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Results of a phase II placebo-controlled randomized trial of minocycline in acute spinal cord injury

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Preclinical studies have attributed neuroprotective properties to the antibiotic minocycline. Animal studies and early clinical trials support its use in several neurological diseases. In animal spinal cord injury models, minocycline improved neurological and histological outcomes, reduced neuronal and oligodendroglial apoptosis, decreased microglial activation and reduced inflammation. A single-centre, human, double-blind, randomized, placebo-controlled study of minocycline administration after spinal cord injury was undertaken for the purposes of dose optimization, safety assessment and to estimate outcome changes and variance. Neurological, functional, pharmacological and adverse event outcomes were compared between subjects administered 7 days of intravenous minocycline ($n = 27$) or placebo ($n = 25$) after acute traumatic spinal cord injury. The secondary outcome used to assess neurological differences between groups that may warrant further investigation was motor recovery over 1 year using the American Spinal Cord Injury Association examination. Recruitment and analyses were stratified by injury severity and injury location *a priori* given the expected influence of these on the sensitivity of the motor exam. Minocycline administered at higher than previously reported human doses produced steady-state concentrations of 12.7 μg/ml (95% confidence interval 11.6–13.8) in serum and 2.3 μg/ml (95% confidence interval 2.1–2.5) in cerebrospinal fluid, mimicking efficacious serum levels measured in animal studies. Transient elevation of serum liver enzymes in one patient was the only adverse event likely related to the study drug. Overall, patients treated with minocycline experienced six points greater motor recovery than those receiving placebo (95% confidence interval −3 to 14; $P = 0.20$, $n = 44$). No difference in recovery was observed for thoracic spinal cord injury ($n = 16$). A difference of 14 motor points that approached significance was observed in patients with cervical injury (95% confidence interval 0–28; $P = 0.05$, $n = 25$). Patients with cervical motor-incomplete injury may have experienced a larger difference (results not statistically significant, $n = 9$). Functional outcomes exhibited differences that lacked statistical significance but that may be suggestive of improvement in patients receiving the study drug. The minocycline regimen established in this study proved feasible, safe and was associated with a tendency towards improvement across several outcome measures. Although this study does not establish the efficacy of minocycline in spinal cord injury the findings are encouraging and warrant further investigation in a multi-centre phase III trial. ClinicalTrials.gov number NCT00559494.

Keywords: spinal cord injury; minocycline; randomized control trial; human
Abbreviations: ASIA = American Spinal Cord Injury Association; SCI = spinal cord injury

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Introduction

Neuroprotection associated with minocycline administration was first demonstrated in a gerbil forebrain ischaemia model (Yrjanheikki *et al.*, 1998). This finding has been reproduced in models of Parkinson's disease, multiple sclerosis, traumatic brain injury, Huntington's disease and amyotrophic lateral sclerosis (Yong *et al.*, 2004). Minocycline has also been shown efficacious in animal models of spinal cord injury (SCI) (Lee *et al.*, 2003; Wells *et al.*, 2003; Stirling *et al.*, 2004; Teng *et al.*, 2004; Festoff *et al.*, 2006; Yune *et al.*, 2007) although two reports failed to reproduce similar benefit (Lee *et al.*, 2010; Pinzon *et al.* 2008). The spectrum of actions of this drug include inhibition of microglial activation and proliferation, reduced excitotoxicity, mitochondrial stabilization resulting in reduced neuronal and oligodendroglial apoptosis, neutralization of oxygen radicals, nitric oxide synthase inhibition, metalloproteinase inhibition, reduced inflammation and Ca^{2+} chelation (Yong *et al.*, 2004).

Minocycline is an appealing agent for translation into clinical trials because of its well-defined human safety record, where it was used primarily over the past 30 years in the treatment of acne (Gough *et al.*, 1996; DTB, 2006). Serious adverse reactions such as drug-induced lupus and hypersensitivity syndrome are rare, occurring in 1/10 000–1 000 000 patients primarily after long-term administration (months to years) (Cunliffe, 1996; Goulden *et al.*, 1996; Shapiro *et al.*, 1997). Clinical trials with minocycline in Huntington's disease (Huntington Study Group, 2004), Parkinson's disease (NINDS NET-PD Investigators, 2008) and amyotrophic lateral sclerosis have demonstrated up to 200 mg/day of oral minocycline to be well tolerated, safe and to exhibit adverse event frequency similar to placebo. In the setting of long-term administration for amyotrophic lateral sclerosis, a mean dose of 387 mg has been reported as the upper limit of oral tolerance; higher doses were associated with increased gastrointestinal side effects and elevated serum blood urea nitrogen and liver enzyme levels (Gordon *et al.*, 2004). Similarly, 100 mg twice-daily for the treatment of relapsing remitting multiple sclerosis did not produce significant toxicity (Metz *et al.*, 2004; Zhang *et al.*, 2008). Notably, these trials employed oral minocycline for long periods (6 weeks to 6 months). Recently, minocycline has been administered at high doses (up to 10 mg/kg) intravenously in stroke patients for 72 h without significant adverse effects (Fagan *et al.*, 2010).

Most clinical studies with minocycline in human neurological disease have been encouraging (Metz *et al.*, 2004; Yong *et al.*, 2004; Lampl *et al.*, 2007; Zhang *et al.*, 2008; Plane *et al.*, 2010). However, one notable exception was a randomized controlled trial in amyotrophic lateral sclerosis where minocycline was associated with clinical deterioration (Gordon *et al.*, 2007). It may be argued that, amyotrophic lateral sclerosis is a chronic degenerative condition that has little in common with acute disorders such as SCI or stroke, or with inflammatory conditions such as multiple sclerosis. Consequently, the efficacy of minocycline may differ across these conditions.

Given the compelling evidence demonstrating benefit in animal models of SCI (Lee *et al.*, 2003; Wells *et al.*, 2003; Stirling *et al.*, 2004; Teng *et al.*, 2004; Festoff *et al.*, 2006; Yune *et al.*, 2007) and an established safety profile in humans, we undertook a study to investigate the utility of minocycline in the management of acute traumatic human SCI. Our objectives were to establish safety and tolerance of an adequate dose in this population, to determine feasibility and to obtain preliminary data towards planning a larger efficacy trial.

