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Myxoma virus derived immune modulating proteins, M-T7 and Serp-1, reduce early inflammation after spinal cord injury in the rat model

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Abstract

Spinal cord injury (SCI)-initiated inflammation was treated with anti-inflammatory reagents. We compared local spinal cord or intraperitoneal infusion of two Myxoma virus derived immune modulating proteins, Serp-1 and M-T7, with dexamethasone (DEX).

Hemorrhage and necrosis after SCI initiate a complex pathogenesis dominated by early, severe and highly destructive inflammatory macrophage infiltration. We examined sustained, 7-day, subdural infusion of either M-T7, a chemokine modulator or Serp-1, a plasminogen activator and factor inhibitor. Mature male rats had epidural balloon crush SCI and sustained subdural infusion of Serp-1, M-T7, DEX or saline for 7 days via the osmotic pump. A separate group of rats with SCI had intra-peritoneal infusion. Clinical evaluation included endpoint monitoring with body weight, hemorrhagic cystitis and bilateral toe pinch response. Sections of the spinal cord were analyzed histologically and macrophage numbers counted by standardized protocol in the cavity of injury (COI).

While the rats administered DEX demonstrated substantial body weight loss, dehydration and dermal atrophy consistent with steroid toxicity, rats infused with Serp-1 and M-T7 had no toxicity. Serp-1 improved withdrawal responses. Subdural infusion of Serp-1, M-T7 and DEX significantly reduced numbers of phagocytic, CD68-positive macrophages. With intraperitoneal infusion only M-T7 reduced macrophage counts, Serp-1 showed only a trend. Local infusion of highly active immune modulating proteins; Serp-1 and M-T7, targeting serine protease and chemokine pathways demonstrated excellent potential for neuroprotection after severe SCI in a rat model, without adverse side effects. Sustained subdural infusion offers an alternative route of administration for treatment of SCI.

Key words: *spinal cord injury, neuroinflammation, subdural infusion, neuroprotection.*

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Introduction

Trauma to the spinal cord initiates a complex disease which is dominated by severe, destructive and prolonged inflammation. This destructive inflammation is characterized by an infiltration of phagocytic macrophages [21-24,37,38]. Macrophages continue to phagocytize myelin, persisting for more than 8 weeks post-spinal cord injury (SCI) [21]. As this inflammatory, phagocytic macrophage infiltrate persists, the cavity of injury (COI) increases in volume [18,35,40] creating a barrier for axonal regeneration [20], while the surrounding spinal cord tissue is irreversibly destroyed. Based on these histopathological findings we have postulated that a reduction in the numbers of macrophages in the COI by pharmacological treatments has the potential to protect the nervous system, a therapy designed to limit tissue destruction and potentially reduce neurological deficits.

An intravenous administration of a very high dose of methylprednisolone succinate used to treat SCI patients [8] has been tested, but while showing early promise, methylprednisolone treatments were not effective in improving neurological functional scores [16]. In addition, high-dose steroids are associated with severe multi-systemic toxicity [32,38]. Because methylprednisolone is unstable in an aqueous solution, it has proven unsuitable for a longer-term infusion in experimental animals. We have thus substituted dexamethasone (DEX) [23,24] infusions as a method to test treatment and to determine anti-inflammatory effects during 1-2-week continuous subdural infusion in a rat model of balloon crush SCI. In this model high doses of DEX did reduce macrophage numbers in the COI in a dose-dependent fashion [23] indicating that DEX and potentially other drug infusions can gain access to the COI from the subdural space. Unfortunately, the highest and the most effective dose, of 4 mg of DEX, when administered over 1 week, was also severely toxic to recipient rats after SCI [24]. Further, the infusion of DEX over a period of 2 weeks did not eliminate inflammation as myelin-rich immunogenic debris persisted in the COI. This persistence of necrotic debris produces an undue risk of re-igniting macrophage-rich inflammatory infiltration after the cessation of treatment [23]. Therefore, we have postulated that non-toxic and potent anti-inflammatory drugs are necessary for prolonged administration after SCI to suppress inflammatory damage and to allow for removal of necrotic debris from the COI by these reduced numbers of macrophages over a long period of time.

