

Neuroprotection by minocycline facilitates significant recovery from spinal cord injury in mice

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Summary

Acute spinal cord injury (SCI) produces tissue damage that continues to evolve days and weeks after the initial insult, with corresponding functional impairments. Reducing the extent of progressive tissue loss ('neuroprotection') following SCI should result in a better recovery from SCI, but treatment options have thus far been limited. In this study, we have tested the efficacy of minocycline in ameliorating damage following acute SCI in mice. This semi-synthetic tetracycline antibiotic has been reported to inhibit the expression and activity of several mediators of tissue injury, including inflammatory cytokines, free radicals and matrix metalloproteinases, making it a suitable candidate for study. Mice were subjected to extradural compression of the spinal cord using a modified aneurysm clip, following which they received treatment with either minocycline

or vehicle beginning 1 h after injury. Behavioural testing of hindlimb function was initiated 3 days after injury using the Basso Beattie Bresnahan locomotor rating scale, and at 1 week using the inclined plane test. Functional assessments demonstrated that minocycline administration significantly improved both hindlimb function and strength from 3 to 28 days after injury compared with vehicle controls. Furthermore, gross lesion size in the spinal cord was significantly reduced by minocycline, and there was evidence of axonal sparing as determined using fluorogold labelling of the rubrospinal tract and by Bielchowsky silver stain. Finally, a comparison of minocycline against the currently approved treatment for acute SCI in humans, methylprednisolone, demonstrated superior behavioural recovery in the minocycline-treated animals.

Keywords: inflammation; minocycline; neuroprotection; recovery; spinal cord injury

Abbreviations: MMP = matrix metalloproteinase; NO = nitric oxide; SCI = spinal cord injury

Introduction

Spinal cord injury (SCI) is a leading cause of permanent disability in young adults (Sekhon and Fehlings, 2001). Current treatment options for significant recovery from SCI are limited. The steroid methylprednisolone has been used to reduce swelling and decrease inflammation (Chikawa *et al.*, 2001; Hall, 2001), but its efficacy is controversial as data from some clinical trials has failed to demonstrate definitive favourable effects (Hurlbert, 2000; Bracken, 2002). Current long-term treatment strategies in SCI are limited to rehabilitative physiotherapy in the hope of restoring function, but the benefit of this is often minimal and highly variable.

Permanent disability in spinal cord trauma results from disruption of white matter tracts, death of oligodendrocytes and extensive neuronal and axonal loss. Approaches to treat SCI include prevention of tissue loss (neuroprotection) and

regeneration of damaged structures. The bulk of tissue destruction develops over days to weeks after the insult, thereby providing a temporal window of opportunity to limit the spread of damage. Another impetus for neuroprotection is that the rescue of as few as 10% of spinal cord axons can be equated with significant functional recovery (Blight, 1983; Fehlings and Tator, 1995).

Specific molecules have been identified that contribute to cell death following SCI. Over-stimulation of glutamate receptors (Liu *et al.*, 1991), resulting in a high influx of calcium ions into the cell, activates a variety of proteases, caspases, phospholipases and endonucleases that promote breakdown of cell constituents including plasma membrane. Inflammation and the elevation of cytokines (Dusart and Schwab, 1994) and matrix metalloproteinases (MMPs)

(Wang *et al.*, 2000) also accounts for tissue vulnerability in SCI. Generation of free radicals, including nitric oxide (NO), likewise plays a significant role in mediating cell death (Diaz-Ruiz *et al.*, 2002). Many previous treatment strategies have targeted individual components or mechanisms in cell injury, and these have proven to be largely unsuccessful when advanced to clinical trials. It is likely that a more effective approach to alleviating SCI will be one that acts simultaneously on the many mechanisms involved in cell injury and death.

Minocycline is a synthetic tetracycline derivative that is used clinically as an antimicrobial agent for the treatment of conditions such as acne. In recent years, it has been shown to have many other actions including the inhibition of caspase-1 and caspase-3 (Chen *et al.*, 2000), which are involved in the generation of interleukin-1 and apoptosis, respectively. Minocycline also inhibits inducible nitric oxide synthase that produces NO (Amin *et al.*, 1996) and attenuates the activation of microglia (Yrjanheikki *et al.*, 1999). A significant literature indicates that minocycline inhibits MMPs, which have many detrimental roles in the CNS (Yong *et al.*, 2001). In culture, minocycline protects neurons from glutamate excitotoxicity (Tikka *et al.*, 2001). Thus, minocycline may serve as a multifaceted agent that targets the multiple processes involved in mediating cell death and the development of secondary injury in SCI. The aim of this study was to determine the effect of minocycline following SCI in mice.

