Animal Studies in Spinal Cord Injury: A Systematic Review of Methylprednisolone

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Summary — The objective of this study was to examine whether animal studies can reliably be used to determine the usefulness of methylprednisolone (MP) and other treatments for acute spinal cord injury (SCI) in humans. This was achieved by performing a systematic review of animal studies on the effects of MP administration on the functional outcome of acute SCI. Data were extracted from the published articles relating to: outcome; MP dosing regimen; species/strain; number of animals; methodological quality; type of injury induction; use of anaesthesia; functional scale used; and duration of follow-up. Subgroup analyses were performed, based on species or strain, injury method, MP dosing regimen, functional outcome measured, and methodological quality. Sixty-two studies were included, which involved a wide variety of animal species and strains. Overall, beneficial effects of MP administration were obtained in 34% of the studies, no effects in 58%, and mixed results in 8%. The results were inconsistent both among and within species, even when attempts were made to detect any patterns in the results through subgroup analyses. The results of this study demonstrate the barriers to the accurate prediction from animal studies of the effectiveness of MP in the treatment of acute SCI in humans. This underscores the need for the development and implementation of validated testing methods.

Key words: *animal, methylprednisolone, steroid, spinal cord injury.*

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Introduction

While animal studies are conducted, in part, to inform human clinical care, several recent reviews have suggested a number of limitations of animal studies that impede the prediction of human outcomes (1–5). These limitations include deficient methodology, lack of standardisation, poor correlation of outcomes with those of clinical studies, and publication bias.

Multiple neuroprotective agents for the treatment of spinal cord injury (SCI) have yielded encouraging results in animal studies, but disappointing results in clinical trials. These disappointing results illustrate translational difficulties in drug testing for SCI treatment. Methylprednisolone (MP) is currently the only neuroprotective agent routinely used clinically for the treatment of acute SCI. The rationale for this intervention, and the recommended dosing regimen, were derived from the National Acute Spinal Cord Injury Study (NASCIS) 2 and 3 trials (6–7). Yet the effectiveness of MP remains in dispute, due to critical subsequent analysis (8–12). Due to limitations and gaps in data from the NASCIS trials, there is no clear consensus on the success rate of the treatment (13).

Perhaps because of the lack of consensus, extensive animal studies were performed to evaluate the effectiveness of MP, both before and after the NASCIS trials, as guides for human intervention. Animal models for assessing MP for the treatment of SCI present a good test case for assessing the merits of animal-based approaches, due to the high number of animal studies and wide variety of animal models and species used. We chose MP *a priori*, because it has been the focus of more animal studies of acute SCI than any other potential neuroprotective agent. In this review, we investigated whether, and to what extent, poor methodological quality and lack of standardisation contribute to difficulties in translating animal research results to clinical use. We also investigated whether, and if so how, other factors such as inter-species and inter-strain differences might contribute to these difficulties. We attempted to address the following two questions: 1) *Are there limitations to the animal models and studies, and if so, what are they?*; and 2) *Can we reliably use the animal models and studies to inform human intervention in acute SCI?*

Methods

Study identification

The literature search was restricted to the published results of animal studies. Studies were

included if they assessed and reported the effects of MP for the treatment of acute SCI in live animals. The exclusion criteria were as follows: a) the language was other than English; b) MP was combined with other treatments, or not directly compared with placebo; c) MP was administered prior to SCI induction, or SCI was non-traumatic (ischaemic SCI); d) only outcomes other than functional recovery were assessed; and e) the study was a duplicate or review of a prior study.

Relevant articles were identified from MEDLINE (PubMed), by using the search terms "methylprednisolone OR glucocorticoid OR steroid" AND "SCI OR spinal cord trauma OR spinal cord injury OR spinal cord". In cases of uncertainty or absence of abstracts, the full articles were reviewed. Reference lists from retrieved articles and from other relevant review articles were searched for additional studies.

Data abstraction

We abstracted the following data from each included study: a) the species and strain (when provided) used; b) the method of injury; c) the dose, timing and duration of MP administration; and d) the type of anaesthesia used during injury induction. Outcome data included: a) the number of animals used for outcome assessment; b) the specific clinical outcome assessed (functional scale used); and c) the duration of any follow-up.

Outcome assessment

For each study, we identified whether beneficial effects (statistically favouring MP treatment) or no effects (statistically not favouring MP treatment) were found. When functional tests were performed at different times, only the final test was included. In cases where no statistics were provided, we reported the original authors' conclusions. In cases of uncertainty, attempts were made to contact the authors for clarification.

Subgroup assessment

During our literature search, we noted substantial variability among studies, such as the dosing and timing regimen of MP, the species and strains used, and duration of MP treatment and follow-up. We therefore prospectively identified the following subgroups for review: a) studies using the same species or strain; b) studies of $\geq$ four weeks (28 days) duration; c) studies using the same injury method (e.g. compression, contusion, or transection); and d) studies that followed a dosing regimen most similar to that currently used in humans (30mg/kg i.v. within eight hours, then 5.4mg/kg/hr for 24–48 hours total; 6, 7).

After data collection, we noted that many studies involved a single MP dose of 30mg/kg, given i.v., either within five minutes of injury or at one hour post-injury. We therefore performed a *post hoc* review, comparing all studies employing either of these regimens. We also performed a *post hoc* review of the following subgroups of studies: a) those that administered MP within one hour and used either the Basso, Beattie, Bresnahan (BBB) locomotor test, the Rivlin and Tator inclined plane test or the Tarlov open field test to assess function (since these functional tests were most consistently used in the studies); and b) those studies that reported both blinding and randomisation, and with a duration of at least four weeks.

Quality assessment

To assess methodological rigour (14–16), we also assessed the data for each study for the following: a) randomisation of intervention; b) blinding of intervention and outcome measurements; c) monitoring and reporting of physiological parameters during injury induction; and d) reporting of housing and handling procedures.

Physiological parameters were defined as pulse, temperature, blood pressure, ECG, haematocrit (Hct), blood gas (pO_2, pCO_2) and pH, and glucose level. We rated the reporting of physiological parameters on the following scale: N = information not stated; P (Poor) = one parameter reported; F (Fair) = two parameters reported; G (Good) = three or more parameters reported.