Materials and methods

This research protocol was approved by the University of Calgary Conjoint Health Research Ethics Board. Between June 2004 and August 2008 all subjects presenting with motor deficit secondary to acute traumatic SCI to the Spine Service at the Foothills Medical Centre in Calgary, Canada were immediately identified to the principal investigators and were assessed and screened for this trial (ClinicalTrials.gov identifier NCT00559494). Those within 12 h of injury who met other inclusion criteria (Table 1) were offered enrolment. Following written informed consent and prior to randomization, patients were stratified into three groups predicted to behave differently within the study: (i) motor-complete SCI (ASIA A or B); (ii) motor-incomplete SCI (ASIA C or D); and (iii) central cord syndrome (ASIA C or D with mean lower extremity motor scores > upper extremity). All subjects received an

Table 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Age 16 or over	Tetracycline hypersensitivity
SCI with ASIA level between C0 and T11, and resulting in a detectable change in the ASIA motor assessment	Elevated liver function tests (AST, ALT, alkaline phosphatase, or total bilirubin greater than 2 times the upper limit of normal)
English speaking subject able to provide informed consent	History of systemic lupus erythematosus (SLE)
Randomization and administration of first dose (drug or placebo) within 12 h of injury	Significant leucopoenia (white blood cell count < 0.5 × lower limit of normal)
	Pre-existing hepatic or renal disease
	Pregnancy or breast feeding
	Presence of systemic disease that might interfere with patient safety, compliance or evaluation of the condition under study (e.g. insulin-dependent diabetes, Lyme disease, clinically significant cardiac disease, HIV, HTLV-1)
	Associated traumatic conditions interfering with informed consent or outcome assessment (e.g. closed head injury)

indwelling lumbar catheter (at L4/5) for CSF sampling and CSF pressure monitoring, a radial arterial line for blood pressure monitoring and a subclavian central venous catheter. Augmentation of spinal cord perfusion, anticipated to be a confounding variable, was controlled through a second randomization as detailed below (results reported separately). Surgical decompression and stabilization was performed within 24 h of injury. Subjects were not treated with corticosteroids. All subjects were screened and enrolled in the Foothills Medical Centre emergency department and were subsequently managed in the intensive care and neurosurgery in-patient ward. Then, they were transferred to the University of Calgary Spinal Cord injury Rehabilitation programme, also housed at the Foothills Medical Centre.

Randomization and masking

Subjects were randomized (1:1) to receive intravenous minocycline (Wyeth Pharmaceuticals) or placebo (equal volume of normal saline) in blocks of 10. For this purpose, sets of 10 random numbers balanced for odd and even integers were computer generated. Sequentially numbered and sealed packaged kits containing drug or placebo were constructed from the randomization codes for each stratified group by an independent individual not otherwise involved in the trial. Patients were administered the next available treatment kit for their appropriate stratum. With the exception of the bedside nurse responsible for study drug administration, all subjects and research personnel were blinded to treatment until the end of the study.

Procedures

The first five subjects randomized to the minocycline group were administered the maximum previously reported human minocycline dose (200 mg twice-daily). Serum and CSF minocycline levels were assayed (Fig. 1) to determine steady-state concentrations. Subsequently, to achieve a steady state more inline with 7–10 μg/ml serum levels recorded in our animal experiments (unpublished observations) and those of others (Popovic *et al.*, 2002), the dose was increased to an 800 mg loading dose tapered by 100 mg at each 12 h administration until 400 mg, then continued for the remainder of the study. Drug or placebo infusions were continued for 14 doses (7 days after injury). All infusions were administered through a subclavian central venous catheter over 30 min.

Spinal CSF pressure was transduced through an indwelling lumbar catheter at L4/5, while mean arterial blood pressure was monitored through an indwelling radial artery catheter. Spinal cord perfusion pressure, the difference between mean arterial pressure and CSF pressure, was calculated and monitored electronically in real time. Subjects whose spinal cord perfusion pressure fell below 75 mmHg at any time during the 7 days of treatment underwent a second randomization assigning them to blood pressure maintenance (control) versus spinal cord perfusion pressure augmentation. Those assigned to the control group received crystalloid fluid and inotrope therapy (norepinephrine) as necessary to maintain mean arterial blood pressure >65 mmHg. Those randomized to active spinal cord perfusion pressure augmentation received crystalloid fluid and if necessary inotrope therapy (norepinephrine) to maintain spinal cord perfusion pressure >75 mmHg. Spinal cord perfusion pressure support was continued until the end of Day 7.

CSF samples (up to 10 ml) were drawn from the indwelling lumbar catheter every 12 h for 7 days as follows: 0.5 h before, 0.5 h after and 6 h after drug infusion in all subjects. Samples were centrifuged at 2000 rpm for 5 min to separate cellular matter. The supernatant was flash frozen and stored at -80°C in aliquots. CSF was analysed for inflammatory and structural proteins (presented elsewhere).

Clinical outcome measures

Neurological function was assessed at intervals using the American Spinal Cord Injury Association (ASIA) standardized neurological examination, including the motor and sensory composites. These examinations were performed by a physical medicine and rehabilitation specialist at Days 1 (time of enrolment), 4, 5 and 7; Weeks 3, 6 and 12; and Months 6 and 12. Day 1 scores were subtracted from each subsequent score to calculate improvement from baseline for graphing purposes.

We chose to use the Day 1 score for baseline comparison acknowledging controversy that such early examinations can be prone to more variability. In order to be enrolled in this study, each subject was required to provide an accurate ASIA neurological exam. Thus, 100% of the enrolled subjects had a Day 1 baseline ASIA score. We considered the alternative of using the Day 4 score to adjust for baseline. However, we found that Day 4 examinations were sometimes not possible, in particular, as subjects were in the ICU and ventilated, often

