in the hydrostatic pressure in the COI. The flow of the cerebrospinal fluid (CSF) from the subarachnoid space into the COI also occurs (5). Since astrocytes widely interconnect with each other *via* gap junctions, the excess water and associated solutes can be moved across the parenchyma *via* any 4 routes.

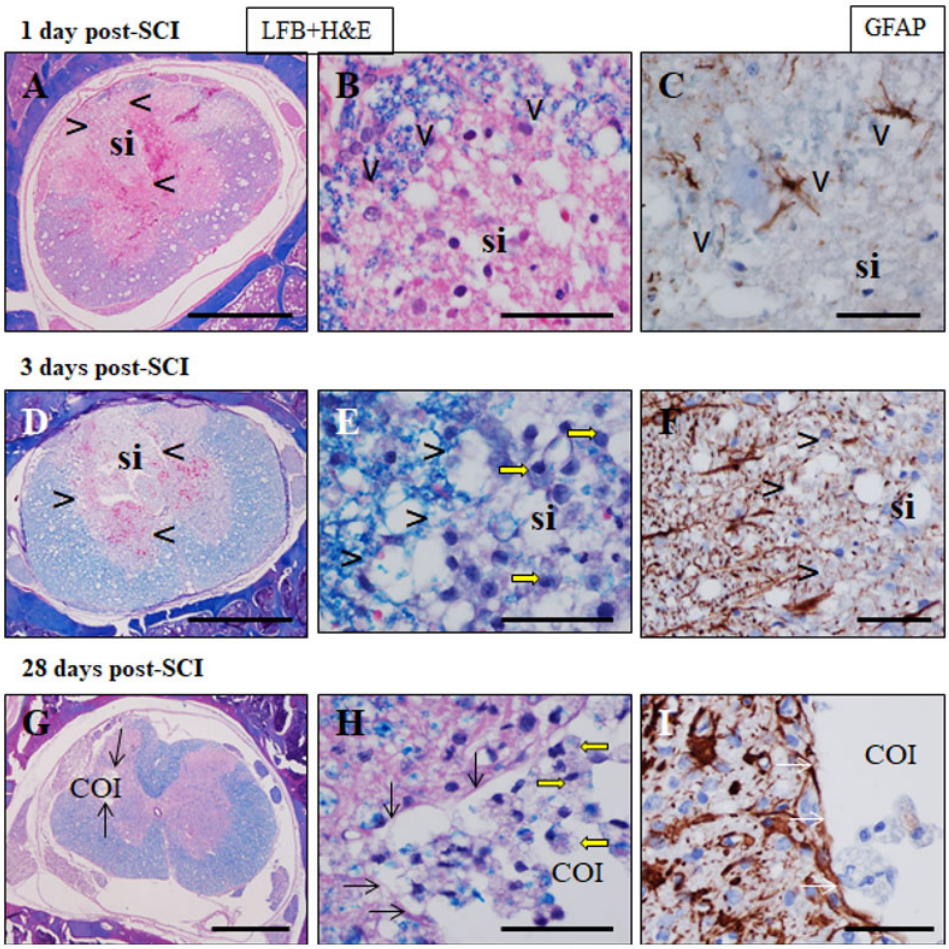

Fig. (2). Reactive astrogliosis involved in the forming cavity of injury following the spinal cord injury. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

LEGEND: An epidural balloon crush injury with a 3Fogarty catheter, of the mid-thoracic spinal cord results in a localized site of injury (si) obliterating most of the dorsal column and surrounding areas with hemorrhages and cellular necrosis indicated by arrowheads. The astrocytes are scattered in the spinal cord around the site of injury at the day 1 post-spinal cord injury (SCI) (C) but appear oriented and hypertrophied at the day 3 post-SCI (F) which coincides with the infiltration of the site of injury by numerous macrophages often laden with blue granules of myelin (yellow arrows in E). At 28 days post-SCI (G-I) a clearly defined cavity of injury (arrows, COI) contains much clear fluid with scattered macrophages containing blue granules of myelin (yellow arrows in H) and is surrounded by a wall of intense astrogliosis (I) with numerous, markedly hypertrophied astrocytes forming a continuous "capsule" around the COI with their processes (arrows in I).

Luxol fast blue counterstained with hematoxylin and eosin (LFB+H&E) (A,B,D,E,G,H). IHC stain with primary anti-GFAP antibody (DAKO kit) - (C,F,I). Size bars: 1 mm (A,D,G), 50 μm (B,C,E,F,H,I).

Although the sub-dural infusion of dexamethasone, Serp-1 or M-T7 resulted in an anti-inflammatory effect of inhibition of macrophage infiltration of the COI [7, 8, 11] the effect of these treatments on edema in the spinal cord surrounding the COI has not been addressed. Dexamethasone appears to have an inhibitory effect on astroglial hypertrophy [7] but Serp-1 and M-T7 do not [11]. It is also evident that successful inhibitory treatment of the severe, destructive inflammation in the COI will require an infusion of effective doses well beyond 1-2 weeks [6-8, 11], not likely achievable with high doses of dexamethasone due to its severe toxicity [7], indicating the need for non-toxic compounds with powerful anti-inflammatory activity with the ability to also inhibit inflammatory edema following the SCI and also, following brain trauma and stroke. Serp-1 and M-T7 infused subdurally fulfill this requirement and recently completed study determined that constant infusion of Serp-1 at 0.2 mg/week for 8 weeks [100] resulted in lowering the numbers of macrophages in the COI to levels seen in untreated SCI until 16 weeks post-SCI [6] indicating a neuroprotective therapeutic effect. Whether inhibition of inflammation in the COI

will result in inhibition of vascular damage and accelerate the evacuation of vasogenic edema remains an important question.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The financial support provided by VPC NeuroPath CONSULTING, Inc. to JMK and KHD and by Medical University of Lublin to WD.