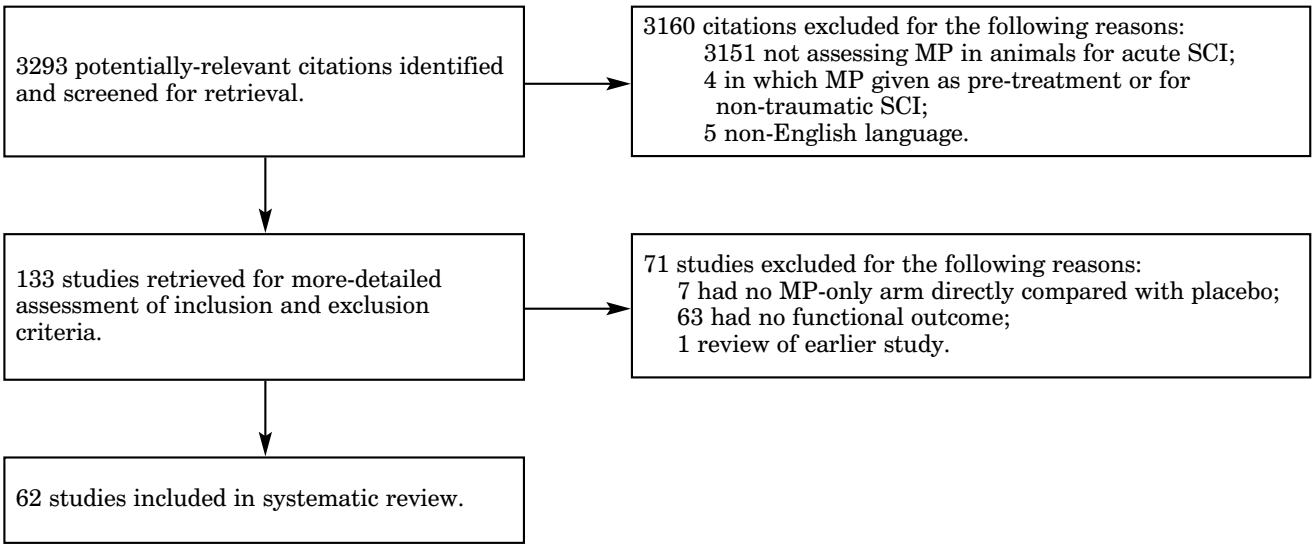
Housing and handling procedures included: number of animals per cage, water and food provisions, ambient lighting, ambient temperature, environmental enrichment, bladder care, and number and type of handlings per day. We rated the reporting of housing and handling procedures on the following scale: N = information not stated; P (Poor) = one procedure or condition reported; F (Fair) = three or more procedures or conditions reported; G (Good) = detailed information provided on three or more procedures or conditions.

Results

Description of the studies

The flowchart outlined in Figure 1 displays the search and article retrieval procedure. Sixty-two studies matched the inclusion criteria and are listed in Table 1 (17–78). A variety of methods, forces, weights, and heights were used to induce injury. SCI ranged from mild to severe, based on

Figure 1: Search and article retrieval procedure



the authors' assessments. Species and strains used were (in decreasing frequency): Sprague-Dawley rats, Wistar rats, cats, Long-Evans rats, dogs, unspecified albino rats, Fischer rats, mice, rabbits, sheep, and monkeys.

A wide range of anaesthetic agents were used. Table 1 lists types of anaesthesia and doses, where reported. Pentobarbital was commonly used at doses from 20mg/kg to 70mg/kg.

Description of assessments performed

A wide variety of scales were used to measure functional outcome, and more than one scale was used in many studies. The BBB locomotor rating scale, the Rivlin and Tator inclined plane test, and the Tarlov or modified Tarlov motor scale were used in 18, 22 and 10 studies, respectively. Functional scales that were unspecified, or were created by the authors, were used in 17 studies. Other functional evaluations included bladder function, thoracolumbar height measurement, and swimming. The follow-up duration ranged from 24 hours to 25 weeks.

The method of MP administration

The dose, timing and duration of MP administration varied widely. Initial MP doses were given at a range of time-points, from "immediately" to up to 48 hours post-injury. The doses of MP administered ranged from 8mg–300mg/kg. The duration of treatment with MP ranged from a single dose to continuous infusion or repeated doses for up to 25 weeks. The most common regimen was a single 30mg/kg dose, administered within one hour of injury (25 studies). Only two studies followed the clinical regimen of 30mg/kg within eight hours of injury, then 5.4mg/kg/hr for 24 hours (6, 7).

Quality of the studies

Tables 2 and 3 describe the quality of the studies. Blinding was reported for functional measurement in 73% of the studies, and randomisation of treatment allocation was reported in 53% (Table 2).

The reporting of physiological parameters monitored was rated as good in 10%, fair in 2%, and poor in 23% of the studies; 66% did not report any parameters (Table 3). The most commonly-reported parameter was body temperature. The reporting of housing and handling procedures was rated as good in 2%, fair in 39%, and poor in 32% of the studies; 27% did not provide any information on housing and handling. Of the 20 studies for which the numbers of animals housed per cage were reported, animals were housed individually in ten studies and in pairs in eight studies. In one study, some animals were housed individually and some in pairs, and in another study, there were four animals per cage.

Results of interventions

Twenty-one (34%) of the studies showed beneficial effects of MP administration, 36 (58%) showed no effects, and 5 (8%) revealed mixed results, depending on the dosing regimen of MP and/or the test used to assess functional outcome.

Among the studies showing a statistically-significant benefit of MP administration, the initial timing of administration ranged from "immediately

Table 1: Summary of included studies

Study (reference)	Species (treated/control [final numbers])	Method of injury (anaesthesia)	Dosing regimen of MP, and timing of dose after trauma	Assessment scales used (duration)	Outcome
Iizuka *et al.* (17)	Wistar rats (11/9)	Compression (pentobarbital 40mg/kg)	30mg/kg at 30 minutes, for 2.5 hours	Residual hindlimb motor function (7 days)	No effect
Ross *et al.* (18)	Wistar rats (6–10/6–11)	Compression (5% halothane)	30mg/kg at 5 minutes, then 5.4mg/kg/h for 8 hours, starting at 15 minutes	Rivlin/Tator inclined plane (7–8 weeks)	No effect
van de Meent *et al.* (19)	Wistar rats (9/12)	Contusion (5% halothane, 1.75% isoflurane)	30mg/kg at 30 minutes	Tarlov open field, thoracolumbar height (TLH) (24 weeks)	Mixed results: benefit for TLH
Lankhorst *et al.* (20)	Wistar rats (12/10)	Contusion (1.75% isoflurane)	30mg/kg at 30 minutes	BBB score (8 weeks)	No effect
Chikawa *et al.* (21)	Wistar rats (8/6)	Compression (pentobarbital 21mg/kg)	30mg/kg at 30 minutes	BBB score (21 days), Tarlov open field (21 days), Rivlin/Tator inclined plane (21 days), KB DBF (6 weeks), KB angle limb rotation (6 weeks), KB hindlimb stride (6 weeks)	All no effect
Taoka *et al.* (22)	Wistar rats (10/10)	Compression (pentobarbital 45mg/kg)	165mg/kg immediately, then, at 45 minutes, 31.5mg/kg/h for 23 hours	Rivlin/Tator inclined plane (56 days), KB DBF (21 days), KB hindlimb stride (21 days)	All benefit
Sharma *et al.* (23)	Wistar rats (MP1 = 10; MP2 = 10; MP3 = 10; control = 10)	Compression (not stated)	*MP1:* 30mg/kg/d, starting at 1 hour, for 3 days, then 15mg/kg/d for 2 days, then 7.5mg/kg/d for 2 days *MP2:* 30mg/kg/d, starting at 8 hours, for 3 days, then 15mg/kg/d for 2 days, then 7.5mg/kg/d for 2 days *MP3:* 30mg/kg/d, starting at 24 hours, for 3 days, then 15mg/kg/d for 2 days, then 7.5mg/kg/d for 2 days	Mean/Anderson mean recovery index (7 days)	Mixed results: benefit with MP1
Jiang *et al.* (24)	Wistar rats (12/12)	Compression (3–5% isoflurane)	60mg/kg at 15 minutes, then 30mg/kg at 2 hours, 4 hours, and 6 hours	BBB score, Rivlin/Tator inclined plane, Gruner foot orienting response, Gruner hindlimb placing response (21 days)	All benefit