Material and methods

Surgery and housing

Male CD-1 mice (Charles River Laboratories, Montreal, Quebec, Canada) of approximately 3 months of age were used. Animals were anaesthetized with a mixture of ketamine (200 mg/kg) and xylazine (10 mg/kg) injected intraperitoneally and immobilized in a stereotactic apparatus. An incision was made in the skin, and the muscle and the tissue overlying the spinal column was bluntly dissected away revealing the laminae. Using the spiny process of T2 as a landmark, laminae 3 and 4 were carefully removed to expose the spinal cord. Extradural compression of the spinal cord at the vertebral level of T3/4 was achieved using a modified aneurysm clip (Joshi and Fehlings, 2002a) with a closing force of 8 g producing mechanical trauma. The clip was left in place for 1 min and then removed. The incision was closed in two layers using nylon suture and the mice were given a subcutaneous injection of 0.5 ml saline to compensate for any blood loss during surgery. All experiments were carried out in accordance with Canadian Council of Animal Care (CCAC) guidelines and approved by the University of Calgary Ethics Committee.

In initial experiments, mice were housed in a room where the ambient temperature was set by a thermostat to 22°C. However, there were unregulated changes in ambient

temperature resulting from drafts when doors were opened, etc., and this therefore became effectively a non-temperature controlled environment. These animals were also heated using overhead lamps until recovery from surgical anaesthesia was complete. Because of the high mortality (see Results) of mice in the non-temperature controlled environment, subsequent experiments utilized animals that were housed in a warm room maintained at 27°C constantly throughout the survival period (henceforth, this is referred to as a temperature controlled environment).

Post-operative care procedures involved providing the mice with drinking water in the cage as well as both softened rat chow and regular pellets placed directly in the cage. Manual expression of the bladder was also required twice daily. In total, 124 mice were used. Of these, 51 animals were kept throughout the survival period in the non-temperature controlled environment, and 73 in the temperature controlled environment.

Pharmacological treatment of animals

Beginning 1 h after injury, mice in both temperature environments were given either an intraperitoneal injection of saline ($n = 41$) or 50 mg/kg minocycline ($n = 43$; Sigma, St Louis, MO, USA). This dose is comparable with that used in the experimental literature (Yrjanheikki *et al.*, 1999; Sanchez Mejia *et al.*, 2001; Brundula *et al.*, 2002). This was followed 24 h later by a second injection of 50 mg/kg. Subsequently, treated mice were injected with a 25 mg/kg dose every 24 h for the next 5 days. All animals were killed at day 28 following SCI. While mice in the non-temperature controlled environment were used for mortality studies, animals in the temperature controlled environment were subjected to behavioural testing for all mice, while a subset of the animals were used for fluorogold and other histological analyses.

The route of intraperitoneal administration for minocycline was chosen for two reasons. First, in the case of acute SCI in humans the incidence of paralytic ileus is quite common and often lasts for several days (Greenberg, 1997). Post-traumatic care often involves the insertion of a naso-gastric tube with frequent suctioning to prevent aspiration of fluids into the lungs (Wilberger, 1996). In this case oral administration of both food and medications is contra-indicated (Wilberger, 1996; Greenberg, 1997). Secondly, most of the experimental literature involving minocycline treatment in neurological diseases cites intraperitoneal injections as the route of administration (Yrjanheikki *et al.*, 1999; He *et al.*, 2001; Arvin *et al.*, 2002; Wu *et al.*, 2002; Zhu *et al.*, 2002).

In a second series of experiments focused only on behavioural recovery, minocycline was tested against methylprednisolone. Groups ($n = 10$ each) consisted of minocycline treatment using the above dosing regimen, vehicle treatment (as above), vehicle (pH 5.0) treatment to mimic the pH of the minocycline solution and a methylprednisolone treatment group. Methylprednisolone (30 mg/kg, intramuscularly; Solu-Medrol; Pharmacia Upjohn, Kalamazoo, MI,

USA) was administered beginning 1 h after surgery and repeated every 6 h for 24 h, for a total of five injections/mouse. This dosing paradigm has been demonstrated to improve directed forepaw reaching in rats with lesions of the dorsal corticospinal tract (Nash *et al.*, 2002).

Behavioural assessments

Basso Beattie Bresnahan locomotor rating scale

Beginning 5 days prior to SCI, animals were acclimated in an open field arena for behavioural testing. Mice were exposed to the open field every day for 5 days. Sessions were 5 min in duration. This procedure was important for minimizing fear and stress within the animals that could negatively influence behaviour during the test sessions.

Three days following SCI, mice were again placed in the open field environment where hindlimb function could be assessed using the Basso Beattie Bresnahan locomotor rating scale (Basso *et al.*, 1995). This is a 22-point scale that systematically details hindlimb function of joint movements, stepping ability, the degree of fine control of coordinated stepping and trunk stability. Test sessions were 4 min in duration and mice were tested twice weekly, from days 3 to 28 post-injury.

Inclined plane task

Spinal cord injured mice were also tested using a second behavioural task, the inclined plane, which assesses an animal's ability to maintain its position on a board which is raised in 5° increments and thus can be used as an index of hindlimb strength (Fehlings and Tator, 1992). The maximum angle at which a mouse is able to maintain its position for at least 5 s constitutes the inclined plane score. Mice were tested once a week throughout the survival period beginning at day 7 post-surgery.