ACKNOWLEDGEMENTS

The authors wish to acknowledge excellent histology service by Mary Jo Smith and Mary Bruni, MIRC Laboratory, McMaster University.

REFERENCES

[1] Krebs, J.; Koch, H.G.; Hartmann, K.; Frotzler, A. The characteristics of posttraumatic syringomyelia. *Spinal Cord,* **2016**, *54*(6), 463-466. http://dx.doi.org/10.1038/sc.2015.218 PMID: 26620880

[2] Bracken, M.B.; Shepard, M.J.; Collins, W.F.; Holford, T.R.; Young, W.; Baskin, D.S.; Eisenberg, H.M.; Flamm, E.; Leo-Summers, L.; Maroon, J.; Marshall, L.F.; Perot, P.L., Jr; Piepneier, J.; Sonntag, V.K.H.; Wagner, F.C.; Wilberger, J.E.; Winn, H.R. A randomized, controlled trial of methylprednisolone or naloxone in the treatment of acute spinal-cord injury. Results of the Second National Acute Spinal Cord Injury Study. *N. Engl. J. Med.,* **1990**, *322*(20), 1405-1411. http://dx.doi.org/10.1056/NEJM199005173222001 PMID: 2278545

[3] Markandaya, M.; Stein, D.M.; Menaker, J. Acute treatment options for spinal cord injury. *Curr. Treat. Options Neurol.,* **2012**, *14*, 175-187. http://dx.doi.org/10.1007/s11940-011-0162-5 PMID: 22302639

[4] Pettiford, J.N.; Bikhchandani, J.; Ostlie, D.J.; St Peter, S.D.; Sharp, R.J.; Juang, D. A review: the role of high dose methylprednisolone in spinal cord trauma in children. *Pediatr. Surg. Int.,* **2012**, *28*(3), 287-294. http://dx.doi.org/10.1007/s00383-011-3012-3 PMID: 21994079

[5] Kwiecien, J.M. Cellular mechanisms of white matter regeneration in an adult dysmyelinated rat model. *Folia Neuropathol.,* **2013**, *51*(3), 189-202. http://dx.doi.org/10.5114/fn.2013.37703 PMID: 24114636

[6] Kwiecien, J.M.; Dabrowski, W.; Dąbrowska-Bouta, B.; Sulkowski, G.; Oakden, W.; Kwiecien-Delaney, C.J.; Yaron, J.R.; Zhang, L.; Schutz, L.; Marzec-Kotarska, B.; Stanisz, G.J.; Karis, J.P.; Struzynska, L.; Lucas, A.R. Prolonged inflammation leads to ongoing damage after spinal cord injury. *PLoS One,* **2020**, *15*(3)e0226584 http://dx.doi.org/10.1371/journal.pone.0226584 PMID: 32191733

[7] Kwiecien, J.; Jarosz, B.; Urdzikova, L.M.; Rola, R.; Dabrowski, W. Subdural infusion of dexamethasone inhibits leukomyelitis after acute spinal cord injury in a rat model. *Folia Neuropathol.,* **2015**, *53*(1), 41-51. http://dx.doi.org/10.5114/fn.2015.49973 PMID: 25909874

[8] Kwiecien, J.M.; Jarosz, B.; Oakden, W.; Klapec, M.; Stanisz, G.J.; Delaney, K.H.; Kotlinska-Hasiec, E.; Janik, R.; Rola, R.; Dabrowski, W. An in *vivo* model of anti-inflammatory activity of subdural dexamethasone following the spinal cord injury. *Neurol. Neurochir. Pol.,* **2016**, *50*(1), 7-15. http://dx.doi.org/10.1016/j.pjnns.2015.10.006 PMID: 26851684

[9] Oakden, W.; Kwiecien, J.M.; O'Reilly, M.A.; Lake, E.M.; Akens, M.K.; Aubert, I.; Whyne, C.; Finkelstein, J.; Hynynen, K.; Stanisz, G.J. A non-surgical model of cervical spinal cord injury induced with focused ultrasound and microbubbles. *J. Neurosci. Methods,* **2014**, *235*, 92-100. http://dx.doi.org/10.1016/j.jneumeth.2014.06.018 PMID: 24970578

[10] Oakden, W.; Kwiecien, J.M.; O'Reilly, M.A.; Dabrowski, W.; Whyne, C.; Finkelstein, J.; Hynynen, K.; Stanisz, G.J. Quantitative MRI in a non-surgical model of cervical spinal cord injury. *NMR Biomed.,* **2015**, *28*(8), 925-936. http://dx.doi.org/10.1002/nbm.3326 PMID: 26053102

[11] Kwiecien, J.M.; Dabrowski, W.; Marzec-Kotarska, B.; Kwiecien-Delaney, C.J.; Yaron, J.R.; Zhang, L.; Schutz, L.; Lucas, A.R. Myxoma virus derived immune modulating proteins, M-T7 and Serp-1, reduce early inflammation after spinal cord injury in the rat model. *Folia Neuropathol.,* **2019**, *57*(1), 41-50. http://dx.doi.org/10.5114/fn.2019.83830 PMID: 31038187

[12] Marmarou, A.; Hochwald, G.; Nakamura, T.; Tanaka, K.; Weaver, J.; Dunbar, J. Brain edema resolution by CSF pathways and brain vasculature in cats. *Am. J. Physiol.,* **1994**, *267*(2 Pt 2), H514-H520. PMID: 8067404