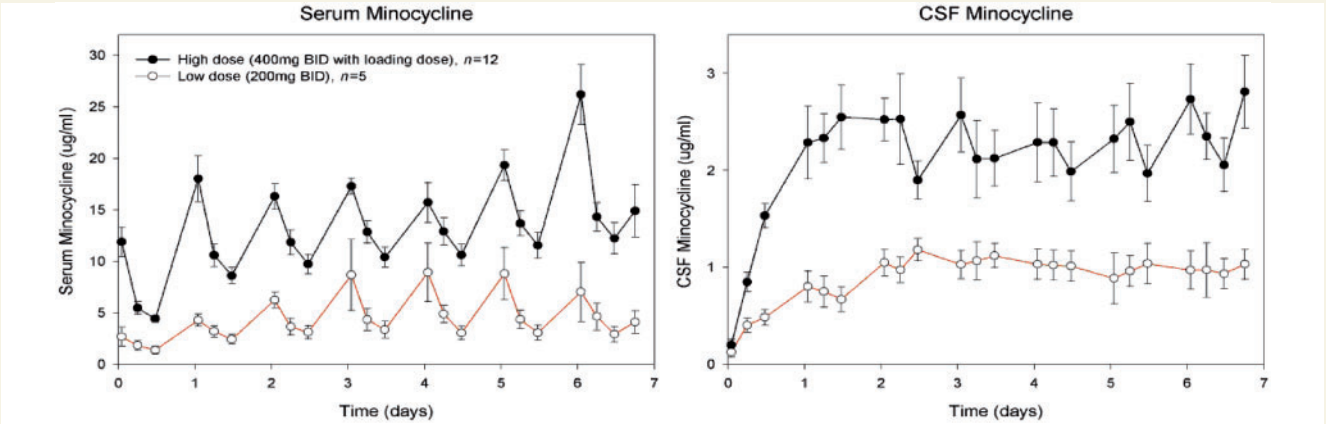
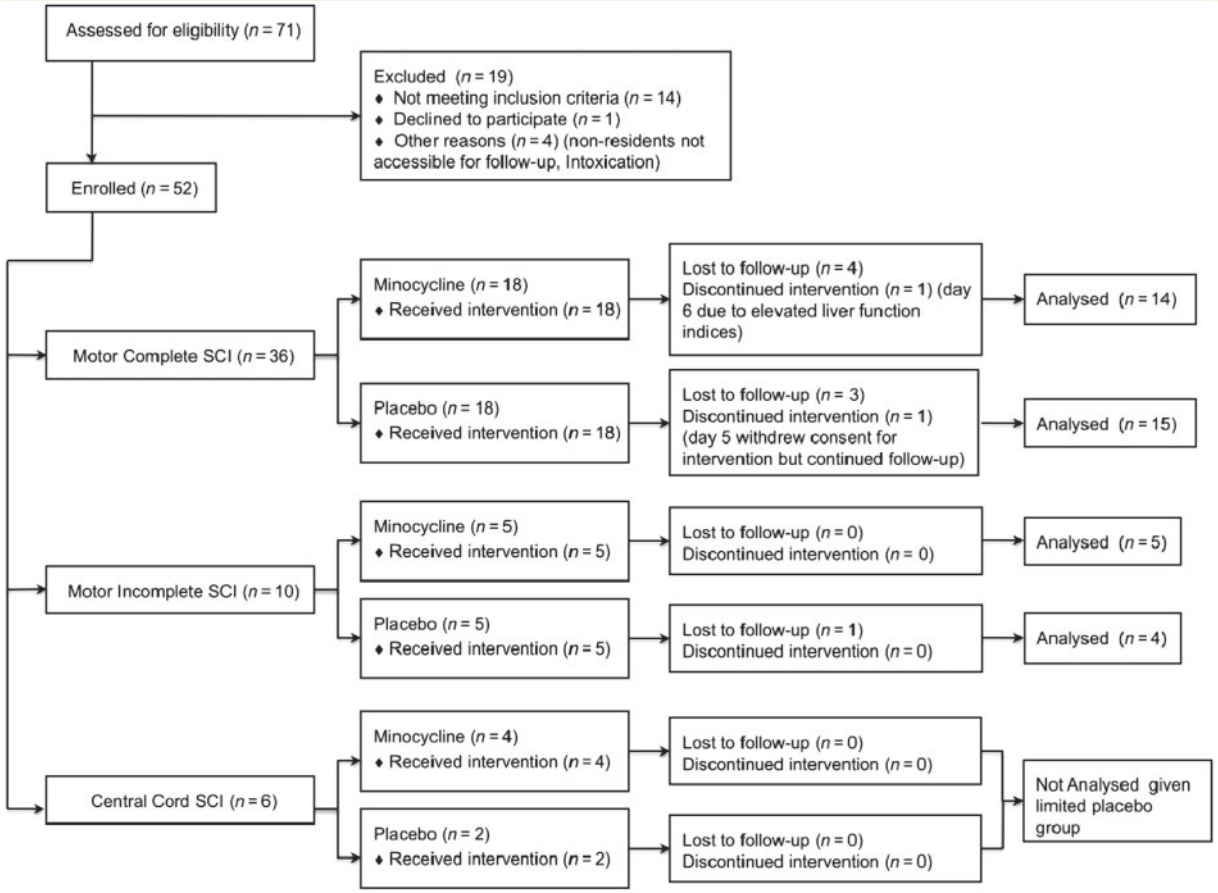


Figure 1 Pharmacokinetics of minocycline in serum and CSF. Mean serum (*left*) and CSF (*right*) minocycline levels versus time (± SEM). CSF was sampled 30 min before, 30 min after and 6 h after each minocycline dose with twice-daily dosing at the doses indicated. Minocycline concentration was determined in the first group of samples in each 24 h period. BID = twice-daily at 12 h intervals.



Subjects were considered lost to follow-up if they lacked data for the ASIA motor examination between 3 and 12 months. Detailed subject follow-up at each time-point [*n* (%)] is presented below:

Time (days)	All subjects (*n* = 52)	Motor-complete (*n* = 36)	Motor-incomplete (*n* = 10)	Central cord injury (*n* = 6)
4	38 (73%)	26 (72)	8 (80)	4 (67)
7	42 (81)	29 (81)	8 (80)	5 (83)
21	46 (88)	32 (89)	8 (80)	6 (100)
42	39 (75)	26 (72)	9 (90)	4 (67)
90	40 (77)	26 (72)	9 (90)	5 (83)
182	39 (75)	27 (75)	8 (80)	4 (67)
365	40 (77)	26 (72)	8 (80)	6 (100)
Mean %	(78)	(76)	(83)	(81)

Figure 2 Enrolment, randomization and follow-up. Consort diagram tracking enrolled subjects through randomization and follow-up (*top*). Table summarizing the detailed follow-up rates at each time-point for outcome data collection (*bottom*).

with sedation. Consequently, only 73% of subjects had data at Day 4 (Fig. 2). Furthermore, it appeared that at Day 4 subject motivation was more likely to influence compliance with the examination. In comparing variability of the Day 1 and 4 examination standard deviations (SD) were 22.68 and 24.73, respectively. The mean change in motor score at Day 4 was −0.368 (SD = 7.964). Sixty-six per cent of subjects displayed a Day 4 motor score within three points of the Day 1 score. Thirteen per cent ($n = 5$) displayed a change >10 points.

Functional outcome was assessed using the Spinal Cord Independence Measure, Functional Independence Measure, London Handicap scale, and Short Form 36 questionnaires administered at 6, 12, 26 and 52 weeks after injury.

Subjects were evaluated for adverse events daily while in hospital and at each clinical evaluation subsequently. All serious adverse events (Table 3) were reviewed promptly by a safety monitoring board composed of clinicians and clinician researchers not otherwise involved in

this study. A summary of all adverse events was reviewed every 6 months.

Statistical analyses

Unadjusted ASIA motor score was compared across time and between groups using repeated measures regression employing data from Days 90, 182 and 365 with baseline score as a covariate using the 'R' statistical package. The model assumed that any change associated with treatment group was constant over these time points. An interaction term between treatment and injury type was included to allow for the possibility that changes associated with treatment group differed among motor-complete and motor-incomplete injured subjects. ASIA sensory scores were similarly evaluated. ASIA motor, pinprick and light scores were evaluated by the Shapiro–Wilk normality test and were consistent with a normal distribution. Functional recovery data were compared over all data points. Adverse events were categorized by system and mean number of events per patient was compared within each system by ANOVA. All statistical tests were two-tailed ($\alpha = 0.05$). *Post hoc*, we calculated the observed number of subjects and the standard deviation for motor recovery (defined as the average of the motor scores at 3, 6 and 12 months when a plateau in recovery was seen) for each subgroup analysed. The Student *t*-test was then used to estimate the between group difference that this study was powered to detect with power = 0.8 and $\alpha = 0.05$.

Results

Subjects

Fifty-two subjects were entered into the study. An additional 19 (27%) subjects with SCI who presented during the enrolment period were not enrolled; 14 (20%) of those did not satisfy the inclusion and exclusion criteria (Fig. 2). The study was terminated upon recruitment of 10 motor-incomplete subjects, a recruitment target defined prior to the trial as a minimum sample size for outcome evaluation in this key subgroup. Average age was 37 years. Seventy-seven per cent of enrolled subjects were male. Thirty-six subjects suffered motor-complete SCI while 10 were motor-incomplete. Six patients presented with central cord syndrome. Baseline demographic and clinical characteristics for the minocycline and placebo groups are summarized in Table 2.