Both haemorrhage and inflammation are believed to contribute to the ongoing phagocytic macrophage activation at sites of spinal cord damage. The thrombotic, clot forming and thrombolytic, clot dissolving serine protease cascades are regulated by serine protease inhibitors, termed serpins. Serp-1 is a 55 kDa glycosylated myxoma virus-derived serpin has been tested for anti-inflammatory activity and therapeutic efficacy in a wide range of inflammatory vascular models from angioplasty injury to aortic transplants, renal transplants and vasculitis in rats and mice [2,5,7,11,14,29,31,36,45,46]. Myxoma virus is host-restricted and not pathogenic in humans. Serp-1 inhibits tissue- and urokinase-type plasminogen activators (tPA and uPA, respectively) in the thrombolytic cascades and factor X and thrombin in the coagulation or thrombotic cascades [36]. Serp-1 reduces haemorrhage and consolidation in a severe inflammatory vasculitis model, vasculitis and pulmonary hemorrhage and consolidation induced by murine gammaherpesvirus-68 (MHV-68) infection in interferon γ receptor deficient mice ($IFN\gamma R^{-/-}$) [3,10,11,47]. Serp-1, as a purified GMP quality protein, has also been successfully tested in FDA-approved Phase 1 and 2 trials in humans [42]. Serp-1 treatment dose-dependently reduced markers of heart damage after coronary stent implants when compared to standard of care treatment and with no major adverse side effects.

Chemokines also mediate inflammatory cell migration to sites of injury and are up-regulated in the spinal cord in neuroinflammation [19,41] and in neuropathic pain [1,17]. M-T7 is a second myxoma virus-derived, secreted purified protein with proven therapeutic potential in disease models. M-T7 is a multi-functional protein; it is an interferon γ receptor homologue that is rabbit species specific (i.e., only functional against rabbit interferon γ), but also inhibits a broad spectrum of C, CC and CXC mouse and human chemokines via their interactions with glycosaminoglycans (GAGs) [4,6,9,15,25,30,34,43]. M-T7 has been tested and found highly effective as an immune modulating therapeutic in aortic and renal allograft rejection [6,9,26]. Thus, these two immune modulating proteins, Serp-1 and M-T7, provide neuroprotective approaches to selectively inhibit two differing inflammatory pathways after

SCI. These two immune modulating proteins provide new approaches to examine the roles of serine proteases in the coagulation cascades and chemokines in the acute inflammatory response forming gradients to guide inflammatory cells to sites of tissue damage.

With these initial studies using a rat model of acute SCI, we have examined the early effects of M-T7, Serp-1 or DEX infusions after balloon crush in a rat model of SCI. This is the first investigation into the potential for beneficial anti-inflammatory activity of these two unique immune modulating proteins. In a sustained administration for 7 days, we determined that subdural infusion of either 0.2 mg of M-T7 or of 0.2 to 1.0 mg Serp-1 significantly reduced macrophage invasion in the COI. The higher Serp-1 dose improved functional testing with no detected adverse effects.

Material and methods

Ethical considerations

Experiments using male, 16-week-old Long Evans rats, 370-410 g, were approved by the Animal Research Ethics Board at McMaster University according to the Guides and Regulations of the Canadian Council of Animal Care.

Serp-1 and M-T7 protein expression and purification

Recombinant Serp-1 was expressed and harvested from a Chinese Hamster Ovary (CHO) cell line (Viron Therapeutics Inc, London, ON, Canada). Serp-1 was purified as previously described as a good manufacturing product (GMP) quality protein for use in patients. Serp-1 protein is estimated to be ~95% purity using Coomassie-stained SDS-polyacrylamide gels and reverse-phase HPLC with no detectable endotoxin [28].