Histological analysis of tissue injury

Retrograde labelling with fluorogold was used to determine the degree of axonal integrity through the lesion site (Joshi and Fehlings, 2002b). Twenty-eight days after injury, minocycline- ($n = 11$) and vehicle-treated mice ($n = 8$) were subjected to transection surgery at T8 and gelfoam soaked in 2% fluorogold was placed at the site. Two days later, animals were perfused with 10% neutral buffered formalin and brains were embedded in paraffin. Sections (6 µm) were cut and fluorogold-positive cells in both red nuclei were counted blind.

Histological analysis was also carried out on spinal cord specimens. Briefly, 6-µm thick longitudinal sections from minocycline ($n = 6$) and vehicle ($n = 5$) treated mice were stained with haematoxylin and eosin. Lesion areas from sections containing the central canal were outlined using Image Pro software and lesion size calculated for statistical

analysis. In addition, serial sections were processed with Bielchowsky silver stain to identify axons. Qualitative analysis of axonal integrity was determined for both minocycline and vehicle-treated groups.

Results

Minocycline decreases the mortality of mice recuperating from SCI in a non-temperature controlled environment

Initial experiments where mice were kept in a non-temperature controlled environment found a significant mortality rate in vehicle-treated animals after SCI (Fig. 1). This was likely the result of the mice being unable to maintain normal body temperature as the ambient temperature fluctuates, and was supported by the finding that many saline-treated animals appeared to be lethargic and exhibited signs of poor grooming and weight loss. Thus, we evaluated the ability of minocycline to influence the survival of animals subjected to SCI. We found that minocycline treatment significantly attenuated mortality (Fig. 1). Over a 3 week post-injury period, 16 out of 26 animals (61.5%) from the vehicle-treated group died, compared with only five of 25 (20%) mice from the minocycline-treated group.

These data indicate that minocycline provides protection against mortality in spinal cord injured mice and thus may ameliorate damage evolving from spinal cord compression.

Minocycline improves behavioural scores following SCI

All subsequent experiments were done on mice housed in a room maintained constantly at 27°C; herein, the mortality rate was low and was not different between the minocycline and vehicle groups.

Behavioural assessments of hindlimb function were conducted twice weekly throughout the experiment using the Basso Beattie Bresnahan locomotor rating scale. Repeated measures analysis revealed a significant treatment effect ($F = 18.283$, $P = 0.0002$) and a day effect ($F = 22.404$, $P < 0.0001$), as well as a significant treatment by day interaction ($F = 2.345$, $P = 0.0251$) where minocycline-treated animals exhibited significant recovery in hindlimb function compared with vehicle-treated mice (Fig. 2A). This difference was observed as early as day 3 post-injury, when minocycline animals exhibited an average score of 5 compared with an average score of 2 for control mice ($P < 0.005$). Some improvements were noted in the vehicle-treated mice over the next week, which then plateaued; however, minocycline-treated mice continued to show improvements in hindlimb function throughout the survival period. Minocycline-treated mice achieved a final average score of 10 (indicative of weight supported plantar stepping) at 4 weeks post-injury compared with only 4.5 (indicative of slight movements of hip, knee and ankle joints) for the vehicle-treated group ($P < 0.005$). These differences in hindlimb function can be

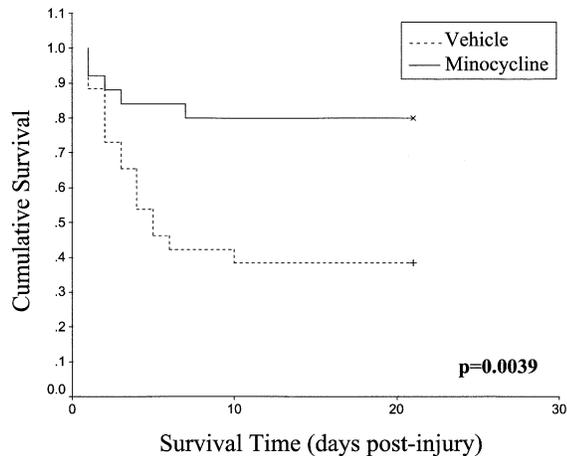


Fig. 1 Minocycline improves survival from SCI when animals were kept in a non-temperature controlled environment. The incidence of mortality in minocycline-treated SCI mice was compared with that in vehicle-treated controls over a 3-week survival period in a non-temperature controlled environment. The cumulative survival refers to the proportion of mice surviving from an initial group size of 26 and 25, respectively, following saline or minocycline treatment. The administration of minocycline beginning 1 h post-injury and every 24 h for 6 days thereafter significantly reduced death.

seen in Fig. 3. Moreover, we found that over half of the animals that received minocycline had scores >10, indicating consistent stepping patterns as well as displaying some evidence of forelimb–hindlimb co-ordination.

The results generated from inclined plane testing, assessing hindlimb strength, also demonstrated a protective effect with minocycline treatment ($F = 5.878$, $P = 0.0249$). Mice treated with this compound exhibited significantly higher scores than vehicle-treated mice at weeks 3 and 4 post-injury ($P < 0.05$, Fig. 2B). Furthermore, there was a significant correlation between Basso Beattie Bresnahan score and inclined plane score over all test days ($r = 0.70$, $P < 0.0001$).