[13] Amiry-Moghaddam, M.; Otsuka, T.; Hurn, P.D.; Traystman, R.J.; Haug, F.M.; Froehner, S.C.; Adams, M.E.; Neely, J.D.; Agre, P.; Ottersen, O.P.; Bhardwaj, A. An alpha-syntrophin-dependent pool of AQP4 in astroglial end-feet confers bidirectional water flow between blood and brain. *Proc. Natl. Acad. Sci. USA,* **2003**, *100*(4), 2106-2111. http://dx.doi.org/10.1073/pnas.0437946100 PMID: 12578959

[14] Amiry-Moghaddam, M.; Lindland, H.; Zelenin, S.; Roberg, B.A.; Gundersen, B.B.; Petersen, P.; Rinvik, E.; Torgner, I.A.; Ottersen, O.P.; Torgner, I.A.; Ottersen, O.P. Brain mitochondria contain aquaporin water channels: evidence for the expression of a short AQP9 isoform in the inner mitochondrial membrane. *FASEB J.,* **2005**, *19*(11), 1459-1467. http://dx.doi.org/10.1096/fj.04-3515com PMID: 16126913

[15] Badaut, J.; Hirt, L.; Granziera, C.; Bogousslavsky, J.; Magistretti, P.J.; Regli, L. Astrocyte-specific expression of aquaporin-9 in mouse brain is increased after transient focal cerebral ischemia. *J. Cereb. Blood Flow Metab.*, **2001**, *21*(5), 477-482. http://dx.doi.org/10.1097/00004647-200105000-00001 PMID: 11333357

[16] Badaut, J.; Regli, L. Distribution and possible roles of aquaporin 9 in the brain. *Neuroscience,* **2004**, *129*(4), 971-981. http://dx.doi.org/10.1016/j.neuroscience.2004.06.035 PMID: 15561412

[17] Boassa, D.; Stamer, W.D.; Yool, A.J. Ion channel function of aquaporin-1 natively expressed in choroid plexus. *J. Neurosci.,* **2006**, *26*(30), 7811-7819. http://dx.doi.org/10.1523/JNEUROSCI.0525-06.2006 PMID: 16870726

[18] Hemley, S.J.; Bilston, L.E.; Cheng, S.; Chan, J.N.; Stoodley, M.A. Aquaporin-4 expression in post-traumatic syringomyelia. *J. Neurotrauma,* **2013**, *30*(16), 1457-1467. http://dx.doi.org/10.1089/neu.2012.2614 PMID: 23441695

[19] Nesic, O.; Lee, J.; Unabia, G.C.; Johnson, K.; Ye, Z.; Vergara, L.; Hulsebosch, C.E.; Perez-Polo, J.R. Aquaporin 1 - a novel player in spinal cord injury. *J. Neurochem.,* **2008**, *105*(3), 628-640. http://dx.doi.org/10.1111/j.1471-4159.2007.05177.x PMID: 18248364

[20] Nielsen, S.; Nagelhus, E.A.; Amiry-Moghaddam, M.; Bourque, C.; Agre, P.; Ottersen, O.P. Specialized membrane domains for water transport in glial cells: high-resolution immunogold cytochemistry of aquaporin-4 in rat brain. *J. Neurosci.,* **1997**, *17*(1), 171-180. http://dx.doi.org/10.1523/JNEUROSCI.17-01-00171.1997 PMID: 8987746

[21] Oshio, K.; Watanabe, H.; Song, Y.; Verkman, A.S.; Manley, G.T. Reduced cerebrospinal fluid production and intracranial pressure in mice lacking choroid plexus water channel Aquaporin-1. *FASEB J.,* **2005**, *19*(1), 76-78. http://dx.doi.org/10.1096/fj.04-1711fje PMID: 15533949

[22] Rash, J.E.; Yasumura, T.; Hudson, C.S.; Agre, P.; Nielsen, S. Direct immunogold labeling of aquaporin-4 in square arrays of astrocyte and ependymocyte plasma membranes in rat brain and spinal cord. *Proc. Natl. Acad. Sci. USA,* **1998**, *95*(20), 11981-11986. http://dx.doi.org/10.1073/pnas.95.20.11981 PMID: 9751776

[23] Escartin, C.; Bonvento, G. Targeted activation of astrocytes: a potential neuroprotective strategy. *Mol. Neurobiol.,* **2008**, *38*(3), 231-241. http://dx.doi.org/10.1007/s12035-008-8043-y PMID: 18931960

[24] Rash, J.E.; Davidson, K.G.; Yasumura, T.; Furman, C.S. Freeze-fracture and immunogold analysis of aquaporin-4 (AQP4) square arrays, with models of AQP4 lattice assembly. *Neuroscience,* **2004**, *129*(4), 915-934. http://dx.doi.org/10.1016/j.neuroscience.2004.06.076 PMID: 15561408

[25] Nesic, O.; Lee, J.; Ye, Z.; Unabia, G.C.; Rafati, D.; Hulsebosch, C.E.; Perez-Polo, J.R. Acute and chronic changes in aquaporin 4 expression after spinal cord injury. *Neuroscience,* **2006**, *143*(3), 779-792. http://dx.doi.org/10.1016/j.neuroscience.2006.08.079 PMID: 17074445

[26] Ko, H.Y.; Kim, W.; Kim, S.Y.; Shin, M.J.; Cha, Y.S.; Chang, J.H.; Shin, Y.B. Factors associated with early onset post-traumatic syringomyelia. *Spinal Cord,* **2012**, *50*(9), 695-698. http://dx.doi.org/10.1038/sc.2012.35 PMID: 22487955