Only subjects presenting within 12 h of injury were included in the study; there were no violations in this regard. The mean time from injury to presentation was 3.6 h. The intervention (minocycline or placebo) was started on average 10.2 h after injury. As part of the protocol, in the setting of ongoing spinal cord compression, surgical decompression was undertaken within 24 h of injury. The mean time to decompression in our cohort was 17.4 h. There were eight (15%) violations to this requirement; four subjects underwent surgery during the 25th hour and two subjects had surgery at 28 h 42 min and 29 h 00 min, respectively. These violations occurred due to operating room triaging that resulted in an unavoidable delay. Two additional violations were due to surgeon non-compliance with the protocol; one subject with a motor-complete SCI (ASIA A) underwent surgery at 5.5 days while the other with central cord syndrome underwent surgery at 11 days.

Table 2 Subject baseline characteristics

Variable	Minocycline (*n* = 27)	Placebo (*n* = 25)
Mean age (years)	40.9	32.1
Sex (%)		
Male	22	17
Female	5	8
Mechanism of Injury (*n*)		
Motor vehicle collision	14	16
Work accident	5	4
Sport injury	6	3
Fall	2	2
Level and severity of injury (*n*)		
Cervical		
Motor-complete	7	13
Motor level C1–4		
ASIA A	(6)	(6)
ASIA B		(2)
Motor level C5–8		
ASIA A	(1)	(5)
ASIA B		
Motor-incomplete	5	4
Motor level C1–4		
ASIA C	(1)	(1)
ASIA D	(1)	(1)
Motor level C5–8		
ASIA C	(3)	(1)
ASIA D		(1)
Central cord injury	4	2
Motor level C1–4		
ASIA C		(1)
ASIA D	(1)	(1)
Motor level C5–8		
ASIA C	(2)	
ASIA D	(1)	
Thoracic		
Motor-complete	11	5
Sensory Level T1–6		
ASIA A	(6)	(3)
Sensory Level T7–12		
ASIA A	(5)	(2)
Motor-incomplete	0	1
Sensory Level T1–6		
ASIA C		(1)
Mean enrolment time (h post-injury of first dose)	10.7	9.6
Mean time to spinal decompression (h)	16.5	18.3
SCPP		
Randomization (*n*)		
SCPP	11	10
Control	11	12
Not randomized*	5	3

*Subjects who did not exhibit spinal cord perfusion pressure <75 mmHg and were therefore not randomized.
SCPP = spinal cord perfusion pressure.

Two subjects did not undergo surgical decompression. One subject with cervical motor-incomplete injury (placebo group) presented with a unilateral facet dislocation that was reduced with traction

within 24 h of the injury. Another subject with cervical motor-incomplete injury (minocycline group) was managed in a hard cervical collar as there was no evidence of spinal cord compression or instability on imaging.

None of the subjects enrolled in this study withdrew before completion of their study intervention. There were three protocol violations related to the interventions. A dose error at one time-point occurred when the full daily minocycline dose was administered at once rather than in two divided doses. In two instances lumbar drain dislodgement occurred requiring replacement. Outcome data were available for ~78% of subjects at each time-point (summarized in Fig. 2).

Minocycline dose and safety

The first five patients to receive minocycline infusions were administered 200 mg twice-daily intravenously, the maximum human dose previously reported (Macdonald *et al.*, 1973; Carney *et al.*, 1974). Serum analyses demonstrated a resulting steady-state concentration of 4.2 μg/ml [95% confidence interval (CI) 3.7–4.7] within 48 h (Fig. 1), while CSF samples revealed a steady-state concentration of 1.0 μg/ml (95% CI 0.9–1.1). Previous work in an animal model of multiple sclerosis (Popovic *et al.*, 2002) as well as in SCI (our unpublished observations) had suggested a therapeutic serum steady-state concentration of 7–10 μg/ml. Consequently, subsequent minocycline infusions included a loading dose of 800 mg sequentially tapered by 100 mg every 12 h until 400 mg was reached and then maintained through Day 7. This dosing schedule achieved a serum steady-state concentration of 12.7 μg/ml (95% CI 11.6–13.8) and a CSF steady state of 2.3 μg/ml (95% CI 2.1–2.5) within 24 h (Fig. 1).

Two subjects died during the study; one early death (Day 20) in a patient suffering high cervical quadriplegia (placebo group) was attributed to multisystem organ failure and fulminate acute respiratory distress syndrome. Another subject (high-dose minocycline group) died of a narcotic drug overdose at 6 months. Adverse events did not vary significantly among the placebo, low-dose (200 mg) or high-dose (400 mg) minocycline groups (Table 3 and Supplementary Table 1). Notably, one subject in the high-dose minocycline group displayed elevated liver enzymes, but was otherwise not symptomatic. These indices promptly normalized following discontinuation of the drug. This was the only adverse event likely related to minocycline observed during the study.

Neurological recovery

Neurological recovery was followed using the ASIA neurological exam. Motor recovery plateaued 3 months after SCI. This pattern of recovery occurred regardless of whether the injury was motor-complete, motor-incomplete or of the central cord type (Supplementary Fig. 1). As expected, patients with motor-incomplete injuries recovered more function than those

Table 3 Adverse events by treatment group

System/Category	Placebo			Low dose			High dose			*P**
	Events/ subject	Number of events	Number of subjects	Events/ subject	Number of events	Number of subjects	Events/ subject	Number of events	Number of subjects	
Cardiac	1.10	22	12	1.00	5	4	0.88	14	9	0.787
Respiratory	0.80	16	13	1.00	5	4	0.81	13	9	0.875
Gastrointestinal	1.45	35	15	2.20	12	5	1.88	36	12	0.439
Genito-urinary	0.40	8	7	0.00	0	0	0.44	7	5	0.326
Musculoskeletal	0.65	14	11	0.60	4	4	0.75	14	13	0.849
Integumentary	1.30	27	14	1.00	5	4	1.13	21	11	0.857
Haematological	0.60	12	9	0.40	2	2	0.63	9	6	0.872
Endocrine	0.05	1	1	0.20	1	1	0.00	0	0	0.205
Psychiatric	0.70	15	9	1.20	8	4	1.13	18	8	0.461
Neurological	1.45	35	13	1.60	11	5	0.56	16	10	0.073
Infectious	2.10	58	19	1.80	12	4	1.38	28	11	0.295
Deep venous thrombosis	0.10	2	2	0.00	0	0	0.06	1	1	0.744
Autonomic dysreflexia	0.10	2	2	0.00	0	0	0.06	1	1	0.744
Pain	2.10	44	18	2.00	10	5	2.00	31	14	0.968
Other	1.05	23	14	2.40	13	5	0.88	19	11	0.019
Serious adverse events[a]	0.30	6	5	0.20	1	1	0.06	1	1	0.312

Placebo = normal saline.
Low dose = minocycline 200 mg IV twice daily for 7 days.
High dose = minocycline 800 mg initial dose tapered by 100 mg at each 12 h administration until 400 mg continued for total 7 days.
a Serious adverse event defined as any untoward medical occurrence that results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity.
*Adverse event/patient in each group was compared by ANOVA.

with motor-complete injuries. Those with central cord injuries appeared to display even greater motor recovery; however, the data for that cohort came from only two placebo subjects. Given this limitation, we did not analyse the central cord syndrome group further.