Minocycline confers neuroprotection in SCI

The functional protection observed in minocycline-treated animals may be due, in part, to tissue sparing, especially since a difference from saline-treated mice was observable by 3 days post-injury. Retrograde labelling of red nucleus neurons (Fig. 4A) indicated that there was a trend towards a greater preservation of the rubrospinal tract in minocycline-treated mice compared with vehicle controls (Fig. 4B). Furthermore, there was a significant correlation between Basso Beattie Bresnahan score at day 28 and neuronal soma counts in the red nucleus ($r = 0.84$, $P < 0.0001$) (Fig. 4C). Finally, preservation of axonal integrity was verified in spinal cord by histological criteria. The analysis of spinal cords from vehicle ($n = 5$) and minocycline ($n = 6$) treated mice stained with

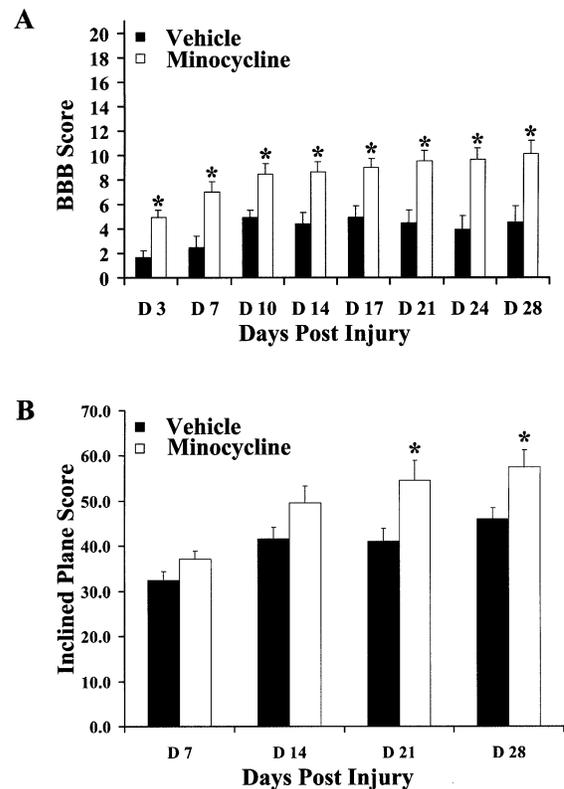


Fig. 2 Minocycline improves Basso Beattie Bresnahan and inclined plane scores. (A) Recovery of hindlimb function following SCI was assessed using the Basso Beattie Bresnahan locomotor rating scale. The data indicate that minocycline-treated mice exhibit significantly higher scores ($*P < 0.005$, unpaired t -test with P -value corrected for multiple comparisons) on each test day. Furthermore, minocycline-treated mice demonstrate continued improvements in hindlimb function reaching a final score of 10, which indicates weight supported plantar stepping. (B) Hindlimb strength was assessed using the inclined plane test. While both groups demonstrated improvements in hindlimb strength with increasing survival time, animals in the minocycline-treated group tended to have higher scores and were significantly different from vehicle-treated controls on days 21 and 28 post-SCI ($*P < 0.05$, unpaired t -test with P -value adjusted for multiple comparisons). The group size was 18 mice in the minocycline group and 15 in the vehicle cohort.

Bielchowsky silver stain, a specific stain for axons, indicated that there were qualitatively more intact axons in minocycline-treated animals compared with vehicle-treated controls (Fig. 5).

The trend towards preservation of rubrospinal neurons and of Bielchowsky axonal profiles indicates that minocycline may have a more global neuroprotective effect on spinal cord tissue including non-axonal elements. Thus, of note, haematoxylin and eosin-stained tissue revealed a significant reduction in lesion size in minocycline-treated mice compared with vehicle-treated controls (1.76 versus 2.80 mm², respectively; $P < 0.001$; see Fig. 6).