[27] Schurch, B.; Wichmann, W.; Rossier, A.B. Post-traumatic syringomyelia (cystic myelopathy): a prospective study of 449 patients with spinal cord injury. *J. Neurol. Neurosurg. Psychiatry,* **1996**, *60*(1), 61-67. http://dx.doi.org/10.1136/jnnp.60.1.61 PMID: 8558154

[28] Backe, H.A.; Betz, R.R.; Mesgarzadeh, M.; Beck, T.; Clancy, M. Post-traumatic spinal cord cysts evaluated by magnetic resonance imaging. *Paraplegia,* **1991**, *29*(9), 607-612. PMID: 1787985

[29] Perrouin-Verbe, B.; Lenne-Aurier, K.; Robert, R.; Auffray-Calvier, E.; Richard, I.; Mauduyt de la Grève, I.; Mathé, J.F. Post-traumatic syringomyelia and post-traumatic spinal canal stenosis: a direct relationship: review of 75 patients with a spinal cord injury. *Spinal Cord,* **1998**, *36*(2), 137-143. http://dx.doi.org/10.1038/sj.sc.3100625 PMID: 9495005

[30] Silberstein, M.; Hennessy, O. Cystic cord lesions and neurological deterioration in spinal cord injury: operative considerations based on magnetic resonance imaging. *Paraplegia,* **1992**, *30*(9), 661-668. PMID: 1408344

[31] Zhang, C.; Morozova, A.Y.; Abakumov, M.A.; Gubsky, I.L.; Douglas, P.; Feng, S.; Bryukhovetskiy, A.S.; Chekhonin, V.P. Precise delivery into chronic spinal cord injury syringomyelic cysts with magnetic nanoparticles MRI visualization. *Med. Sci. Monit.,* **2015**, *21*, 3179-3185. http://dx.doi.org/10.12659/MSM.895624 PMID: 26486048

[32] Karimi-Abdolrezaee, S.; Billakanti, R. Reactive astrogliosis after spinal cord injury-beneficial and detrimental effects. *Mol. Neurobiol.,* **2012**, *46*(2), 251-264. http://dx.doi.org/10.1007/s12035-012-8287-4 PMID: 22684804

[33] Inman, D.M.; Steward, O. Physical size does not determine the unique histopathological response seen in the injured mouse spinal cord. *J. Neurotrauma,* **2003**, *20*(1), 33-42. http://dx.doi.org/10.1089/08977150360517164 PMID: 12614586

[34] Reddy, K.K.; Del Bigio, M.R.; Sutherland, G.R. Ultrastructure of the human posttraumatic syrinx. *J. Neurosurg.,* **1989**, *71*(2), 239-243. http://dx.doi.org/10.3171/jns.1989.71.2.0239 PMID: 2746347

[35] Seki, T.; Fehlings, M.G. Mechanistic insights into posttraumatic syringomyelia based on a novel in *vivo* animal model. Laboratory investigation. *J. Neurosurg. Spine,* **2008**, *8*(4), 365-375. http://dx.doi.org/10.3171/SPI/2008/8/4/365 PMID: 18377322

[36] Levine, D.N. The pathogenesis of syringomyelia associated with lesions at the foramen magnum: a critical review of existing theories and proposal of a new hypothesis *J. Neurol. Sci.,* **2004**, *220*, 3-21.

[37] Najafi, E.; Bilston, L.E.; Song, X.; Bongers, A.; Stoodley, M.A.; Cheng, S.; Hemley, S.J. Longitudinal measurements of syrinx size in a rat model of posttraumatic syringomyelia. *J. Neurosurg. Spine,* **2016**, *24*(6), 941-948. http://dx.doi.org/10.3171/2015.10.SPINE15538 PMID: 26918577

[38] Delaney, K.H.; Kwiecien, J.M.; Wegiel, J.; Wisniewski, H.M.; Percy, D.H.; Fletch, A.L. Familial dysmyelination in a Long Evans rat mutant. *Lab. Anim. Sci.,* **1995**, *45*(5), 547-553. PMID: 8569154

[39] Kwiecien, J.M.; O'Connor, L.T.; Goetz, B.D.; Delaney, K.H.; Fletch, A.L.; Duncan, I.D. Morphological and morphometric studies of the dysmyelinating mutant, the Long Evans shaker rat. *J. Neurocytol.,* **1998**, *27*(8), 581-591. http://dx.doi.org/10.1023/A:1006922227791 PMID: 10405025

[40] Kwiecien, J.M. Cellular compensatory mechanisms in the CNS of dysmyelinated rats. *Comp. Med.,* **2010**, *60*(3), 205-217. PMID: 20579436

[41] O'Connor, L.; Goetz, B.; Kwiecien, J.M.; Delaney, K.; Fletch, A.; Duncan, I.D. Insertion of a retrotransposon into the myelin basic protein gene causes CNS dysmyelination in the Long Evans shaker (LES) rat. *J. Neurosci.,* **1999**, *19*, 3404-3413. PMID: 10212300

[42] Eftekharpour, E.; Karimi-Abdolrezaee, S.; Sinha, K.; Velumian, A.A.; Kwiecien, J.M.; Fehlings, M.G. Structural and functional alterations of spinal cord axons in adult Long Evans Shaker (LES) dysmyelinated rats. *Exp. Neurol.,* **2005**, *193*(2), 334-349. http://dx.doi.org/10.1016/j.expneurol.2005.01.019 PMID: 15869936

[43] Brodbelt, A.R.; Stoodley, M.A. Post-traumatic syringomyelia: a review. *J. Clin. Neurosci.,* **2003**, *10*(4), 401-408. http://dx.doi.org/10.1016/S0967-5868(02)00326-0 PMID: 12852875