End-point motor recovery for each patient was defined by the plateau in motor function observed in the study population (i.e. 3–12 month outcome data). In the 44 subjects with data available beyond 3 months, this recovery was 6 (95% CI −3 to 14; $P = 0.20$) motor points greater in patients treated with minocycline than in those receiving placebo. Those with thoracic SCI ($n = 17$) did not show any benefit associated with treatment. However, in the setting of cervical SCI ($n = 25$), minocycline administration was associated with a 14 point (95% CI 0–28; $P = 0.05$) difference in motor score over that seen with placebo that approached significance. In Table 2, among subjects with cervical injury the distribution of motor-complete injury (versus motor incomplete) differed between the treatment (44%) and placebo (68%) groups possibly affecting this observed difference. However, the difference was maintained on subgroup analysis (although not statistically significant) suggesting that the effect of baseline differences in injury severity was not enough to explain this observation. The difference appeared less pronounced in the cervical motor-complete subjects (10 points; 95% CI −9 to 28; $P = 0.29$, $n = 16$) than motor-incomplete patients (22 points; 95% CI −7 to 52; $P = 0.12$, $n = 9$; Fig. 3). However, notable in the cervical motor-incomplete group was one subject administered placebo who experienced exceptionally poor recovery. This subject exhibited a C3 injury level, with an ASIA motor score of 2 initially and 0 on subsequent exams. On exclusion of this subject, an augmented difference between minocycline and placebo in motor-incomplete subjects became less clear (13 points; 95% CI −6 to 31; $P = 0.135$, $n = 8$). Comparison of the low- and high-dose minocycline groups suggested a greater difference with higher doses (Supplementary Fig. 2).

Motor recovery (defined by the plateau in motor function) in the subgroup with cervical SCI was evaluated for the distribution of recovery that differed between the minocycline and placebo groups. We compared motor recovery with and without minocycline in the upper extremities and in the lower extremities (Fig. 4). We also compared gain of ASIA motor scores in the zone of partial preservation (myotomes with motor score <5 and >0 at baseline) and in new segments in the cervical motor-complete group where these zones could be defined (Fig. 4). These comparisons suggested that the majority of the difference seen between the treatment groups occurred in the lower extremity and in new segment scores. Little difference was seen in the upper extremities or in the zone of partial preservation.

In an attempt to diminish the potential confounding effect of timing to surgical decompression, we undertook surgery within 24 h of injury. Exceptions to this prerequisite are detailed above. We also examined the relationship between timing to surgical decompression and motor recovery using scatter plots and the Person Product-Moment Correlation Coefficient. We did not observe a correlation between the timing of surgical decompression and motor recovery in any subgroup. The correlation coefficients were as follows: all subjects 0.156, cervical SCI 0.025, thoracic SCI 0.295, motor-complete SCI 0.130, motor-incomplete SCI −0.019, cervical motor-complete SCI −0.023 and cervical motor-incomplete SCI −0.019.

Sensory recovery followed a time-course and pattern similar to motor recovery (Fig. 5). The degree of recovery appeared greater in minocycline-treated patients compared to placebo; however, this was not statistically significant; nine pinprick points (95% CI −3 to 22; $P = 0.15$); seven light-touch points (95% CI −6 to 20; $P = 0.27$). No difference in sensory scores was apparent between patients with thoracic SCI given minocycline or placebo. A tendency towards a higher pinprick and light-touch scores was observed in minocycline-treated patients with cervical motor-incomplete injuries, but this did not reach statistical significance; 14 pinprick points (95% CI −32 to 60, $P = 0.49$) and 20 light-touch points (95% CI −14 to 54, $P = 0.20$).

Functional recovery

Functional recovery was assessed using standardized outcome scales: Functional Independence Measure, Spinal Cord Independence Measure, London Handicap Scale and Short Form 36. Similar to ASIA motor and sensory recovery, functional outcome as assessed by these scales appeared greater in the minocycline-treated group but the difference was not statistically significant (Fig. 6). This difference was most apparent in the Spinal Cord Independence Measure and Functional Independence Measure outcome scales. Spinal Cord Independence Measure and Functional Independence Measure are more disease specific and concentrate on performance of particular tasks (e.g. activities of daily living, sphincter management and respiration) while London Handicap scale, as well as the Short Form 36, further emphasize social functioning (e.g. occupation, social integration and economic self-sufficiency). While a plateau in recovery was generally seen in all scales, it was apparent at 6 months in distinction to the 3-month plateau seen with motor recovery. Similar to neurological recovery using ASIA motor and sensory scores, the difference between the minocycline-treated and placebo-treated groups was more apparent in subjects with cervical motor-incomplete SCI (Fig. 6). These differences in functional outcome were not statistically significant.

Discussion

Minocycline is a synthetic tetracycline. Its only current clinical indications revolve around its antimicrobial properties. However, significant animal data have provided impetus for study of its neuroprotective properties in a variety of neurodegenerative and acute neural insults (reviewed by Yong *et al.*, 2004; Matsukawa *et al.*, 2009; Plane *et al.*, 2010). Animal work also supports its application in SCI (Lee *et al.*, 2003; Wells *et al.*, 2003; Stirling *et al.*, 2004; Teng *et al.*, 2004; Festoff *et al.*, 2006; Yune *et al.*, 2007).

Several promising SCI therapies have been translated into clinical trials, but none have yet proven to be of significant benefit in the human (Tator, 2006). Failure of translation may be attributed to several factors not the least of which is the greater