Benefit = statistically beneficial result of MP vs placebo; no effect = no statistically-significant difference between MP and placebo; mixed outcome = both beneficial results and no effect of MP noted (see text for details). LE = Long-Evans; SD = Sprague-Dawley; BBB = Basso, Beattie, Bresnahan locomotor test; KB = Kunkel-Bagden/Bregnan; DBF = distance between hindfeet.

Table 1: continued

Study (reference)	Species (treated/control [final numbers])	Method of injury (anaesthesia)	Dosing regimen of MP, and timing of dose after trauma	Assessment scales used (duration)	Outcome
Kalayci *et al.* (25)	Wistar rats (6/12)	Compression (pentobarbital 20mg/kg)	30mg/kg immediately	Rivlin/Tator inclined plane + reaction to painful stimulus (24 hours)	No effect
Weaver *et al.* (26)	Wistar rats (motor only group = 6; pain/ motor group = 7, control = 13)	Compression (4% halothane)	30mg/kg at 2 hours, then 15mg/kg at 24 hours and 48 hours	Mechanical allodynia (10 and 12 weeks), BBB score (6 and 12 weeks)	All no effect
Gül *et al.* (27)	Wistar rats (10/20)	Compression (pentobarbital 20mg/kg)	30mg/kg "after trauma"	Rivlin/Tator inclined plane + reaction to painful stimulus (24 hours)	Benefit
Boran *et al.* (28)	Wistar rats (20/20)	Contusion (ketamine 60mg/kg, fentanyl 0.05mg/kg)	30mg/kg within 60 minutes	Swimming (14 days)	No effect
Kaptanoglu *et al.* (29)	Wistar rats (8/8)	Contusion (xylazine 10mg/kg, ketamine 60mg/kg)	30mg/kg immediately	Rivlin/Tator inclined plane (24 hours)	No effect
Kanter *et al.* (30)	Wistar rats (6/6)	Compression (pentobarbital 20mg/kg)	30mg/kg immediately	Rivlin/Tator inclined plane + reaction to painful stimulus (24 hours)	No effect
Yucel *et al.* (31)	Wistar rats (6/6)	Contusion (xylazine 10mg/kg, ketamine 50mg/kg)	30mg/kg immediately	Modified Gale motor function score (6 weeks), Rivlin/Tator inclined plane (6 weeks)	All benefit
Ates *et al.* (32)	Wistar rats (9/9)	Contusion (xylazine 10mg/kg, ketamine 50mg/kg)	30mg/kg immediately	Modified Gale motor function score (6 weeks), Rivlin/Tator inclined plane (6 weeks)	All benefit
Gok *et al.* (33)	Wistar rats (7/7)	Contusion (xylazine 10mg/kg, ketamine 60mg/kg)	30mg/kg immediately	Rivlin/Tator inclined plane (24 hours)	No effect
Okutan *et al.* (34)	Wistar rats (8/8)	Contusion (xylazine 10mg/kg, ketamine 60mg/kg)	30mg/kg immediately	BBB score (24 hours)	No effect

Benefit = statistically beneficial result of MP vs placebo; no effect = no statistically-significant difference between MP and placebo; mixed outcome = both beneficial results and no effect of MP noted (see text for details). LE = Long-Evans; SD = Sprague-Dawley; BBB = Basso, Beattie, Bresnahan locomotor test; KB = Kunkel-Bagden/Bregnan; DBF = distance between hindfeet.

Table 1: continued

Study (reference)	Species (treated/control [final numbers])	Method of injury (anaesthesia)	Dosing regimen of MP, and timing of dose after trauma	Assessment scales used (duration)	Outcome
O'Callaghan & Speakman (35)	SD rats (75/75)	Transection (not stated)	8mg "at operation", then every 2 weeks, up to 25 weeks	Motor/sensory assessment (up to 25 weeks)	No effect
Holtz *et al.* (36)	SD rats (20/10)	Compression (4.25% chloral hydrate, 1% pentobarbital, 3% halothane)	30mg/kg at 60 minutes	Rivlin/Tator inclined plane (4 days)	Benefit
Benzel *et al.* (37)	SD rats (MP1 = 11; MP2 = 10; MP3 = 12; MP4 = 10; control = 11)	Compression (halothane, ketamine 50mg/kg)	*MP1:* 30mg/kg at 30 minutes and 120 minutes, then daily for 9 days *MP2:* 15mg/kg at 30 minutes and 120 minutes *MP3:* 30mg/kg at 30 minutes, then 15mg/kg at 120 minutes *MP4:* 60mg/kg at 30 minutes	Rivlin/Tator inclined plane (1 month)	All no effect
Holtz & Gerdin (38)	SD rats (8/8)	Compression (1% pentobarbital, 4.25% chloral hydrate)	30mg/kg at 1 hour	Rivlin/Tator inclined plane (4 days)	No effect
Iwai *et al.* (39)	SD rats (MP1 = 12; MP2 = 16; control = 28)	Thermal injury (1.5% fluothane)	*MP1:* 30mg/kg at 2 hours, then at 3 hours. 5.4mg/kg/h for 24 hours *MP2:* 30mg/kg at 2 hours, then 16.2mg/kg at 3 hours, then 5.4mg/kg/h for 3 hours	Modified LeMay neurological impairment score (5 hours and 23 hours), modified Gale corrected combined behavioural score (5 hours and 23 hours)	All no effect
Behrmann *et al.* (40)	SD rats (total MP groups = 104; control = 39)	Contusion (ketamine 80mg/kg, xylazine 10mg/kg)	Numerous doses and regimens: includes 30mg/kg × 4 doses (at 5 minutes, 2 hours, 4 hours, and 6 hours); 45mg/kg × 4 doses; 60mg/kg × 4 doses; 90mg/kg × 4 doses; 120mg/kg × 4 doses; 300mg/kg × 4 doses; 30mg/kg at 5 minutes, then 15mg/kg at 2 hours, 4 hours, and 6 hours	Tarlov open field (29 days and 43 days), Rivlin/Tator inclined plane (29 days and 43 days), grid walking (29 days and 43 days), KB stride length (43 days), toe spread (43 days), KB base of support (43 days), KB number of footprints/ft walked (43 days)	Mixed results: benefit with MP regimens of 30mg/kg × 4 doses and 60mg/kg × 4 doses (inclined plane, Tarlov); and with 30mg/kg at 5 minutes, then 15mg/kg at 2 hours, 4 hours, and 6 hours (Tarlov); and with 60mg/kg × 1 dose, then 30mg/kg ×3 doses (inclined plane)