M-T7 was expressed and purified as previously described. In brief, M-T7 was transformed into DH10Bac bacteria (Invitrogen, Carlsbad, CA), and blue/white screened on LB+Kan+Tet+Gen+IPTG+X-gal plates. Bacmids were purified and used to transfect Sf9 insect cells with Cellfectin II (Invitrogen, Carlsbad, California). Baculovirus supernatants were collected to infect High Five insect cells. M-T7 was purified by sequential column purification as previously described [4].

Stock protein aliquots were diluted in saline at 0.1 mg/ml for M-T7 and 0.1 or 0.5 mg/ml for Serp-1. Dexamethasone was diluted in saline to obtain a concentration of 0.5 mg/ml. The 2ML1 osmotic pumps (Alzet) were loaded with 2 ml of saline dilutions of each treatment or with saline alone.

Neurosurgery – rat balloon crush SCI model

The surgical procedure involved in the balloon crush SCI and subdural (SD) infusion has been previously described [22-24]. Briefly, 33 rats were induced for SCI surgery in 5% isoflurane in 95% oxygen and maintained under 4% isoflurane in 96% oxygen. The laminectomy was created between L1 and L2 vertebrae and a 3Fogarty catheter inserted cranial over the dura to position the balloon over the caudal thoracic spinal cord. The balloon was inflated with 20 ml saline for 5 seconds, deflated and removed. For SD infusion (N = 20 rats), a small cut was created in the dura over the dorsal spinal cord in the laminectomy and a rat intrathecal catheter inserted over the spinal cord cranially to approximate the catheter tip with the site of the crush injury. The other end of the catheter was connected to the osmotic pump placed under the skin of the flank.

In a separate group of rats with laminectomy and SCI, osmotic pumps were placed in the peritoneal cavity (N = 13 rats) via an incision in the midline of the ventral abdominal wall. Osmotic pumps for the subdural infusion were preloaded with saline (n = 3), 1 mg Serp-1 (n = 4), 0.2 mg Serp-1 (n = 4), 0.2 mg M-T7 (n = 5), or 1.0 mg DEX (n = 4). For intraperitoneal (IP) administration the osmotic pumps were loaded with saline (n = 3) or 0.2 mg Serp-1 (n = 5) or 0.2 M-T7 (n = 5). The osmotic pumps 2ML1 secrete at the rate of 10 µl per hour according to the manufacturer.

Prior to awakening from anaesthetic, all rats received injection of 0.4 ml ketoprofen analgesic (10 mg/ml, Anafen, Merial Canada, Inc., Baie d'Urfe, Quebec, Canada) and 5 ml saline subcutaneously.

Post-surgical period

Endpoint description: rats with hind end paralysis, with urinary bladder distended with hemorrhagic urine, moderate to marked dehydration, lethargy and reduced body temperature were to be humanely euthanized prior to the completion of the study, 7 days, and not included in the study (n = 2) [22].

During the two days post-SCI ketoprofen analgesic was administered to all experimental animals and rats with hemorrhagic cystitis were treated with an intramuscular injection of 50 ml enrofloxacin

(50 mg/ml, Baytril®, Mississauga, CANADA) for 3-5 days until blood cleared from urine. All SCI rats developed paraplegia and most of them had distended urinary bladder indicating paralysis of its function and requiring gentle manual voiding 1-2 times a day. A large proportion of rats with distended urinary bladder had also micturition leading to soiling of the perineum and hind legs. The body weight was taken at 3 days post-SCI and also before the perfusion at day 7 post-SCI. For functional testing, pinching of toes in both hind limbs was performed in all rats daily starting from day one post-SCI. The withdrawal response to toe pinch was recorded as none = 0, mild = 1, moderate = 2, strong/normal = 3 resulting in the full flexion of the hip and knee joints with a considerable force.