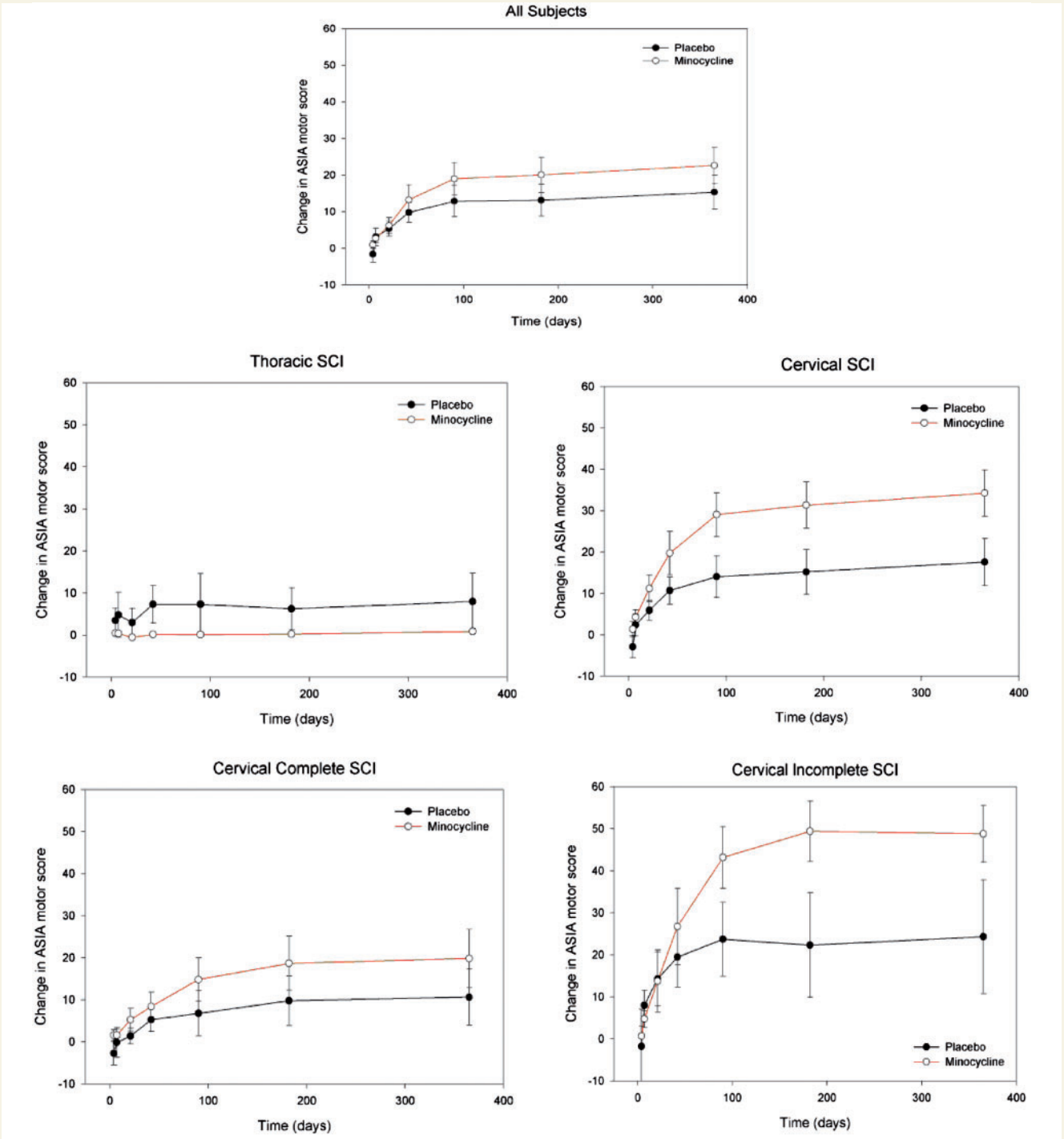


Figure 3 Change in ASIA motor score in the minocycline and placebo groups. Graphic depiction of the change in ASIA motor score (baseline score subtracted from measured score at each time-point ± SEM) over time in all subjects, thoracic injured subjects, cervical injured subjects, subjects with cervical motor-complete and cervical motor-incomplete injury as indicated in subheadings. For cervical SCI $P = 0.05$, all other groups did not achieve or approach statistical significance.

heterogeneity of human SCI when compared to experimental animal models and the resulting variability in spontaneous neurological recovery (Fawcett *et al.*, 2007). In addition, outcome measures are not uniformly sensitive in all human patients; smaller therapeutic effects, as can be expected for most single agent therapies, may be diluted by subjects not responding on a chosen outcome scale. Finally, it must be recognized that animal models do not fully represent the human condition and secondary injury mechanisms may vary in importance and timing among species. These observations underscore the need for future human SCI studies to focus on subpopulations most likely to demonstrate clinical benefit so as to guard against masking of

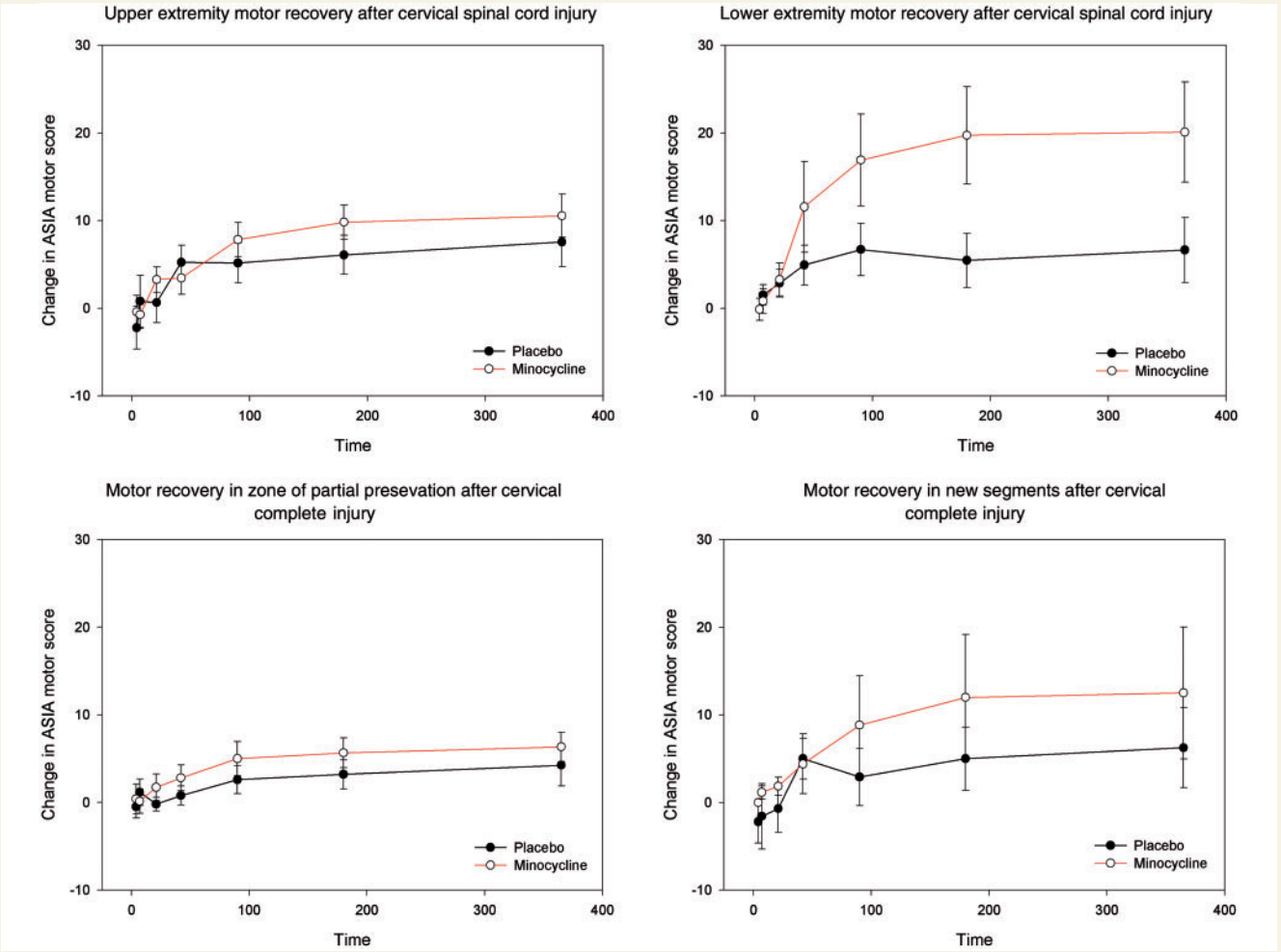


Figure 4 Changes in upper and lower extremity ASIA motor score after cervical SCI in the minocycline and placebo groups. Graphic depiction of the change in ASIA motor score (baseline score subtracted from measured score at each time-point ± SEM) in the upper and lower extremities over time in cervically injured subjects and ASIA motor recovery in the zone of partial preservation and in new segments after motor-complete cervical SCI as indicated in the subheadings.

an effective treatment. In particular, the ASIA motor examination is not sensitive to segmental recovery in thoracic SCI. Furthermore, as injury severity increases beyond that threshold required to eliminate neurological function (i.e. complete SCI), clinically detectable recovery necessitates anatomical recovery to and beyond that point. Thus, we would expect the ASIA neurological examination to be less sensitive in complete-injured subjects and following thoracic SCI, as suggested in this study.