Benefit = statistically beneficial result of MP vs placebo; no effect = no statistically-significant difference between MP and placebo; mixed outcome = both beneficial results and no effect of MP noted (see text for details). LE = Long-Evans; SD = Sprague-Dawley; BBB = Basso, Beattie, Bresnahan locomotor test; KB = Kunkel-Bagden/Bregnan; DBF = distance between hindfeet.

Table 1: continued

Study (reference)	Species (treated/control [final numbers])	Method of injury (anaesthesia)	Dosing regimen of MP, and timing of dose after trauma	Assessment scales used (duration)	Outcome
Farooque *et al.* (41)	SD rats (6?/6?)	Compression (fluanisone, midazolam)	30mg/kg at 60 minutes	Rivlin/Tator inclined plane (21 days)	No effect
Perez-Espejo *et al.* (42)	SD rats (10/9)	Compression (4% halothane)	30mg/kg "after compression"	Days to micturition recovery, Rivlin/Tator inclined plane (28 days), modified Tarlov open field (28 days), Gale toe spread (28 days)	Mixed results: benefit for Tarlov and for inclined plane
Haghighi *et al.* (43)	SD rats (8/8)	Compression (4% halothane)	30mg/kg immediately	Rivlin/Tator inclined plane, Tarlov open field, Gale toe spread (all 49 days)	All no effect
Haghighi *et al.* (44)	SD rats (10/10)	Compression (4% isoflurane)	30mg/kg immediately, then at 2, 3, 4, 5, 7, and 14 days	Rivlin/Tator inclined plane, modified Tarlov open field, Gale toe spread (all 56 days)	All no effect
Hara *et al.* (45)	SD rats (7/7)	Compression (pentobarbital 50mg/kg)	30mg/kg at 5 minutes, 2 hours, 4 hours, and 6 hours	Inclined plane, swimming, open field, toe spread, contact place response, withdrawal reflex, righting reflex, modified Gale combined behavioural score (all 4 weeks)	All no effect
Legos *et al.* (46)	SD rats (3/5)	Compression (1–2% isoflurane)	30mg/kg at 1 minute	BBB score (28 days), days to micturition recovery	All no effect
Nash *et al.* (47)	SD rats (10/10)	Transection (5% isoflurane)	30mg/kg at 1 hour, then every 6 hours × 4 doses	Directed forepaw reaching score (6 weeks)	Benefit
Rabchevsky *et al.* (48)	SD rats (6/6)	Contusion (ketamine 80mg/kg, xylazine 10mg/kg)	60mg/kg at 5 minutes, then 5.4mg/kg/hr for 24 hours	BBB score (6 weeks)	No effect
Lixin *et al.* (49)	SD rats (4/3)	Contusion (ketamine 60mg/kg, xylazine 10mg/kg)	60mg/kg immediately, then 30mg/kg at 4 hours	BBB score (10 weeks)	No effect
Yu *et al.* (50)	SD rats (?/?)	Contusion (pentobarbital 50mg/kg)	*MP1:* 30mg/kg at 5 minutes *MP2:* 30mg/kg at 8 hours *MP3:* 30mg/kg at 5 minutes, 2 hours, 4 hours, and 6 hours	Incidence of bladder disability (1 day), BBB score ("acute" period)	Mixed results: benefit with MP1 & MP3 (bladder function)

Benefit = statistically beneficial result of MP vs placebo; no effect = no statistically-significant difference between MP and placebo; mixed outcome = both beneficial results and no effect of MP noted (see text for details). LE = Long-Evans; SD = Sprague-Dawley; BBB = Basso, Beattie, Bresnahan locomotor test; KB = Kunkel-Bagden/Bregnan; DBF = distance between hindfeet.

Table 1: continued

Study (reference)	Species (treated/control [final numbers])	Method of injury (anaesthesia)	Dosing regimen of MP, and timing of dose after trauma	Assessment scales used (duration)	Outcome
Kim & Jahng (51)	SD rats (5/4)	Contusion (ketamine 60mg/kg, xylazine 10mg/kg)	60mg/kg immediately, then 30mg/kg at 4 hours	BBB score (10 weeks)	No effect
Gorio *et al.* (52)	SD rats (18/18)	Contusion (halothane)	30mg/kg at 30 minutes	BBB score (28 days)	No effect
Lee *et al.* (53)	SD rats (6/8)	Contusion (pentobarbital 50–70mg/kg)	30mg/kg at 15 minutes	BBB score (8 weeks)	Benefit
Lopez-Vales *et al.* (54)	SD rats (18/18)	Photochemical injury (pentobarbital 40mg/kg)	30mg/kg at 30 minutes	BBB score, Rivlin/Tator inclined plane (14 days)	All no effect
Cetin *et al.* (55)	SD rats (8/8)	Compression (thiopental sodium 40mg/kg)	30mg/kg at 5 minutes, then 5.4mg/kg/hr for 8 hours	Swimming test (48 hours)	Benefit
Zileli *et al.* (56)	Albino rats (10/10)	Contusion (pentobarbital 30mg/kg)	15mg/kg at 45 minutes	Lower extremity motor function (14 days)	No effect
Cayli *et al.* (57)	Albino rats (18/18)	Contusion (chloral hydrate 400mg/kg)	30mg/kg immediately	Modified Gale motor function score, Rivlin/Tator inclined plane (10 days)	All benefit
Cayli *et al.* (58)	Albino rats (12/12)	Contusion (chloral hydrate 400mg/kg)	30mg/kg immediately	Modified Gale motor function score, Rivlin/Tator inclined plane (6 weeks)	All benefit
Takami *et al.* (59)	Fischer rats (8/9)	Contusion (1–2% halothane)	30mg/kg at 5 minutes, 2 hours, and 4 hours	BBB score (10 weeks)	No effect
Guizar-Sahagun *et al.* (60)	Fischer rats (6/12)	Contusion (ketamine 77.5mg/kg, xylazine 12.5mg/kg)	30mg/kg immediately, at 2 hours and 4 hours, then 60mg/kg at 8 hours and 24 hours	Modified Tarlov open field (8 weeks)	No effect
Mu *et al.* (61)	LE rats (9/9)	Contusion (pentobarbital 40mg/kg)	30mg/kg at 2 hours and 4 hours	BBB score (6 weeks)	No effect
Ji *et al.* (62)	LE rats (8/8)	Transection (midazolam 2.5mg/kg, 2–3% fluothane)	30mg/kg immediately, then at 4 hours and 8 hours	BBB score, sub-BBB score (4 weeks)	All benefit