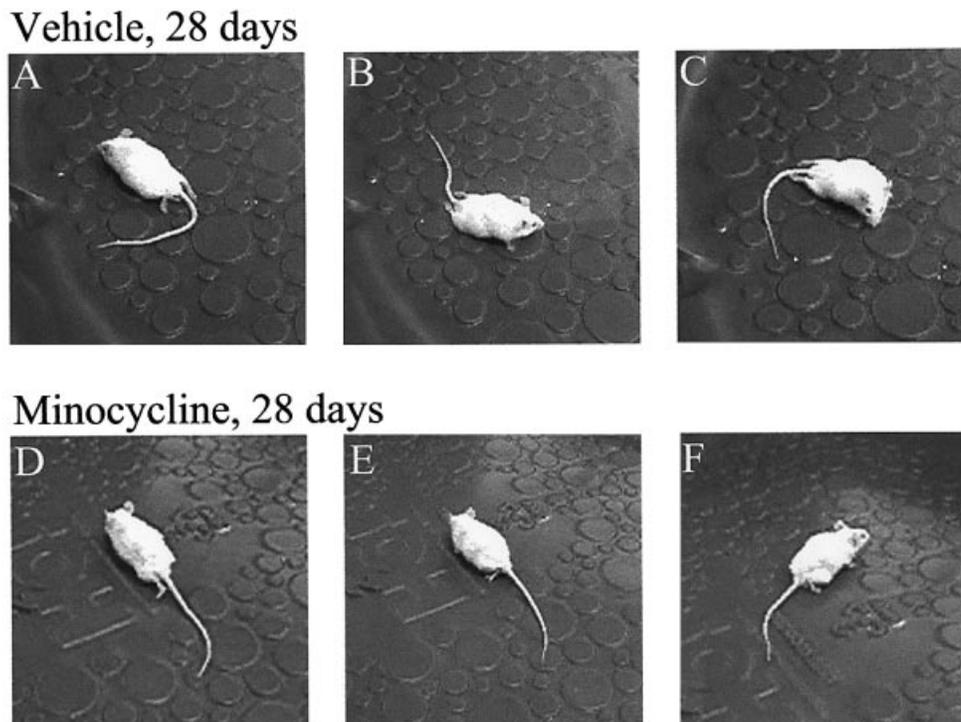


Fig. 3 Representative hindlimb movements of mice at 28 days post-injury. (A–C) Non-contiguous sequences demonstrating the extension of the hindlimbs with little stepping motion typical of vehicle-treated mice. D–F are from a minocycline mouse: (D and E) contiguous sequences showing stepping of the left hindlimb; (F) is another example of stepping motions.

Minocycline is more effective than methylprednisolone in facilitating behavioural recovery

In a final series of experiments, we compared the extent of the functional recovery elicited by minocycline against methylprednisolone. We also controlled for the possibility that the pH (5.0) of the minocycline solution might have generated a stress response, by including a vehicle group where the pH of the saline solution was lowered to 5.0 with HCl. While minocycline treatment resulted in significant recovery of hindlimb function, methylprednisolone injections did not lead to behavioural improvement compared with saline controls (Fig. 7). Repeated measures analysis revealed a significant treatment effect ($F = 6.020$, $P = 0.003$) and a day effect ($F = 57.362$, $P < 0.001$), as well as a significant treatment by day interaction ($F = 2.169$, $P = 0.004$). Multiple comparison *post hoc* analysis using Tukey's uncovered significant differences between minocycline-treated mice and mice from all other treatment groups [P -values <0.01 , <0.05 and <0.05 for vehicle, vehicle (pH 5.0) and methylprednisolone, respectively]. Furthermore, the pH 5.0 vehicle group did not differ from saline controls.

Discussion

Traumatic injury to the spinal cord typically results in axonal damage and cell death and leaves individuals with varying

degrees of functional impairments. The extent of these impairments is dependent upon both the severity of the injury as well as the level at which the injury occurred. Damage arising from acute SCI is generally described as two distinct pathophysiological events. The damage incurred at the time of injury is termed primary injury and typically results from direct mechanical disruption of cord integrity. In contrast, secondary injury occurs in a delayed yet progressive fashion and involves cellular and biochemical events that initiate cascades culminating in tissue damage and cell death (Dumont *et al.*, 2001). It is well recognized that the evolution of secondary tissue damage spreads away from the injury epicenter, incorporating tissue both rostral and caudal to the primary lesion with increasing functional deficits. The fact that damage continues to develop over time in the days and weeks following acute SCI provides an opportunity to intervene. Neuroprotective strategies aimed at preventing damage arising from secondary injury processes provide some hope for tissue sparing and improved functional outcome. This in conjunction with the fact that current treatment options are limited hastens the need to find novel therapeutic agents.

In these experiments, we found that minocycline facilitated significant long-term recovery of mice from SCI. This was true for both hindlimb function and strength. Three days following SCI both minocycline- and vehicle-treated mice exhibited severe deficits in hindlimb function and locomoted using forelimbs and dragging hindlimbs. The only observed