As a therapeutic strategy, minocycline harbours several advantages for translation. From a pharmacological perspective, it is clinically available for human use, crosses the blood–brain barrier, and can be safely administered over long periods. From a mechanistic perspective, it has an influence on multiple biochemical pathways associated with secondary injury. It has been shown to be of benefit in humans with multiple sclerosis (Metz *et al.*, 2004; Zhang *et al.*, 2008) and stroke (Lampl *et al.*, 2007), but not in Huntington's disease (Huntington Study Group, 2004). Indeed, it may be associated with worsening outcome in amyotrophic lateral sclerosis. While differing pathophysiology among these conditions is likely contributory, the dose and route of minocycline administration are also significant variables. The present study employed an unprecedented intravenous loading and maintenance dose of minocycline (800 mg tapering to 400 mg every 12 h) to achieve serum levels similar to those efficacious in animal models of SCI (Popovic *et al.*, 2002) (and our unpublished observations). Animal studies suggest that rapid and sufficient dosing is probably critical to minocycline's efficacy in SCI (Lee *et al.*, 2003; Festoff *et al.*, 2006). With this dosing protocol, we observed only one adverse event likely related to minocycline; elevated serum hepatic enzymes that normalized after discontinuation of the drug. We are aware of only one other study in the literature that examined a short course of high-dose minocycline (Fagan *et al.*, 2010). In that study, 60 stroke subjects were administered up to 700 mg of minocycline IV in twice-daily divided doses infused over 1 h for 3 days. Similar to our study, only one subject was thought to have experienced an adverse event related to minocycline, specifically

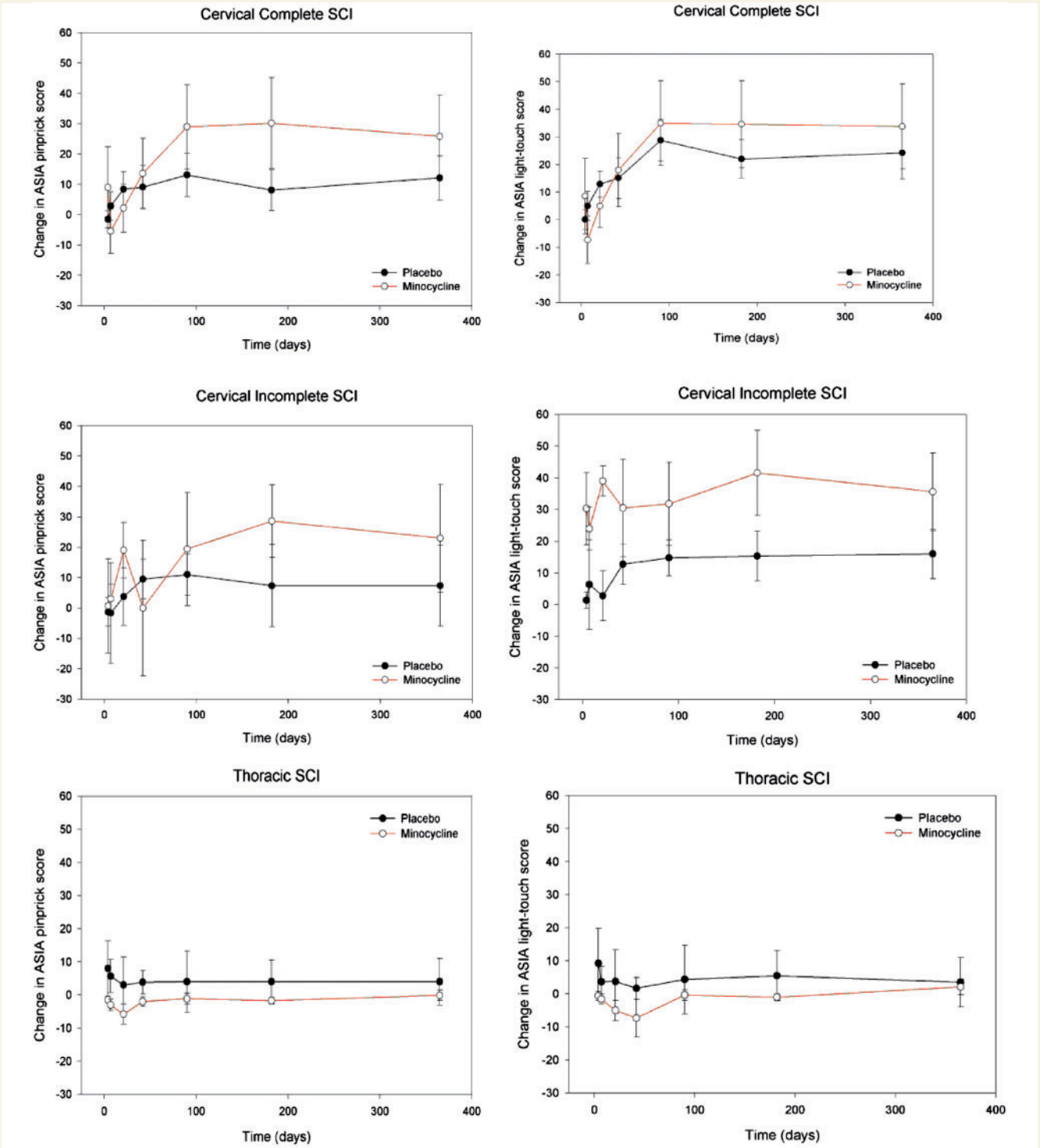


Figure 5 Changes in ASIA sensory scores in the minocycline and placebo groups. Graphic depiction of the change in ASIA light touch sensory and ASIA pinprick sensory scores (baseline score subtracted from measured score at each time-point ± SEM) over time in subjects with cervical motor-complete and cervical motor-incomplete injury as indicated in the subheadings.

elevated hepatic enzymes. By comparison, in our study, 27 subjects received 800 mg daily after loading in twice-daily doses over 30 min. Fagan *et al.* (2010) and the present study point to an increased risk of hepatic injury with high-dose minocycline and possibly in patients with stroke and SCI (upper limit of the 95% CI for the observed incidence is ~8–18%) (Newman, 1995). Notably, SCI itself has been associated with evidence of liver injury as demonstrated by serum liver function tests in humans (Shepard and Bracken, 1994). This may suggest a greater vulnerability to minocycline toxicity, and furthermore, that pharmacokinetic data in different disease states