Benefit = statistically beneficial result of MP vs placebo; no effect = no statistically-significant difference between MP and placebo; mixed outcome = both beneficial results and no effect of MP noted (see text for details). LE = Long-Evans; SD = Sprague-Dawley; BBB = Basso, Beattie, Bresnahan locomotor test; KB = Kunkel-Bagden/Bregnan; DBF = distance between hindfeet.

Table 1: continued

Study (reference)	Species (treated/control [final numbers])	Method of injury (anaesthesia)	Dosing regimen of MP, and timing of dose after trauma	Assessment scales used (duration)	Outcome
Guizar-Sahagun *et al.* (63)	LE rats (8/8)	Contusion (ketamine 80mg/kg, xylazine 8mg/kg)	30mg/kg at 20 minutes, 4 hours, 10 hours, and 20 hours	BBB score (8 weeks)	No effect
Wells *et al.* (64)	CD-1 mice (10/20)	Compression (ketamine 200mg/kg, xylazine 10mg/kg)	30mg/kg at 1 hour, then every 6 hours for 24 hours	BBB score (28 days)	No effect
Sheng *et al.* (65)	C57BL/6J mice (25/25)	Compression (5% halothane)	30mg/kg at 5 minutes, then 5.4mg/kg/h for 24 hours	Fixed rotarod, accelerated rotarod, screen grasping (21 days)	All no effect
Anghelescu *et al.* (66)	Rabbits (40?/20?)	Contusion (ketamine 50mg/kg)	15mg/kg at 30 minutes, 120 minutes, 24 hours, and 48 hours	Posterior limb movements (14 days)	Benefit
Okonkwo *et al.* (67)	New Zealand White rabbits (10/10)	Contusion via 10g from 6cm at mid-thoracic (ketamine, xylazine, halothane)	30mg/kg at 10 minutes	Tarlov open field (48 hours)	No effect
Means *et al.* (68)	Cats (16/8)	Compression (pentobarbital 30mg/kg, succinylcholine 1mg/kg)	15mg/kg/d for 3 days, starting at 1 hour, then 7.5mg/kg/d for 3 days, then 3.75mg/kg/d for 3 days	Recovery index (8 weeks)	Benefit
Demopoulos *et al.* (69)	Cats (10/10)	Contusion (pentobarbital 25mg/kg)	15mg/kg at 45 minutes and at 5 hours, then 15mg/kg twice daily, for 7 days	Functional outcome (14 weeks)	Benefit
Faden *et al.* (70)	Cats (6/8)	Contusion (pentobarbital 25mg/kg, gallamine triethiodide 4mg/kg/h)	22.5mg/kg × 2 doses on day 1, beginning at 1 hour, then tapered over 9 days	Tarlov open field (6 weeks)	No effect
Anderson *et al.* (71)	Cats (10/7)	Compression (pentobarbital 30mg/kg, succinylcholine 1mg/kg)	30mg/kg at 30 minutes, then 15mg/kg at 2 hours and 6 hours, then 15mg/kg every 6 hours, for 42 hours	Recovery index (4 weeks)	Benefit

Benefit = statistically beneficial result of MP vs placebo; no effect = no statistically-significant difference between MP and placebo; mixed outcome = both beneficial results and no effect of MP noted (see text for details). LE = Long-Evans; SD = Sprague-Dawley; BBB = Basso, Beattie, Bresnahan locomotor test; KB = Kunkel-Bagden/Bregnan; DBF = distance between hindfeet.

Table 1: continued

Study (reference)	Species (treated/control [final numbers])	Method of injury (anaesthesia)	Dosing regimen of MP, and timing of dose after trauma	Assessment scales used (duration)	Outcome
Braughler *et al.* (72)	Cats (10/7)	Compression (pentobarbital 30mg/kg, succinylcholine 1mg/kg)	30mg/kg at 30 minutes, then 15mg/kg at 2 hours and 6 hours, then 2.5mg/kg/h for 42 hours	Recovery index (4 weeks)	Benefit
Young *et al.* (73)	Cats (14/20)	Contusion (pentobarbital 25mg/kg)	15mg/kg at 45 minutes	Locomotor recovery (6 weeks)	Benefit
Ducker & Hamit (74)	Dogs (12/12)	Contusion (pentobarbital 30mg/kg)	8mg/kg at 3 hours	Modified Tarlov open field (6 weeks)	Benefit
Coates *et al.* (75)	Dogs (4/10)	Compression (1.5% isoflurane, thiopental 15mg/kg, atropine 0.4mg/kg)	30mg/kg at 30 minutes, then 15mg/kg at 2 hours and 6 hours, then 10mg/kg every 4 hours, for 42 hours	Functional classification score (21 days)	No effect
Yang *et al.* (76)	Dogs (5/5)	Compression (thiopental 15mg/kg, isoflurane)	30mg/kg, starting at 48 hours, every 6 hours × 6 doses, then 2mg/kg oral prednisolone, twice daily until tapered	Recovery of conscious proprioception, Recovery of extensor postural thrust (1 month)	All benefit
Yeo *et al.* (77)	Sheep (11/15)	Contusion (not stated)	40mg within 1 hour	Motor/sensory assessment (13 weeks)	No effect
Green *et al.* (78)	Monkeys (MP1 = 5; MP2 = 5; control = 5)	Contusion (pentobarbital)	*MP1*: 30mg/kg at 1 hour, then at 7 hours, 15mg/kg every 6 hours × 4 doses, then 7.5mg/kg every 6 hours × 4 doses, then 3.5mg/kg every 6 hours × 4 doses, then 1.5mg/kg evey 6 hours × 4 doses *MP2*: 30mg/kg at 5 hours, then as for MP1	Motor function score (12 weeks)	All benefit

Benefit = statistically beneficial result of MP vs placebo; no effect = no statistically-significant difference between MP and placebo; mixed outcome = both beneficial results and no effect of MP noted (see text for details). LE = Long-Evans; SD = Sprague-Dawley; BBB = Basso, Beattie, Bresnahan locomotor test; KB = Kunkel-Bagden/Bregnan; DBF = distance between hindfeet.