[44] Bowes, A.L.; Yip, P.K. Modulating inflammatory cell responses to spinal cord injury: all in good time. *J. Neurotrauma,* **2014**, *31*(21), 1753-1766. http://dx.doi.org/10.1089/neu.2014.3429 PMID: 24934600

[45] Schoenfeld, A.J.; Laughlin, M.D.; McCriskin, B.J.; Bader, J.O.; Waterman, B.R.; Belmont, P.J., Jr Spinal injuries in United States military personnel deployed to Iraq and Afghanistan: an epidemiological investigation involving 7877 combat casualties from 2005 to 2009. *Spine,* **2013**, *38*(20), 1770-1778. http://dx.doi.org/10.1097/BRS.0b013e31829ef226 PMID: 23759821

[46] Shank, C.D.; Walters, B.C.; Hadley, M.N. Current topics in the management of acute traumatic spinal cord injury. *Neurocrit. Care,* **2019**, *30*(2), 261-271. http://dx.doi.org/10.1007/s12028-018-0537-5 PMID: 29651626

[47] Hurlbert, R.J.; Hadley, M.N.; Walters, B.C.; Aarabi, B.; Dhall, S.S.; Gelb, D.E.; Rozzelle, C.J.; Ryken, T.C.; Theodore, N. Pharmacological therapy for acute spinal cord injury. *Neurosurgery,* **2013**, *72*(Suppl. 2), 93-105. http://dx.doi.org/10.1227/NEU.0b013e31827765c6 PMID: 23417182

[48] Ahuja, C.S.; Martin, A.R.; Fehlings, M. Recent advances in managing a spinal cord injury secondary to trauma. *F1000 Res.,* **2016**, *5*, 1017. http://dx.doi.org/10.12688/f1000research.7586.1 PMID: 27303644

[49] Brodbelt, A.R.; Stoodley, M.A.; Watling, A.M.; Tu, J.; Jones, N.R. Fluid flow in an animal model of post-traumatic syringomyelia. *Eur. Spine J.,* **2003**, *12*(3), 300-306. http://dx.doi.org/10.1007/s00586-002-0492-9 PMID: 12800004

[50] Gardner, W.J. Hydrodynamic mechanism of syringomyelia: Its relationship to myelocele. *J. Neurol. Neurosurg. Psychiatry,* **1965**, *28*, 247-259. http://dx.doi.org/10.1136/jnnp.28.3.247 PMID: 14345682

[51] Greitz, D. Unraveling the riddle of syringomyelia. *Neurosurg. Rev.,* **2006**, *29*(4), 251-263. http://dx.doi.org/10.1007/s10143-006-0029-5 PMID: 16752160

[52] Josephson, A.; Greitz, D.; Klason, T.; Olson, L.; Spenger, C. A spinal thecal sac constriction model supports the theory that induced pressure gradients in the cord cause edema and cyst formation. *Neurosurgery,* **2001**, *48*(3), 636-645. http://dx.doi.org/10.1097/00006123-200103000-00039 PMID: 11270555

[53] Stoodley, M.A.; Gutschmidt, B.; Jones, N.R. Cerebrospinal fluid flow in an animal model of noncommunicating syringomyelia. *Neurosurgery,* **1999**, *44*(5), 1065-1075. http://dx.doi.org/10.1097/00006123-199905000-00068 PMID: 10232540

[54] Austin, J.W.; Afshar, M.; Fehlings, M.G. The relationship between localized subarachnoid inflammation and parenchymal pathophysiology after spinal cord injury. *J. Neurotrauma,* **2012**, *29*(10), 1838-1849. http://dx.doi.org/10.1089/neu.2012.2354 PMID: 22655536

[55] Graham, D.I.; Adams, J.H.; Nicoll, J.A.; Maxwell, W.L.; Gennarelli, T.A. The nature, distribution and causes of traumatic brain injury. *Brain Pathol.,* **1995**, *5*(4), 397-406. http://dx.doi.org/10.1111/j.1750-3639.1995.tb00618.x PMID: 8974622

[56] Kimelberg, H.K. Current concepts of brain edema. Review of laboratory investigations. *J. Neurosurg.,* **1995**, *83*(6), 1051-1059. http://dx.doi.org/10.3171/jns.1995.83.6.1051 PMID: 7490620

[57] Papadopoulos, M.C.; Krishna, S.; Verkman, A.S. Aquaporin water channels and brain edema. *Mt. Sinai J. Med.,* **2002**, *69*(4), 242-248. PMID: 12357265

[58] Unterberg, A.W.; Stover, J.; Kress, B.; Kiening, K.L. Edema and brain trauma. *Neuroscience,* **2004**, *129*(4), 1021-1029. http://dx.doi.org/10.1016/j.neuroscience.2004.06.046 PMID: 15561417

[59] Boldin, C.; Raith, J.; Fankhauser, F.; Haunschmid, C.; Schwantzer, G.; Schweighofer, F. Predicting neurologic recovery in cervical spinal cord injury with postoperative MR imaging. *Spine,* **2006**, *31*(5), 554-559. http://dx.doi.org/10.1097/01.brs.0000201274.59427.a4 PMID: 16508551

[60] Miyanji, F.; Furlan, J.C.; Aarabi, B.; Arnold, P.M.; Fehlings, M.G. Acute cervical traumatic spinal cord injury: MR imaging findings correlated with neurologic outcome-prospective study with 100 consecutive patients. *Radiology,* **2007**, *243*(3), 820-827. http://dx.doi.org/10.1148/radiol.2433060583 PMID: 17431129