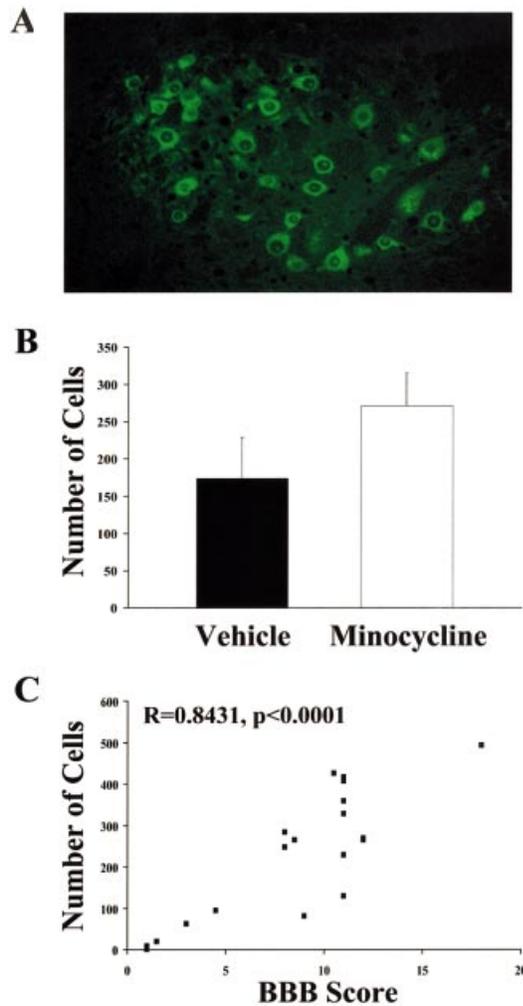


Fig. 4 A trend towards preservation of the rubrospinal tract was obtained following minocycline treatment compared with vehicle injections. (A) Representative section of red nucleus labelled with fluorogold that was administered distal to the injury in the spinal cord. (B) Labelled neurons were counted across the entire red nucleus from minocycline ($n = 11$) and vehicle ($n = 8$) treated mice following retrograde fluorogold labelling. Minocycline treatment preserved some white matter tracts compared with vehicle, as indicated by a greater number of cells within the red nuclei that were fluorogold positive. However, this did not arrive at statistical significance ($P = 0.09$, unpaired t -test). (C) Correlation analysis between Basso Beattie Bresnahan score on day 28 post-injury and cell counts from red nuclei. The significant correlation indicates that axonal sparing through the lesion site is, in part, responsible for improved hindlimb function in the minocycline group.

hindlimb movements consisted of slight-extensive movements of the hindlimb joints, with no evidence of weight support. While both groups showed some improvement throughout the duration of the study, recovery in the vehicle group was limited. In contrast, minocycline-treated mice showed continual and significant improvements over the 4-week recovery period. Twenty-eight days post-injury the average group score for the Basso Beattie Bresnahan test was

10, with a majority of mice exhibiting scores >10 . In these animals, consistent weight supported plantar stepping was observed, and some showed evidence of forelimb-hindlimb coordination.

Since the functional recovery of minocycline-treated mice can be differentiated from vehicle controls from the earliest time point studied (3 days), the mechanism of minocycline is likely one of neuroprotection, rather than tissue regeneration. In support, minocycline provided histological savings in terms of lesion size as well as spared axons. Axonal sparing was observed qualitatively in tissue sections stained with Bielchowsky silver stain, as well as inferred from the fluorogold counts where only intact axons could transport this marker through the lesion site. Fluorogold-labelled cells were counted in the red nucleus, since neurons of the red nucleus project descending axons to form the rubrospinal tract. It can be argued that the axonal sparing inferred from the fluorogold data contributed to the improved hindlimb function seen in the minocycline-treated animals, as the rubrospinal tract of rodents is heavily involved in motor function (Muir and Whishaw, 2000; Kuchler *et al.*, 2002). In keeping with this a strong correlation was demonstrated between fluorogold counts and Basso Beattie Bresnahan scores 28 days post-SCI ($P < 0.0001$). Although the difference between the two groups in terms of cell counts was not statistically significant, the difference was $>10\%$. As mentioned previously, the literature indicates that rescue of as few as 10% of the long fibre tracts in the spinal cord can be sufficient for locomotive ability (Blight, 1983).

The pathophysiological events contributing to SCI are thought to involve free radical production, lipid peroxidation, eicosanoid and prostaglandin production, protease activity, excitotoxic molecules such as glutamate, and intracellular increases in Ca^{2+} (Dumont *et al.*, 2001). In addition, several components of inflammation are also thought to be involved (Bethea, 2000). There is an increasing body of evidence that minocycline has many other effects independent of its antimicrobial actions. Many of these actions include the inhibition of several of the mediators of secondary injury described above. For example, Yrjanheikki and colleagues showed that in primary neuron cultures treated with minocycline, cell death due to glutamate toxicity was significantly attenuated (Yrjanheikki *et al.*, 1999). Furthermore, these investigators demonstrated that minocycline was also able to inhibit the induction of interleukin-1-converting enzyme (caspase-1), and reduce cyclo-oxygenase-2 expression as well as prostaglandin E2 production in a model of focal ischaemia in the rat (Yrjanheikki *et al.*, 1999). Minocycline has also been shown to inhibit the upregulation of caspase-1 and -3 mRNA expression in a mouse model of Huntington's disease, as well as to decrease iNOS activity (Chen *et al.*, 2000). In a recent study, minocycline was demonstrated to inhibit the release of cytochrome *c* from mitochondria, an effect that decreased apoptosis of cells (Zhu *et al.*, 2002). Finally, minocycline has been reported to reduce the production of MMPs as well as inhibit their activity