Pathology

At day 7 post-SCI, the rats were overdosed with sodium pentobarbital, 80 mg/kg body weight and whole body perfusion performed with buffered formalin [22]. The spine together with the spinal cord was removed and post-fixed in formalin for 24 hours, then placed in a decalcifying solution; formalin supplemented with 4% EDTA, pH 7.2, placed on a rotating shaker. The decalcifying solution was replaced twice a week and after 6-7 weeks, the bones of the spine were soft. Three-mm-thick consecutive cross sections of the spine with spinal cord, 16 per rat, were cut to include the site of laminectomy and the site of the balloon crush. The tissues were processed in rising concentrations of ethyl alcohol and xylene and embedded in paraffin wax. For histologic analysis 5-mm-thick sections were mounted on the glass slide, stained with luxol fast blue, counterstained with haematoxylin and eosin (LFB + H&E) and cover-slipped.

For immunohistochemical analysis, glass-mounted sections were de-waxed, blocked with 2.5% normal horse serum, exposed to a 1 : 100 dilution of anti-CD68 antibody (Abcam, Cambridge, MA, USA) and the brown color developed with DAB (Novolink, Leica, Concord, ON, Canada).

Macrophage counts in the cavity of injury

Sections of the spine with the spinal cord stained with LFB + H&E were analyzed under a Nikon Eclipse 50*i* microscope and representative sections were photographed. For macrophage counts, 40× magnification photographs of the edge of the cavity of injury (COI) containing approximately 20% of the spinal cord tissue and 80% of its content were selected from micrograph files for each rat. A rectangular photograph at 40× of LFB + H&E-stained spinal cord resulted in a field of 225 × 300 mm and was used to count large cells with oval, often indented nuclei containing abundant vacuolated cytoplasm. In the COI, phagocytic cells often contained LFB-positive, blue granules of myelin and/or red blood cells. A large proportion of these cells were CD68-positive and were interpreted as macrophages.

Macrophages were counted in fields from different sections, 3-13 per rat, cell counts were averaged and these averages used to calculate mean and standard deviation for each treatment group.

Statistical analysis

Mean values for functional responses (weight, toe pinch withdrawal) were calculated for each group of rats and each treatment were calculated and compared to measured histopathology scores, incorporating mononuclear cell count in the COI. Findings were assessed for statistical significance using analysis of variance (ANOVA) with secondary Fisher's PLSD or Student's unpaired, two-tailed *T*-test.

Results

Clinical observations

After the surgery and recovery from anaesthesia rats were paraplegic and with dilated urinary bladders with haemorrhagic urine, presumed hemorrhagic cystitis, requiring injections of Baytril® antibiotic for 3-5 days until the blood cleared from urine. Out of 20 rats infused subdurally, only 3 rats had no dilated urinary bladder and out of 13 rats infused IP, 6 rats had no dilated bladder.

A simple functional test for neurologic recovery, the withdrawal reaction to toe pinch, indicated early benefit with the higher dose Serp-1 treatment (Fig. 1A). Withdrawal after toe pinch administered individually to the right and left paws and bladder function was tested over 7 days' follow-up post-SCI. Serp-1 (1.0 mg)-treated rats had a significant improvement ($p < 0.05$) in withdrawal while other treatments demonstrated only a trend. Bladder function was not significantly altered by either treatment. IP infusion of either M-T7 or Serp-1 had no effect on functional recovery. The M-T7 and DEX subdurally infused rats displayed a trend toward improved functional recovery but this was not significant.