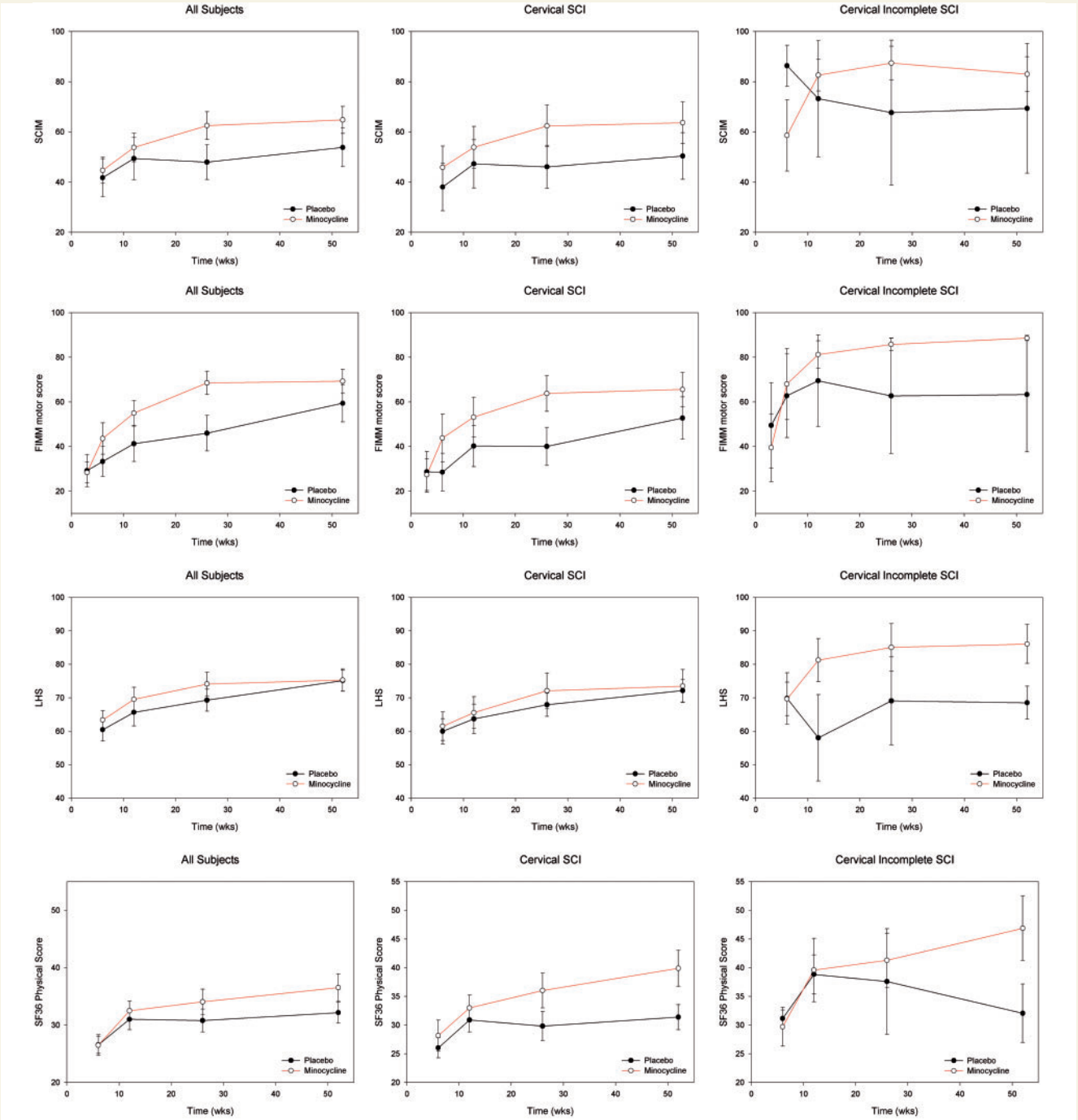


Figure 6 Changes in functional outcome scales in the minocycline and placebo groups. Graphic depiction of functional outcome scores ± SEM. *Top row:* Spinal Cord Independence Measure (SCIM); *second row:* Functional Independence Measure motor subtotal score (FIMM); *third row:* London Handicap score (LHS); and *bottom row:* Short Form 36 (SF36) physical score versus time.

may not be transferable to the SCI patient as minocycline clearance is predominantly hepatic.

The purpose of this single-centre study was to demonstrate feasibility and to investigate variance of and changes in ASIA scores and functional outcome scores after minocycline administration for the treatment of human SCI in order to plan future trials of efficacy, if warranted. The results demonstrate high protocol compliance and that the dose required to mimic prior laboratory work appears well tolerated in human patients. Furthermore, differences in neurological and functional outcomes warranting further investigation may exist for treated patients with cervical SCI where the ASIA motor exam is thought to be more sensitive (motor recovery $P = 0.05$, sensory and functional outcomes not significant). However, this study is limited by its small sample size. Although the change in motor outcome associated with minocycline treatment appeared to approach significance in

sub-analyses (cervical SCI group, $n = 25$), this finding cannot be over interpreted and must be re-examined in a properly powered study. Clinical outcome data were collected in this study only with the intention of estimating variance and examining changes in outcome associated with the treatment groups (i.e. a phase II trial) in order to plan a formal trial of efficacy. *Post hoc* power analysis revealed that using the observed number of subjects (n) and standard deviation (SD) in each subgroup, this study was powered to detect a group difference in motor recovery as follows: all subjects $n = 46$, SD = 19.419, difference of 12 ASIA motor recovery points; cervical SCI $n = 29$, SD = 20.752, difference of 16 points; cervical motor-incomplete injury $n = 9$, SD = 19.344, difference of 28 points; and cervical motor-complete $n = 20$, SD = 15.838, difference of 15 points (power = 0.8 and $\alpha = 0.05$).

It is also notable that smaller studies such as this are prone to baseline differences among treatment groups (Table 2). Our minocycline group was on average older and had fewer females compared to placebo. There were more motor-complete injured subjects with thoracic injury but less with cervical injury in the minocycline group. Subgroup analysis in the cervical group suggested that the difference in outcome between treatment groups was maintained in the motor-complete and -incomplete subgroups but those differences were not statistically significant. These results set the stage for a well-powered multi-centre effort with the purpose of confirming efficacy in a larger group of patients where such limitations have less influence.

We conclude as a result of this study that minocycline given intravenously within 12 h of SCI and for 7 days resulted in steady-state serum concentrations reaching target values suggested by animal studies without significant adverse events. In a randomized, double-blind manner, the treatment was associated with an apparent improvement in neurological and functional outcomes compared with placebo, warranting further formal investigations of efficacy.

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All authors contributed to study design. V.W. Yong performed all CSF and serum studies. S. Casha, I. Bains and R.J. Hurlbert enrolled subjects. S. Casha and R.J. Hurlbert collaborated with the admitting surgeon for patient management. D. Zygun oversaw intensive care management. D. McGowan performed data collection. I. Bains monitored adverse events. S. Casha maintained the database and performed data analysis with Dr. J. Singer and Dr. T. Lee of the CIHR Canadian HIV Trials Network, Vancouver, British Columbia, who undertook an independent statistical analysis. S. Casha, V.W. Yong and R.J. Hurlbert performed data interpretation and manuscript preparation. None of the authors or collaborators had any competing interests.

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Supplementary material

Supplementary material is available at *Brain* online.

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