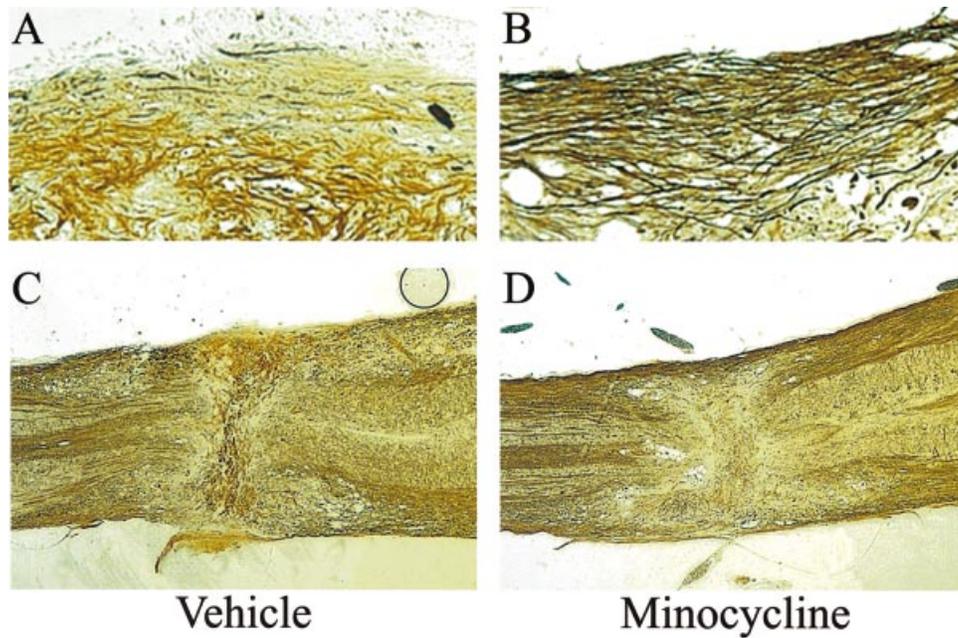


Fig. 5 Axonal tracts through the site of spinal cord compression are relatively preserved following minocycline administration compared with saline treatment. **A** and **B** are higher magnification micrographs of the upper area of the spinal cord displayed in **C** and **D**, respectively. These are representative results (from five vehicle and six minocycline mice) of spinal cord processed for Bielchowsky silver stain demonstrating axons in spinal cords from minocycline (**B**, **D**) and vehicle (**A**, **C**) treated mice. Qualitatively, there is better preservation of axonal integrity in the white matter of minocycline-treated mice compared with vehicle controls. Magnification **C**, **D** 25× and **A**, **B** 100×.

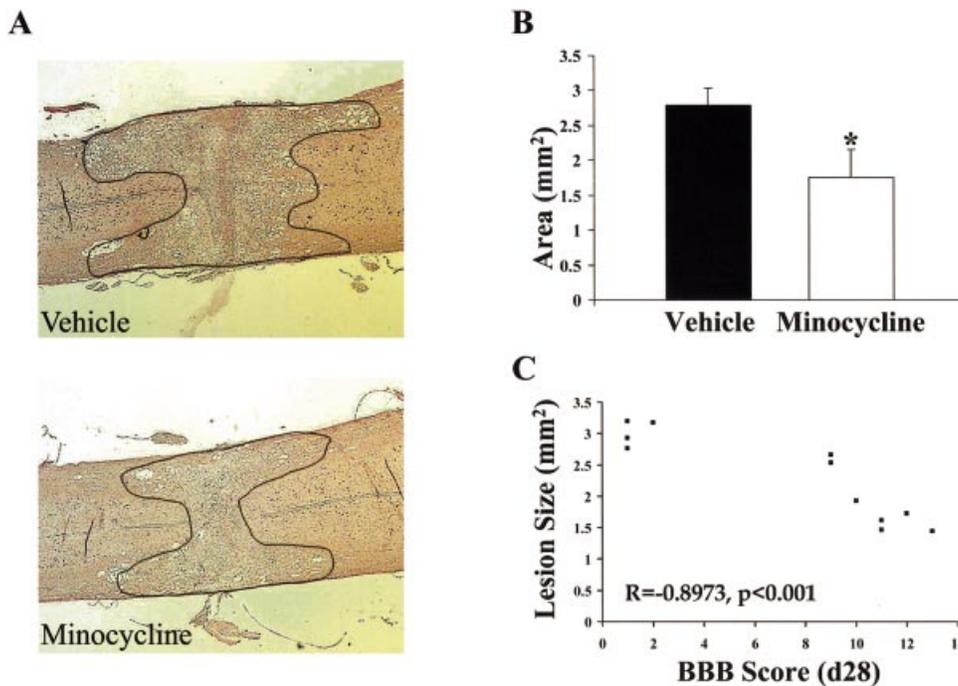


Fig. 6 Preservation of spinal cord tissue following minocycline treatment. Histological analyses were conducted of longitudinal sections encompassing the lesion area and containing the central canal. Lesion areas (**A**) were outlined using Image Pro software and tabulated. In correspondence with behavioural indices, minocycline treatment resulted in a significant decrease in lesion area compared with saline-treated controls ($*P < 0.001$) (**B**). There was a significant correlation between lesion size thus analysed and the Basso Beattie Bresnahan score of 11 mice analysed (**C**).