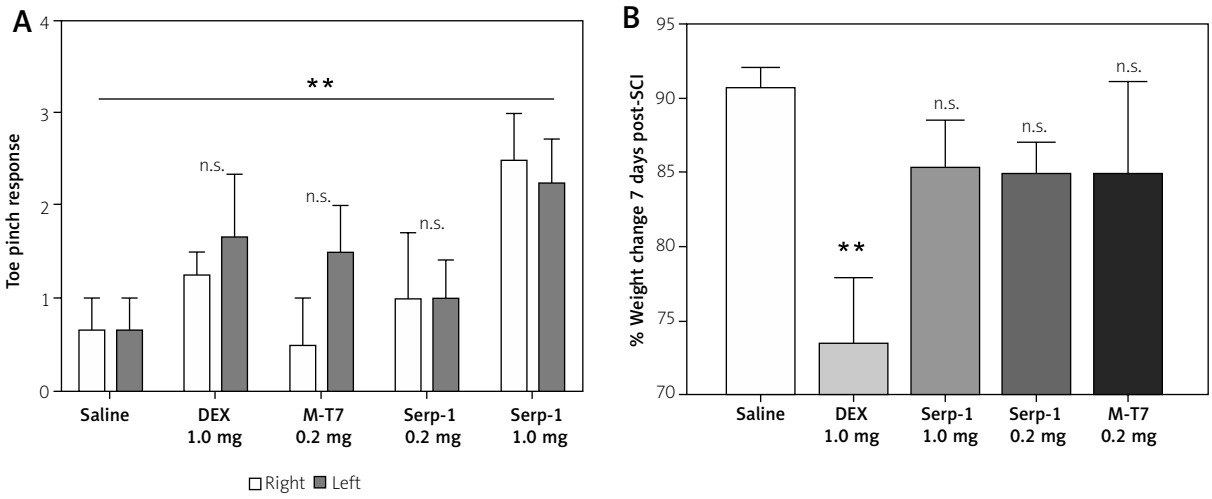

Fig. 1. Clinical findings. **A**) Reaction to the hind leg toe pinch in rats with the spinal cord injury. A functional testing with a simple toe pinch administered to the right and left hind leg. The scores of the strength of the withdrawal reaction to the toe pinch; 0 – none, 1 – mild, 2 – moderate, 3 – strong/normal were recorded over the duration of the study, 6 daily measurements and averaged for the treatment groups. ANOVA with Fisher LSD, $p < 0.01$. **B**) Body weight changes in the spinal cord injury rats administered with subdural infusion of anti-inflammatory treatments. Infusion of 1.0 mg dexamethasone led to significant weight loss, while the infusion of Serp-1 treatment at doses of 0.2-1.0 mg and M-T7 at 0.2 mg resulted in stable weight at 7 days post-SCI. ANOVA with Fisher LSD, $p < 0.01$.

There was apparent toxicity with the DEX subdural infusion. There was significant weight loss with DEX treatment ($p < 0.01$), but treatment with the M-T7 subdural infusion (0.2 mg) and the Serp-1 infusions (either 0.2 or 1.0 mg) did not cause weight loss (Fig. 1B). Rats infused with DEX after SCI also had marked decline in body condition, showing signs of dehydration and developing remarkably thin skin (dermal atrophy) and poor body condition consistent with a Cushinoid appearance after 7 days of treatment.

Histopathology

Balloon crush injury induced SCI in rats resulted in a large lesion obliterating most of the dorsal column and surrounding areas of the gray and white matter at 7 days' follow-up (Fig. 2). The injured spinal cord contained large amounts of necrotic debris including luxol fast blue (LFB) positive myelin and red blood cells. Large numbers of CD68-positive macrophages infiltrated the spinal cord tissue around the lesion which converted into a cavity of injury (COI) and a wide inflamed and necrotic area in saline-infused rats. On the LFB + H&E stain, there were numerous large cells with large oval, sometimes indented nucleus and abundant, vacuolated cytoplasm. The vacuolated cytoplasm in the macrophage cells often encompassed red blood cells and/or blue granules of myelin. These cells were interpreted as macrophages, and were the only cells observed within the COI. Infiltrating cells were tightly packed leaving little un-phagocytized necrotic debris and red blood cells in the periphery of the COI.

The subdural infusion of M-T7, Serp-1 and DEX had a dramatic effect on reducing the numbers of macrophages that were scattered among large amounts of necrotic debris including blue-stained damaged myelin and numerous red blood cells in the periphery of the lesion (Fig. 3C-E). The numbers of CD68-positive cells were also remarkably reduced within the lesion and in the tissue of the spinal cord surrounding the lesion (Fig. 2H-J).