[61] Sharma, H.S.; Badgaiyan, R.D.; Alm, P.; Mohanty, S.; Wiklund, L. Neuroprotective effects of nitric oxide synthase inhibitors in spinal cord injury-induced pathophysiology and motor functions: an experimental study in the rat. *Ann. N. Y. Acad. Sci.,* **2005**, *1053*, 422-434. http://dx.doi.org/10.1196/annals.1344.037 PMID: 16179549

[62] Bradshaw, K.; Smith, M. Disorders of sodium balance after brain injury. *Contin. Educ. Anaesth. Crit. Care Pain,* **2008**, *8*, 129. http://dx.doi.org/10.1093/bjaceaccp/mkn019

[63] Human, T.; Cook, A.M.; Anger, B.; Bledsoe, K.; Castle, A.; Deen, D.; Gibbs, H.; Lesch, C.; Liang, N.; McAllen, K.; Morrison, C.; Parker, D., Jr; Rowe, A.S.; Rhoney, D.; Sangha, K.; Santayana, E.; Taylor, S.; Tesoro, E.; Brophy, G. Treatment of hyponatremia in patients with acute neurological injury. *Neurocrit. Care,* **2017**, *27*(2), 242-248. http://dx.doi.org/10.1007/s12028-016-0343-x PMID: 28054290

[64] Agre, P.; King, L.S.; Yasui, M.; Guggino, W.B.; Ottersen, O.P.; Fujiyoshi, Y.; Engel, A.; Nielsen, S. Aquaporin water channels-from atomic structure to clinical medicine. *J. Physiol.,* **2002**, *542*(Pt 1), 3-16. http://dx.doi.org/10.1113/jphysiol.2002.020818 PMID: 12096044

[65] Amiry-Moghaddam, M.; Hoddevik, E.H.; Ottersen, O.P. Aquaporins: multifarious roles in brain. *Neuroscience,* **2010**, *168*(4), 859-861. http://dx.doi.org/10.1016/j.neuroscience.2010.04.071 PMID: 20450960

[66] Badaut, J.; Lasbennes, F.; Magistretti, P.J.; Regli, L. Aquaporins in brain: distribution, physiology, and pathophysiology. *J. Cereb. Blood Flow Metab.,* **2002**, *22*(4), 367-378. http://dx.doi.org/10.1097/00004647-200204000-00001 PMID: 11919508

[67] Tait, M.J.; Saadoun, S.; Bell, B.A.; Papadopoulos, M.C. Water movements in the brain: role of aquaporins. *Trends Neurosci.,* **2008**, *31*(1), 37-43. http://dx.doi.org/10.1016/j.tins.2007.11.003 PMID: 18054802

[68] Verkman, A.S. Mammalian aquaporins: diverse physiological roles and potential clinical significance. *Expert Rev. Mol. Med.,* **2008**, *10*e13 http://dx.doi.org/10.1017/S1462399408000690 PMID: 18482462

[69] Nesic, O.; Guest, J.D.; Zivadinovic, D.; Narayana, P.A.; Herrera, J.J.; Grill, R.J.; Mokkapati, V.U.; Gelman, B.B.; Lee, J. Aquaporins in spinal cord injury: the janus face of aquaporin 4. *Neuroscience,* **2010**, *168*(4), 1019-1035. http://dx.doi.org/10.1016/j.neuroscience.2010.01.037 PMID: 20109536

[70] Papadopoulos, M.C.; Manley, G.T.; Krishna, S.; Verkman, A.S. Aquaporin-4 facilitates reabsorption of excess fluid in vasogenic brain edema. *FASEB J.,* **2004**, *18*(11), 1291-1293. http://dx.doi.org/10.1096/fj.04-1723fje PMID: 15208268

[71] Papadopoulos, M.C.; Verkman, A.S. Aquaporin-4 and brain edema. *Pediatr. Nephrol.,* **2007**, *22*(6), 778-784. http://dx.doi.org/10.1007/s00467-006-0411-0 PMID: 17347837

[72] Huang, F.; Li, Y.N.; Yin, F.; Wu, Y.T.; Zhao, D.X.; Li, Y.; Zhang, Y.F.; Zhu, Q.S. Ginsenoside Rb1 inhibits neuronal apoptosis and damage, enhances spinal aquaporin 4 expression and improves neurological deficits in rats with spinal cord ischemia□reperfusion injury. *Mol. Med. Rep.,* **2015**, *11*(5), 3565-3572. http://dx.doi.org/10.3892/mmr.2015.3162 PMID: 25573543

[73] Mao, L.; Wang, H.D.; Pan, H.; Qiao, L. Sulphoraphane enhances aquaporin-4 expression and decreases spinal cord oedema following spinal cord injury. *Brain Inj.,* **2011**, *25*(3), 300-306. http://dx.doi.org/10.3109/02699052.2010.542432 PMID: 21280976

[74] Papadopoulos, M.C.; Verkman, A.S. Potential utility of aquaporin modulators for therapy of brain disorders. *Prog. Brain Res.,* **2008**, *170*, 589-601. http://dx.doi.org/10.1016/S0079-6123(08)00446-9 PMID: 18655912

[75] Jung, J.S.; Bhat, R.V.; Preston, G.M.; Guggino, W.B.; Baraban, J.M.; Agre, P. Molecular characterization of an aquaporin cDNA from brain: candidate osmoreceptor and regulator of water balance. *Proc. Natl. Acad. Sci. USA,* **1994**, *91*(26), 13052-13056. http://dx.doi.org/10.1073/pnas.91.26.13052 PMID: 7528931

[76] Neely, J.D.; Christensen, B.M.; Nielsen, S.; Agre, P. Heterotetrameric composition of aquaporin-4 water channels. *Biochemistry,* **1999**, *38*(34), 11156-11163. http://dx.doi.org/10.1021/bi990941s PMID: 10460172