Table 2: The quality of the studies: blinding and randomisation

Study (reference)	B	R
Iizuka *et al.* (17)	N	N
Ross *et al.* (18)	Y	N
van de Meent *et al.* (19)	Y	Y
Lankhorst *et al.* (20)	Y	Y
Chikawa *et al.* (21)	Y	N
Taoka *et al.* (22)	Y	N
Sharma *et al.* (23)	N	N
Jiang *et al.* (24)	Y	Y
Kalayci *et al.* (25)	N	N
Weaver *et al.* (26)	Y	N
Gül *et al.* (27)	N	N
Boran *et al.* (28)	Y	Y
Kaptanoglu *et al.* (29)	Y	Y
Kanter *et al.* (30)	N	N
Yucel *et al.* (31)	Y	Y
Ates *et al.* (32)	Y	Y
Gok *et al.* (33)	Y	Y
Okutan *et al.* (34)	Y	Y
O'Callaghan & Speakman (35)	N	N
Holtz *et al.* (36)	N	N
Benzel *et al.* (37)	Y	Y
Holtz & Gerdin (38)	N	N
Iwai *et al.* (39)	Y	N
Behrmann *et al.* (40)	Y	Y
Farooque *et al.* (41)	N	N
Perez-Espejo *et al.* (42)	Y	Y
Haghighi *et al.* (43)	Y	Y
Haghighi *et al.* (44)	Y	Y
Hara *et al.* (45)	Y	Y
Legos *et al.* (46)	Y	Y
Nash *et al.* (47)	Y	Y
Rabchevsky *et al.* (48)	Y	Y
Lixin *et al.* (49)	Y	Y
Yu *et al.* (50)	Y	N
Kim & Jahng (51)	Y	N
Gorio *et al.* (52)	Y	N
Lee *et al.* (53)	N	Y
Lopez-Vales *et al.* (54)	Y	N
Cetin *et al.* (55)	N	Y
Zileli *et al.* (56)	N	N
Cayli *et al.* (57)	N	Y
Cayli *et al.* (58)	Y	Y
Takami *et al.* (59)	Y	N
Guizar-Sahagun *et al.* (60)	Y	Y
Mu *et al.* (61)	Y	Y
Ji *et al.* (62)	Y	N
Guizar-Sahagun *et al.* (63)	Y	Y
Wells *et al.* (64)	N	N
Sheng *et al.* (65)	Y	Y
Anghelescu *et al.* (66)	N	N
Okonkwo *et al.* (67)	Y	Y
Means *et al.* (68)	Y	N
Demopoulos *et al.* (69)	Y	N
Faden *et al.* (70)	Y	N
Anderson *et al.* (71)	N	N
Braughler *et al.* (72)	Y	Y
Young *et al.* (73)	Y	Y
Ducker & Hamit (74)	Y	Y
Coates *et al.* (75)	Y	N
Yang *et al.* (76)	N	Y
Yeo *et al.* (77)	Y	N
Green *et al.* (78)	Y	Y
Total number reporting	**45**	**33**
(%)	(73)	(53)

B = reporting of blinding; R = reporting of randomisation.

after" injury up to 48 hours post-injury, with doses ranging from 8–165mg/kg (Table 1). Among the studies showing no effects, the initial timing of MP administration ranged from "immediately after" injury up to 24 hours post-injury, with doses ranging from 8mg–300mg/kg.

Benefits were obtained both with a single dose of MP, administered within five minutes of injury, and with repeated doses administered for up to nine days. Results showing no effects were also obtained both with a single dose of MP, administered within five minutes, and with repeated doses administered for up to 25 weeks.

Table 4 classifies the results of the studies by species and strain. Overall, there were more studies showing benefits of MP treatment in cats (five of six studies) and dogs (two of three studies). Both of the mouse studies which were assessed showed no effects of MP administration. The results in rats varied between and within strains, although there were, overall, more studies showing no effects (30 of 47 studies) than beneficial effects. Five studies in rats showed mixed results. One rabbit study found beneficial effects of MP treatment, and the other found no effects. The single study on monkeys showed that MP treatment was beneficial,

and the single study on sheep found no effects. Table 5 classifies the results of the studies by injury method. The results varied within and between the different injury methods.

In an effort to detect any patterns in the results, we further narrowed the criteria of the analysed studies. We looked at the studies in which MP was administered as a single 30mg/kg dose, within one hour of injury. With this common MP dosing regimen, seven out of these 25 studies (28%) found beneficial effects, 15 (60%) showed no effects, and three (12%) showed mixed results.

Among these 25 common MP dosing regimen studies, one of the eight which assessed BBB scores (13%) found beneficial effects, and seven of these eight (88%) showed no effects. Seven of the 16 which performed Rivlin and Tator inclined plane tests (44%) found beneficial effects, and nine of these 16 (56%) found no effects. One of the five which used the Tarlov or modified Tarlov open-

Table 3: The quality of the studies: Reporting of housing and handling procedures, and monitoring of physiological parameters