Macrophage counts

Invading macrophage cell counts were measured using a standardized approach in the periphery of the COI lesion. Numbers of positively stained cells in 3-13 40× high power fields were counted for each rat at 7 days' follow-up for each treatment condition (presented in Fig. 3). In saline-infused rats the num-

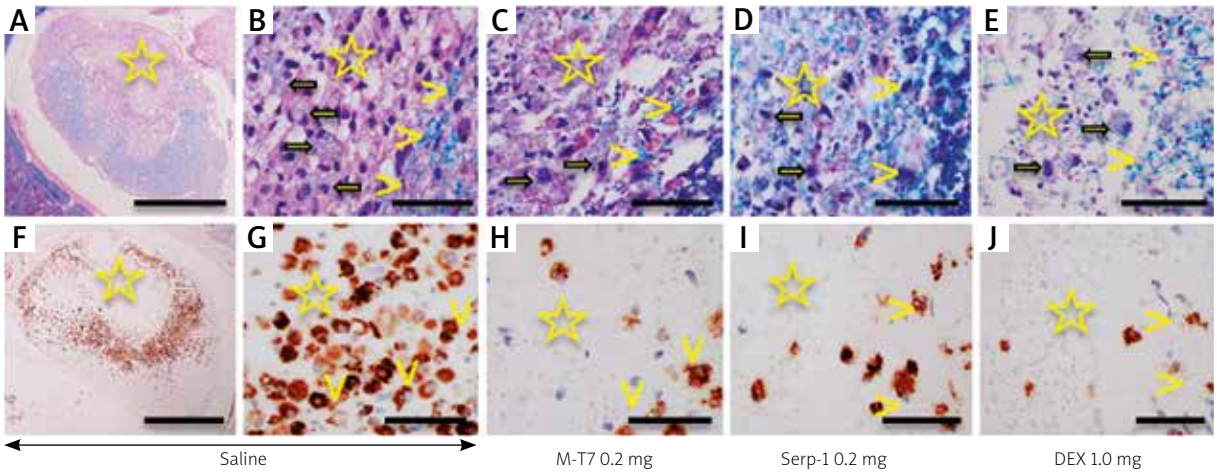

Fig. 2. Histologic analysis of the effect of the anti-inflammatory treatment on the severity of macrophage infiltration in the COI. The balloon crush injury of the spinal cord in rats was infused subdurally for one week with saline (**A, B, F, G**), 0.2 mg M-T7 (**C, H**), 0.2 mg (**D, I**) or dexamethasone (**E, J**). The area of the cavity of injury (COI) is indicated with the star and the margin of the COI is indicated by open arrowheads. While in saline-infused rats, large, vacuolated cells, often containing blue granules of myelin and red blood cells (small yellow arrows), interpreted as macrophages, are numerous, they are rare in the COI of rats infused with M-T7 (**C, H**), Serp-1 (**D, I**) and dexamethasone (**E, J**) infused rats. A large proportion of macrophages stain with anti-CD68 antibody and there is a remarkable reduction in CD-68 positive cells both in the COI and in the surrounding tissue of the spinal cord. Luxol fast blue counterstained with haematoxylin and eosin (LFB + H&E); **A-E**, anti-CD68 antibody; **F-J**. Size bars – 1,000 mm; **A, F,** 50 mm; **B-E, G-J**.

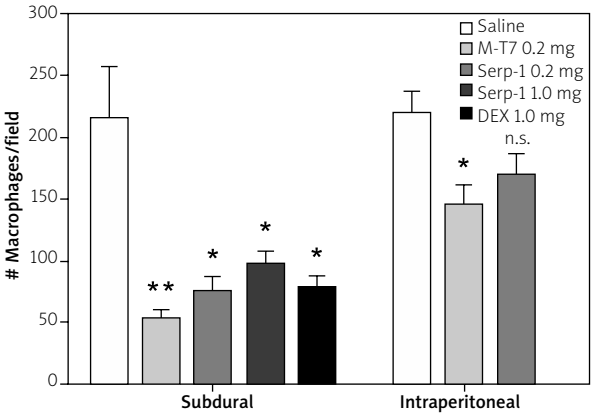

Fig. 3. Macrophage counts in COI. While the subdural and intraperitoneal administration of saline resulted in virtually the same high counts per standard field, 225 × 300 µm, the subdural infusion of all treatments was associated with a significant reduction in macrophages. In contrast, intraperitoneal infusion resulted in much less reduction, with the M-T7 infusion having an effect on a significant reduction but the Serp-1 infusion having no remarkable impact.