[77] Neely, J.D.; Amiry-Moghaddam, M.; Ottersen, O.P.; Froehner, S.C.; Agre, P.; Adams, M.E. Syntrophin-dependent expression and localization of Aquaporin-4 water channel protein. *Proc. Natl. Acad. Sci. USA,* **2001**, *98*(24), 14108-14113. http://dx.doi.org/10.1073/pnas.241508198 PMID: 11717465

[78] Klatzo, I. Evolution of brain edema concepts. *Acta Neurochir. Suppl. (Wien),* **1994**, *60*, 3-6. PMID: 7976571

[79] Bloch, O.; Papadopoulos, M.C.; Manley, G.T.; Verkman, A.S. Aquaporin-4 gene deletion in mice increases focal edema associated with staphylococcal brain abscess. *J. Neurochem.,* **2005**, *95*(1), 254-262. http://dx.doi.org/10.1111/j.1471-4159.2005.03362.x PMID: 16181429

[80] Ma, T.; Yang, B.; Gillespie, A.; Carlson, E.J.; Epstein, C.J.; Verkman, A.S. Generation and phenotype of a transgenic knockout mouse lacking the mercurial-insensitive water channel aquaporin-4. *J. Clin. Invest.,* **1997**, *100*(5), 957-962. http://dx.doi.org/10.1172/JCI231 PMID: 9276712

[81] Manley, G.T.; Fujimura, M.; Ma, T.; Noshita, N.; Filiz, F.; Bollen, A.W.; Chan, P.; Verkman, A.S. Aquaporin-4 deletion in mice reduces brain edema after acute water intoxication and ischemic stroke. *Nat. Med.,* **2000**, *6*(2), 159-163. http://dx.doi.org/10.1038/72256 PMID: 10655103

[82] Manley, G.T.; Binder, D.K.; Papadopoulos, M.C.; Verkman, A.S. New insights into water transport and edema in the central nervous system from phenotype analysis of aquaporin-4 null mice. *Neuroscience,* **2004**, *129*(4), 983-991. http://dx.doi.org/10.1016/j.neuroscience.2004.06.088 PMID: 15561413

[83] Kimura, A.; Hsu, M.; Seldin, M.; Verkman, A.S.; Scharfman, H.E.; Binder, D.K. Protective role of aquaporin-4 water channels

after contusion spinal cord injury. *Ann. Neurol.,* **2010**, *67*(6), 794-801.
http://dx.doi.org/10.1002/ana.22023 PMID: 20517941

[84] Saadoun, S.; Papadopoulos, M.C. Aquaporin-4 in brain and spinal cord oedema. *Neuroscience,* **2010**, *168*(4), 1036-1046.
http://dx.doi.org/10.1016/j.neuroscience.2009.08.019 PMID: 19682555

[85] Xu, W.B.; Gu, Y.T.; Wang, Y.F.; Lu, X.H.; Jia, L.S.; Lv, G. Bradykinin preconditioning modulates aquaporin-4 expression after spinal cord ischemic injury in rats. *Brain Res.,* **2008**, *1246*, 11-18.
http://dx.doi.org/10.1016/j.brainres.2008.09.087 PMID: 18951883

[86] Auguste, K.I.; Jin, S.; Uchida, K.; Yan, D.; Manley, G.T.; Papadopoulos, M.C.; Verkman, A.S. Greatly impaired migration of implanted aquaporin-4-deficient astroglial cells in mouse brain toward a site of injury. *FASEB J.,* **2007**, *21*(1), 108-116.
http://dx.doi.org/10.1096/fj.06-6848com PMID: 17135365

[87] Saadoun, S.; Papadopoulos, M.C.; Watanabe, H.; Yan, D.; Manley, G.T.; Verkman, A.S. Involvement of aquaporin-4 in astroglial cell migration and glial scar formation. *J. Cell Sci.,* **2005**, *118*(Pt 24), 5691-5698.
http://dx.doi.org/10.1242/jcs.02680 PMID: 16303850

[88] Halassa, M.M.; Fellin, T.; Takano, H.; Dong, J-H.; Haydon, P.G. Synaptic islands defined by the territory of a single astrocyte. *J. Neurosci.,* **2007**, *27*(24), 6473-6477.
http://dx.doi.org/10.1523/JNEUROSCI.1419-07.2007 PMID: 17567808

[89] Haydon, P.G.; Carmignoto, G. Astrocyte control of synaptic transmission and neurovascular coupling. *Physiol. Rev.,* **2006**, *86*(3), 1009-1031.
http://dx.doi.org/10.1152/physrev.00049.2005 PMID: 16816144

[90] Østby, I.; Øyehaug, L.; Einevoll, G.T.; Nagelhus, E.A.; Plahte, E.; Zeuthen, T.; Lloyd, C.M.; Ottersen, O.P.; Omholt, S.W. Astrocytic mechanisms explaining neural-activity-induced shrinkage of extraneuronal space. *PLOS Comput. Biol.,* **2009**, *5*(1)e1000272
http://dx.doi.org/10.1371/journal.pcbi.1000272 PMID: 19165313

[91] Tsai, H.H.; Li, H.; Fuentealba, L.C.; Molofsky, A.V.; Taveira-Marques, R.; Zhuang, H.; Tenney, A.; Murnen, A.T.; Fancy, S.P.; Merkle, F.; Kessaris, N.; Alvarez-Buylla, A.; Richardson, W.D.; Rowitch, D.H. Regional astrocyte allocation regulates CNS synaptogenesis and repair. *Science,* **2012**, *337*(6092), 358-362.
http://dx.doi.org/10.1126/science.1222381 PMID: 22745251