Study (reference)	H/H (no. per cage)	PM
Iizuka *et al.* (17)	N	N
Ross *et al.* (18)	F (1)	G
van de Meent *et al.* (19)	P	P
Lankhorst *et al.* (20)	F (2)	N
Chikawa *et al.* (21)	P	N
Taoka *et al.* (22)	N	N
Sharma *et al.* (23)	N	N
Jiang *et al.* (24)	F (2)	N
Kalayci *et al.* (25)	F (1)	N
Weaver *et al.* (26)	P (1 & 2)	N
Gül *et al.* (27)	F (1)	N
Boran *et al.* (28)	P	N
Kaptanoglu *et al.* (29)	N	N
Kanter *et al.* (30)	G (1)	N
Yucel *et al.* (31)	P	N
Ates *et al.* (32)	F	N
Gok *et al.* (33)	N	N
Okutan *et al.* (34)	N	N
O'Callaghan & Speakman (35)	N	N
Holtz *et al.* (36)	P	G
Benzel *et al.* (37)	N	N
Holtz & Gerdin (38)	P	G
Iwai *et al.* (39)	P (1)	P
Behrmann *et al.* (40)	F (2)	P
Farooque *et al.* (41)	F (1)	G
Perez-Espejo *et al.* (42)	P	P
Haghighi *et al.* (43)	F (1)	P
Haghighi *et al.* (44)	F (1)	P
Hara *et al.* (45)	F (2)	G
Legos *et al.* (46)	F (2)	P
Nash *et al.* (47)	P	N
Rabchevsky *et al.* (48)	P (2)	N
Lixin *et al.* (49)	P	N
Yu *et al.* (50)	N	N
Kim & Jahng (51)	P (2)	N
Gorio *et al.* (52)	F (2)	P
Lee *et al.* (53)	F	P
Lopez-Vales *et al.* (54)	N	N
Cetin *et al.* (55)	F	N
Zileli *et al.* (56)	N	N
Cayli *et al.* (57)	P	N
Cayli *et al.* (58)	F (4)	N
Takami *et al.* (59)	F	P
Guizar-Sahagun *et al.* (60)	F (1)	N
Mu *et al.* (61)	P	N
Ji *et al.* (62)	P	N
Guizar-Sahagun *et al.* (63)	F (1)	P
Wells *et al.* (64)	F	N
Sheng *et al.* (65)	N	P
Anghelescu *et al.* (66)	N	N
Okonkwo *et al.* (67)	P	N
Means *et al.* (68)	N	P
Demopoulos *et al.* (69)	N	N
Faden *et al.* (70)	N	F
Anderson *et al.* (71)	N	N
Braughler *et al.* (72)	P	N
Young *et al.* (73)	F	N
Ducker & Hamit (74)	F	N
Coates *et al.* (75)	P	G
Yang *et al.* (76)	P	N
Yeo *et al.* (77)	F	P
Green *et al.* (78)	F	N
Total ratings: as number (%)*		
No information	17 (27)	41 (66)
Poor	20 (32)	14 (23)
Fair	24 (39)	1 (2)
Good	1 (2)	6 (10)

*H/H = reporting of housing and handling procedures; PM = monitoring of physiological parameters; P = poor; F = fair; G = good (see text for definitions); N = no information. *Total may not equal 100% due to rounding.*

Table 4: Results of the studies by species/strain

Species/ strain	No. of studies	Benefit n (%)	No effect n (%)	Mixed results n (%)
Wistar rat	18	5 (28)	11 (61)	2 (11)
SD rat	21	4 (19)	14 (67)	3 (14)
Albino rat	3	2 (67)	1 (33)	0 (0)
Fischer rat	2	0 (0)	2 (100)	0 (0)
LE rat	3	1 (33)	2 (67)	0 (0)
Mouse	2	0 (0)	2 (100)	0 (0)
Rabbit	2	1 (50)	1 (50)	0 (0)
Cat	6	5 (83)	1 (17)	0 (0)
Dog	3	2 (67)	1 (33)	0 (0)
Sheep	1	0 (0)	1 (100)	0 (0)
Monkey	1	1 (100)	0 (0)	0 (0)

SD = Sprague-Dawley; LE = Long-Evans.

field tests (20%) found beneficial effects, and four of these five (80%) found no effects.

The results also varied among those studies giving a single dose of 30mg/kg MP "immediately after" injury, or within five minutes. Five of these 14 (36%) studies found beneficial effects, seven (50%) showed no effects, and two (14%) showed mixed results. Among the 39 studies assessing functional outcome for at least 28 days, 15 (38%) found beneficial effects, 21 (54%) showed no effects, and three (8%) showed mixed results. Among the 22 studies reporting both randomisation and blinding, and with durations of at least 28 days, eight (36%) found beneficial effects, 11 (50%) showed no effects, and three (14%) showed mixed results. The two studies following the current standard clinical dose and regimen of MP (6, 7) showed no effects.

Discussion

Variability in the results

The most conspicuous pattern to emerge from our analysis is the pronounced variability in the animal study results, and thus the inability of these results to predict human clinical outcomes. In rodents (a total of 47 studies), more studies showed no effect than showed overall benefit, but studies on cats (six studies), dogs (three studies) and monkeys (one study) revealed more benefit than non-benefit from MP treatment.

Results showing both benefit and non-benefit were found with the same initial dose and timing of MP administration, with the same functional assessment scale, and with the same duration of follow-up. However, our study was not a meta-analysis. Disparate intervention regimens, durations, and outcome assessments, and the absence of detailed statistical reporting in many studies, preclude the pooling of results.

Variability in study design and implementation

What factors might lead to such variability in results? Inter-laboratory and intra-laboratory differences in study design and quality could be involved. Only slightly greater than half of the studies reported randomisation of intervention, which, even if implemented, may not have the standardisation benefits intended in human studies. Many studies lacked any blinding of functional evaluation, which can also affect outcomes. Very few studies reported physiological parameters, such as blood pressure, heart rate and blood gases, during injury induction. These parameters may play a role in spinal cord lesion volume (79).

The anaesthesia used during injury induction varied widely in both type and dose. Anaesthetic agents may affect the response to SCI by altering physiological parameters and having intrinsic neuroprotective effects (79). In Sprague-Dawley rats, for example, sevoflurane provided protection against cerebral ischaemia (80), and bupivacaine protected against extended spinal cord lesions after injury (81). The studies also varied with respect to the functional scale used and the duration of follow-up. Variability in any one of these parameters could cause differences in outcomes.

Differences in housing and handling procedures can also affect study outcomes. Animal housing conditions, and handling by personnel, can affect metabolic parameters such as cortisone secretion, cholesterol level, heart rate and blood pressure, all of which may induce neurological and other changes and thus alter physical function (14, 82–84). Enriched housing stimulates neurogenesis, changes in synaptic signalling, and altered gene expression, across a variety of species and strains (14).

Table 5: Results of the studies by injury

Injury method	No. of studies	Benefit n (%)	No effect n (%)	Mixed results n (%)
Compression*	27	9 (33)	16 (59)	2 (7)
Contusion	30	10 (33)	17 (57)	3 (10)
Transection*	3	2 (67)	1 (33)	0 (0)
Other	2	0 (0)	2 (100)	0 (0)

**Total not equal to 100% due to rounding.*

Routine procedures and environmental conditions, such as cage size, cage movement, handling, and type of cage ventilation, lead to significant, lasting changes in physiological parameters and behaviour (83, 85, 86). Animals caged in groups and/or enriched environments tend to score differently in functional assessments than animals housed individually and in standard cages (82). Enriched housing improves function in animals after SCI or other central nervous system lesions (87–89), and can induce significant changes in physiology, behaviour, and anatomical development (85). Changes in physiological parameters as a result of housing and handling procedures, can also be species-specific or strain-specific (90). As demonstrated by this review, the reporting of these procedures is grossly deficient, which hampers any cross-study comparisons.

Animal research is intended, in part, to inform research into human conditions and their interventions, particularly when the animal research aims to address potential human responses to treatments. The variability in animal research results renders the prediction of human outcomes problematic. Due to pronounced inter-laboratory and intra-laboratory variations in injury method, species, anaesthesia, duration, functional outcome tests, and housing and handling procedures, there have been calls for standardisation in SCI research protocols (91–93).