bers of macrophages were similar for the subdural and for the IP infusions; averaging 217 and 221 per field, respectively (n = 3 for both routes of administration).

Subdural infusion of M-T7, 0.2 mg, significantly reduced the number of macrophages to an average of 55 (n = 5), and subdural infusion of Serp-1, at 0.2 mg to an average of 77 (n = 4), while Serp-1 1.0 mg subdural infusion reduced macrophage counts to an average of 98 (n = 4). Subdural infusion of 1.0 mg DEX reduced the number of macrophages to 80 (n = 4) per standard 40× field. All subdural treatments resulted in a significant reduction of macrophages, $p < 0.01$ for 0.2 mg M-T7 and $p < 0.05$ for both doses of Serp-1, 0.2 and 1.0 mg and for DEX 1.0 mg. IP infusion of M-T7 0.2 mg or of Serp-1 0.2 mg produced a smaller reduction in the numbers of macrophages in the COI, 147 and 171 per standard field, respectively. While the counts for the M-T7 IP infusion were statistically lower ($p < 0.05$), the counts with the Serp-1 IP treatment were not significant.

Discussion

With this short, pilot study we have investigated the potential for two immune modulating proteins to reduce early inflammation and damage after SCI in a rat balloon crush injury model. Treatment with Serp-1, a *serpin*, or M-T7, a broad spectrum inhibitor of chemokine to GAG interactions was tested for efficacy after SCI. We have demonstrated that a subdural infusion of these anti-inflammatory treatments led to a remarkable inhibition of early inflammatory infiltration with promise for neuroprotective activity. The higher dose Serp-1 treatment also significantly improved foot withdrawal to toe pinch. Both treatments whether given as subdural infusions or as IP infusion did not cause significant side effects or weight loss.

Spinal cord trauma initiates a severe and prolonged inflammatory response [21-24] with destruction of the spinal cord tissue surrounding the site of injury and causing a gradual expansion of the cavity of injury (COI) [18,35,40]. Phagocytic macrophages loaded with luxol fast blue-positive myelin granules have been observed in SCI lesions beyond 8 weeks post-injury [21] and are considered central mediators of this destructive inflammation. A reduction in macrophage invasion at sites of SCI are thus considered to have potential for neural protection. In the previous research, depletion of macrophages with dichloromethylene diphosphonate-loaded (clodronate) liposomes reduced Wallerian degeneration [28] and the administration of tamoxifen reduced pro-inflammatory activation of microglia [27]. In our previous studies, subdural infusion of dexamethasone for 1-2 weeks also produced a dose-dependent suppression of macrophage numbers in the SCI lesion [23,24], but rats treated with the highest and the most effective dose of DEX, 4 mg per week, had signs of severe steroid toxicity. However, these experiments did demonstrate that the anti-inflammatory action of water-soluble compounds can be readily evaluated by subdural infusion into necrotic and semi liquid COI lesions [24]. Therefore, a simple diffusion of an active anti-inflammatory compound such as DEX from the subdural space into the aqueous milieu of the COI can alter inflammatory mechanisms and inhibit macrophage taxis.