(Paemen *et al.*, 1996; Brundula *et al.*, 2002). Although the precise molecular mechanisms by which minocycline facili-

tates recovery from SCI remain to be elucidated, it is tempting to speculate that it simultaneously interferes with

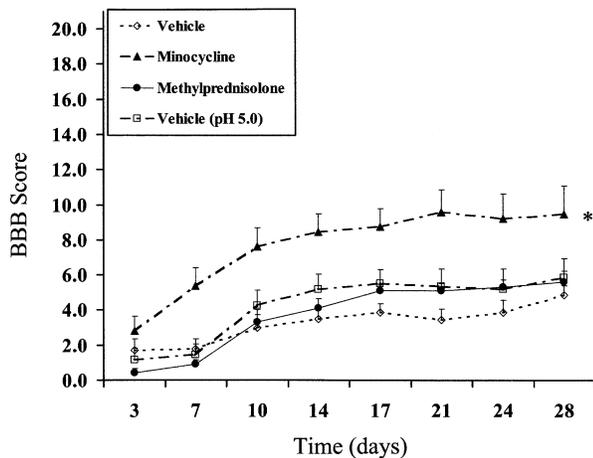


Fig. 7 Superior recovery with minocycline compared with methylprednisolone. The neuroprotective efficacy of minocycline was compared with methylprednisolone. Behavioural assessments of hindlimb function demonstrated significantly improved recovery in minocycline-treated animals compared with those treated with methylprednisolone ($P < 0.05$). Furthermore, there were no significant differences between methylprednisolone and the two control groups, vehicle and vehicle (pH 5.0), indicating that methylprednisolone is not effective in this model of acute spinal cord injury.

the inflammatory cascades, free radicals, MMPs, glutamate and other molecules that contribute to primary and secondary mechanisms of injury. This is unlike conventional therapies of the past (i.e. glutamate antagonists, gangliosides, Ca^{2+} channel blockers), which have generally been highly specific, targeting only one element in the cascade. This approach often leaves other damaging processes intact to mediate further injury. While animal studies involving these compounds have demonstrated some efficacy, they have proven largely unsuccessful when advanced to clinical trial. To date, methylprednisolone is the only drug currently approved for use in the treatment of acute SCI, and only in some centres. The use of methylprednisolone in acute SCI therapy is highly controversial due to a lack of conclusive evidence of efficacy from both animals studies and clinical trials (Hurlbert, 2000, 2001; Molano *et al.*, 2002; Rabchevsky *et al.*, 2002; Takami *et al.*, 2002). When minocycline treatment was compared with methylprednisolone treatment in this model of spinal cord compression, functional benefits were observed only after minocycline administration. The dosing regimen used here is similar to that used by Nash and colleagues, who demonstrated significantly improved directed forepaw reaching in rats with lesions of the dorsal corticospinal tract (Nash *et al.*, 2002). Reasons for the difference in the neuroprotective efficacy of methylprednisolone in that study and the results reported here may include the different species used or the severity of the injury produced; that is, compression of the whole cord in this study versus lesion of the dorsal funiculus (including corticospinal tract) only in the study by Nash *et al.* (2002).

Concerns have been raised in the literature regarding the acidity of the minocycline solutions used parenterally (Nessler *et al.*, 2002). We controlled for the possibility that the mildly acidic solution (pH 5.0) of minocycline used intraperitoneally could have generated discomfort that then evoked physiological changes such as a stress response. In this regard, the use of saline vehicle with pH 5.0 did not evoke behavioural recovery from normal saline controls (Fig. 7). Moreover, inspections of the peritoneal cavity when mice were killed at the end of the experimental period did not reveal any fibrosis.

Regardless of the ultimate mechanisms of minocycline, a clinical trial to test the effectiveness of minocycline in recovery from SCI seems warranted. In this regard, minocycline has already been demonstrated to have a good safety record during prolonged use in humans to treat infections associated with acne (Seukeran *et al.*, 1997; Shapiro *et al.*, 1997). Moreover, minocycline is small (494 Da protein) and highly lipophilic, so it readily penetrates the blood–brain barrier to enter the CNS (Saivin and Houin, 1988). Furthermore, the serum levels of minocycline resulting from the intraperitoneal injections in mice in this study (5–10 $\mu\text{g/ml}$) are comparable to those obtained after oral dosing of 200 mg in humans (J.Wells, V.Simon, B.Herman, A.Lyon and V.W.Yong, unpublished results). Another argument in favour of minocycline treatment for SCI is that this drug is proving to have widespread efficacy in limiting the pathology of animal models of CNS diseases including Parkinson's disease (Wu *et al.*, 2002), Huntington's disease (Chen *et al.*, 2000), stroke (Yrjanheikki *et al.*, 1999), multiple sclerosis (Brundula *et al.*, 2002; Popovic *et al.*, 2002) and amyotrophic lateral sclerosis (Zhu *et al.*, 2002).

In summary, we demonstrate that the use of minocycline in a mouse model of SCI significantly preserves axonal integrity, prevents tissue loss and leads to behavioural improvements. While the clinical score of the minocycline-treated SCI mice is remarkable compared with vehicle controls, it is possible that further recovery could be facilitated by rehabilitative treatments such as physiotherapy. We propose that the current results warrant further investigations to determine whether minocycline may be of benefit for the treatment of acute SCI in humans.

Acknowledgements

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