[92] Walz, W. Role of astrocytes in the clearance of excess extracellular potassium. *Neurochem. Int.,* **2000**, *36*(4-5), 291-300.
http://dx.doi.org/10.1016/S0197-0186(99)00137-0 PMID: 10732996

[93] Simard, M.; Nedergaard, M. The neurobiology of glia in the context of water and ion homeostasis. *Neuroscience,* **2004**, *129*(4), 877-896.
http://dx.doi.org/10.1016/j.neuroscience.2004.09.053 PMID: 15561405

[94] Lee, I.H.; Lindqvist, E.; Kiehn, O.; Widenfalk, J.; Olson, L. Glial and neuronal connexin expression patterns in the rat spinal cord during development and following injury. *J. Comp. Neurol.,* **2005**, *489*(1), 1-10.
http://dx.doi.org/10.1002/cne.20567 PMID: 15977163

[95] Theriault, E.; Frankenstein, U.N.; Hertzberg, E.L.; Nagy, J.I. Connexin43 and astrocytic gap junctions in the rat spinal cord after acute compression injury. *J. Comp. Neurol.,* **1997**, *382*(2), 199-214.
http://dx.doi.org/10.1002/(SICI)1096-9861(19970602)382:2<199::AID-CNE5>3.0.CO;2-Z PMID: 9183689

[96] Horner, P.J.; Power, A.E.; Kempermann, G.; Kuhn, H.G.; Palmer, T.D.; Winkler, J.; Thal, L.J.; Gage, F.H. Proliferation and differentiation of progenitor cells throughout the intact adult rat spinal cord. *J. Neurosci.,* **2000**, *20*(6), 2218-2228.
http://dx.doi.org/10.1523/JNEUROSCI.20-06-02218.2000 PMID: 10704497

[97] Yang, H.; Lu, P.; McKay, H.M.; Bernot, T.; Keirstead, H.; Steward, O.; Gage, F.H.; Edgerton, V.R.; Tuszynski, M.H. Endogenous neurogenesis replaces oligodendrocytes and astrocytes after primate spinal cord injury. *J. Neurosci.,* **2006**, *26*(8), 2157-2166.
http://dx.doi.org/10.1523/JNEUROSCI.4070-05.2005 PMID: 16495442

[98] Zai, L.J.; Wrathall, J.R. Cell proliferation and replacement following contusive spinal cord injury. *Glia,* **2005**, *50*(3), 247-257.
http://dx.doi.org/10.1002/glia.20176 PMID: 15739189

[99] MacVicar, B.A.; Feighan, D.; Brown, A.; Ransom, B. Intrinsic optical signals in the rat optic nerve: role for K(+) uptake *via* NKCC1 and swelling of astrocytes. *Glia,* **2002**, *37*(2), 114-123.
http://dx.doi.org/10.1002/glia.10023 PMID: 11754210

[100] Kwiecien, J.M.; Dabrowski, W.; Kwiecien-Delaney, B.J.; Kwiecien-Delaney, C.J.; Siwicka-Gieroba, D.; Yaron, J.R.; Zhang, L.; Delaney, K.H.; Lucas, A.R. Neuroprotective effect of subdural infusion of Serp-1 in spinal cord trauma. *Biomedicines,* **2020**, *8*, 372.

[101] Kangwantas, K.; Pinteaux, E.; Penny, J. The extracellular matrix protein laminin-10 promotes blood-brain barrier repair after hypoxia and inflammation *in vitro*. *J. Neuroinflammation,* **2016**, *13*, 25.
http://dx.doi.org/10.1186/s12974-016-0495-9 PMID: 26832174

[102] Askenase, M.H.; Sansing, L.H. Stages of the inflammatory response in pathology and tissue repair after intracerebral hemorrhage. *Semin. Neurol.,* **2016**, *36*(3), 288-297.
http://dx.doi.org/10.1055/s-0036-1582132 PMID: 27214704

[103] Kumar, H.; Ropper, A.E.; Lee, S.H.; Han, I. Propitious therapeutic modulators to prevent blood-spinal cord barrier disruption in spinal cord injury. *Mol. Neurobiol.,* **2017**, *54*(5), 3578-3590.
http://dx.doi.org/10.1007/s12035-016-9910-6 PMID: 27194298

[104] Dai, E.; Guan, H.; Liu, L.; Little, S.; McFadden, G.; Vaziri, S.; Cao, H.; Ivanova, I.A.; Bocksch, L.; Lucas, A. Serp-1, a viral anti-inflammatory serpin, regulates cellular serine proteinase and serpin responses to vascular injury. *J. Biol. Chem.,* **2003**, *278*(20), 18563-18572.
http://dx.doi.org/10.1074/jbc.M209683200 PMID: 12637546

[105] Dai, E.; Viswanathan, K.; Sun, Y.M.; Li, X.; Liu, L.Y.; Togonu-Bickersteth, B.; Richardson, J.; Macaulay, C.; Nash, P.; Turner, P.; Nazarian, S.H.; Moyer, R.; McFadden, G.; Lucas, A.R. Identification of myxomaviral serpin reactive site loop sequences that regulate innate immune responses. *J. Biol. Chem.,* **2006**, *281*(12), 8041-8050.
http://dx.doi.org/10.1074/jbc.M509454200 PMID: 16407226

[106] Kwiecien, J.M.; Zhang, L.; Yaron, J.R.; Schutz, L.N.; Kwiecien-Delaney, C.J.; Enkidia, A.; Awo, E.A.; Burgin, M.; Dabrowski, W.; Lucas, A.R. Local chitosan-serpin injection after spinal cord injury reduces inflammatory damage and improves neurologic function. . *Clin. Med.,* **2020**, *9*, E1221.