The current study, using two unique immune modulating protein treatments, and the prior studies with DEX [23,24] resulted in lowering the numbers of macrophages but did not eliminate the inflammation. One would predict that discontinuing an anti-inflammatory infusion may initiate a severe and destructive macrophage infiltration given a large amount of myelin-rich necrotic debris and numerous red blood cells, both of potent immunogenic potential. We would predict that it may be of therapeutic necessity to continue infusions of treatments on a long-term basis. It is also possible that allowing the presence of low numbers of macrophages in the COI will act to remove necrotic debris and damaged red blood cells while causing little or no damage to the surrounding spinal cord tissue. The sustained treatment to achieve the elimination of the inflammation post-SCI however, will need to have a much longer duration than one week as in the present study, or even two weeks [23] but the determination of the duration of the treatment needs to be performed empirically, based on chronic infusion studies with drugs of low toxicity.

Although we used a relatively novel route of administration, a sustained subdural infusion and utilized the formation of the COI in the injured spinal cord to access its liquid content with infused compounds via a simple diffusion, more conventional methods of drug administration in SCI patients involve the intravenous route [8]. We sought to determine whether sustained IP administration, which is systemic and similar to intravenous infusion, of M-T7 and Serp-1 would result in lowering the numbers of macrophages in the COI. In the prior work, subcutaneous infusion by an osmotic pump for Serp-1 [7] and repeated, daily IP administration of Serp-1 [5,12] and M-T7 [6,9,26] were effective in achieving therapeutic doses. In the IP infusion of M-T7 there was a noticeable reduction in macrophage numbers ($p < 0.05$) which however, was not detected in Serp-1 IP infusion. In subdural infusion however, the reduction in macrophages was much more pronounced for both viral proteins. The potential reasons for a greater therapeutic success of proteins infused subdurally include: (1) the proximity of the end of the intrathecal catheter delivering the drugs to the lesion in the spinal cord allowing for its sustained high concentration as opposed to systemic distribution via IP infusion and the dilution of an administered compound throughout the circulation, (2) the small volume of the CSF in the subarachnoid space of the spinal cord, a small fraction of the total volume of the CSF in an adult rat, 300-330 µl [13] which allowed for little dilution of the infused drugs, (3) direct communication of the COI with the CSF of

the subdural space where the drug was infused allowing for a simple diffusion of a high concentration of the drug into the COI, (4) circumvention of the blood spinal cord barrier.

The current findings with Serp-1 and M-T7 support a beneficial effect of subdural anti-inflammatory protein infusions after SCI in a rat model. In particular, targeting the serine proteases in the thrombotic and thrombolytic cascades (tPA, uPA plasmin, fX and thrombin) at a higher dose of Serp-1 markedly reduced inflammation at both doses. M-T7 also reduced inflammation when given by subdural infusion at the lower dose. IP infusion was only effective for M-T7 treatment. For both proteins, Serp-1 and M-T7 macrophage counts were reduced. This finding is consistent with the prior work on aortic allograft transplant where Serp-1 and M-T7 were capable of significantly reducing macrophage cell counts after transplant [5,6,9,26,33,44]. In renal allografts, Serp-1 but not M-T7 reduced F4/80 macrophage counts [5,6,9]. The specific subclasses of macrophages as well as T cells, and neutrophil counts have not as yet been measured. In the future work we would plan to examine M-1 and M-2 macrophages as well as total T cell counts together with Th1, Th2, Treg and T17 counts for both protein treatments.

Future, long-term studies in rats and in other, larger animal models with more extensive testing will need to be performed to determine the feasibility of the use of the subdural infusion as a routine method of administration of therapeutic compounds to treat SCI and other diseases of the spinal cord. Given the peculiar propensity of the injured CNS to form the COI, an intralesional infusion of neuroprotective compounds should be considered as a potential route of sustained administration in traumatic brain injury and in stroke.

Data archiving

The endpoint monitoring data, clinical observations and histology photographs generated and/or analysed during the current study are available from the corresponding authors on reasonable request. Purified Serp-1 and M-T7 proteins are available upon request if adequate stores are available.

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Serp-1 was expressed and purified by Viron Therapeutics, Inc (previously London, ON, Canada; this company is not currently active). M-T7 was expressed and purified in the Lucas lab.

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Disclosure

The authors report no conflict of interest.

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