This is probably not an attainable goal. For example, the designers of the New York University (NYU) impactor model mandated certain conditions in experimental procedures in an attempt to standardise SCI. Yet, as Kwon *et al.* (94) state, "in practice… it would appear that not all these stringent conditions (e.g. anaesthetic doses, rat strain) are adhered to by every centre that possesses an NYU impactor". In addition to the injury procedures, there might simply be too many variables to achieve putative standardisation. Many of these variables are intrinsic to the studies themselves, and thus are unavoidable. In addition, animals might still react differently to the same controlled conditions. In terms of mouse behaviour, at least, standardisation has proved elusive (95, 96).

Inter-species and inter-strain differences

Even if the standardisation of all study design variables and procedures could be accomplished, would there still be translational difficulties for pharmacological studies in SCI? While we were not able to control for all the factors, we found that, even when many of these factors were controlled for, variability in results remained. This suggests that: a) the uncontrolled factors (such as housing and handling procedures) account for significant variation in results; and/or b) other factors inherent to the animal models themselves might account for some of the variability in results.

Differences in spinal cord neuroanatomy, physiology and reaction to injury, both among and within species and strains, could further hamper extrapolation to humans. For example, in response to acute SCI, mice develop a significant connective tissue matrix and minimal central cavities, in comparison to rats (97). Astrocytic response, lesion size, and neurofilament crossing into the lesion site, all differ substantially among hamsters, mice, and rats (98). Dogs, guinea-pigs, and rats have increased collateralisation of spinal cord blood vessels compared to cats and rabbits (99). Both qualitative and quantitative differences in inflammatory response, neurodegeneration, and other pathological features of secondary injury and wound healing mechanisms, vary among different strains of mice and rats (100–102).

All these inherent and immutable physiological differences make translation of acute SCI animal research results to humans problematic. As a practical matter, these differences also suggest that much more than animal model selection, study design and performance criteria, stand between these animal results and human applications (103).

Limitations of systematic reviews

Systematic reviews are vulnerable to various forms of bias. Our search was limited to published studies referenced in an electronic database; there may also be relevant unpublished studies. If such a publication bias against negative animal studies exists, then it is possible that our review would demonstrate more studies showing no effect, if unpublished studies were included. In addition, the quality of this review is inherently influenced by the quality of each individual study. Possible bias in individual studies, due to lack of randomisation or blinding of assessment outcome, will be reflected in this review. We tried to minimise these biases by performing a subgroup analysis of only those studies which reported both randomisation and blinding.

Alternative approaches to the understanding of human SCI

Currently, most of the pre-clinical work in SCI involves animal models. The barriers to successful translation of animal SCI studies to human clinical relevance underscore the need to develop and use more validated human-based testing methods. It is troubling that there is a dearth of research to further the understanding of the pathology of human spinal cord injury in comparison to that in other animals (104). The use of human cadavers,

imaging techniques and electrodiagnostic studies, to unravel the pathophysiological changes after human spinal cord injury, need to be given greater priority in SCI research.

Many *in vitro* and *in silico* models have been developed, examples of which include: a model of the glial scar that develops after spinal cord injury (105); an *in vitro* model of axonal injury (106); and an *in silico* model of axonal injury and repair (107). These models provide the opportunity to define the mechanisms and intracellular signalling pathways associated with SCI and SCI treatment. Many of these *in vitro* models simulate the various forms of SCI. One model system involves the use of a laser to simulate transection injury (108, 109). By using this model, a defined physiological process in a single cell can be determined at a precise distance from the cell body and transected. Other *in vitro* models include the simulation of contusion injury by the dropping of weights onto cultured spinal tissue (110), and the deformation of cultured neural cells by the manipulation of an adherent silicone plate, to simulate stretch injury (111). These and other *in vitro* models have been particularly instrumental in increasing understanding of genetic, biochemical and pathological responses to spinal cord injury and for the testing of neuroprotective agents. For example: a transection laser model revealed the depolarisation of neuronal cell bodies after injury, and also the factors that affected this depolarisation (108); in a study of dendrites that were transected from spinal cells via laser cell surgery, researchers noted a relation between neuronal death and increases in *jun* gene expression (109); and research with an *in vitro* mechanical injury model showed that lazaroids and other inhibitors of lipid peroxidation inhibited cell death (112).

Many of these techniques involve the use of animal, rather than human, neural cells and tissues. Thus, inter-species barriers to extrapolation are likely to be factors which limit their effectiveness. In contrast, in 2000, researchers developed a technique to immortalise human motoneurons and, in 1999, immortalised human cells that differentiate into sensory neurons with nociceptive properties were produced (113, 114). Researchers at the University of Miami are collaborating on the Human Spinal Cord Injury Model Project, which correlates a patient's neurological function with neurophysiological status, imaging studies and histopathology (115).

While they show great promise as tools to understand human SCI and to test novel neuroprotective agents, these models are still in their early stages of development and validation. Thus, their effectiveness cannot be evaluated at this time. However, potential limitations to some or all of the above methods include: a) a lack of correlation between the cellular model and human *in vivo* neurophysiology; b) unpredictable transformation of cell lines, to allow *in vitro* growth; c) a lack of active metabolites or metabolic activation, required for some of the investigational drugs; and d) an insufficient modelling of the spinal microenvironment in *in vitro* models, particularly with respect to vasculature. The use of a combination of human-specific models, such as various *in vitro* models, imaging studies, histopathology studies, and *in silico* models, may provide the most complete understanding of human SCI.

Conclusion

This review demonstrates the barriers to the prediction of the effectiveness of MP treatment in human SCI based on animal studies. The effectiveness of MP treatment differed, both among and within species. There are several possible explanations for these differences, which include variability in study design and poor methodological quality. However, the complete elimination of these variables is unlikely, and even if it were achieved, it would not fully address the demonstrated inconsistency of results within subgroups with similar study design and improved methodology. This suggests that other immutable factors, such as inter-species and inter-strain differences in neurophysiology and in responses to injury and treatment, account for some of the discrepancies.

Preclinical studies are used to inform clinical trials. It is unlikely that the animal experimental data will be further used to inform clinical use of MP. However, the animal studies of novel neuroprotective agents still involved in pre-clinical testing, will be used to inform clinical trials. This analysis of MP, used as a test case, illustrates the numerous barriers to reliably using the animal studies to inform human intervention in SCI. The results of these animal tests may theoretically be more reliable, if the testing is focused only on relevant and validated model(s), where known human parameters were included in the validation process. However, there is no such model, nor is it likely that any such a model could be developed, due to inter-species and intra-species differences. Therefore, research emphasis should be on the development and use of validated human-based methods.

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