BMJ Open

SCISSOR—Spinal Cord Injury Study on Small molecule-derived Rho inhibition: a clinical study protocol

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To cite: Kopp MA, Liebscher T, Watzlawick R, *et al*. SCISSOR—Spinal Cord Injury Study on Small molecule-derived Rho inhibition: a clinical study protocol. *BMJ Open* 2016;**6**: e010651. doi:10.1136/bmjopen-2015-010651

► Prepublication history for this paper is available online. To view these files please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2015-010651).

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Received 23 November 2015
Revised 25 February 2016
Accepted 19 May 2016

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ABSTRACT

Introduction: The approved analgesic and anti-inflammatory drugs ibuprofen and indometacin block the small GTPase RhoA, a key enzyme that impedes axonal sprouting after axonal damage. Inhibition of the Rho pathway in a central nervous system-effective manner requires higher dosages compared with orthodox cyclooxygenase-blocking effects. Preclinical studies on spinal cord injury (SCI) imply improved motor recovery after ibuprofen/indometacin-mediated Rho inhibition. This has been reassessed by a meta-analysis of the underlying experimental evidence, which indicates an overall effect size of 20.2% regarding motor outcome achieved after ibuprofen/indometacin treatment compared with vehicle controls. In addition, ibuprofen/indometacin may also limit sickness behaviour, non-neurogenic systemic inflammatory response syndrome (SIRS), neuropathic pain and heterotopic ossifications after SCI. Consequently, 'small molecule'-mediated Rho inhibition after acute SCI warrants clinical investigation.

Methods and analysis: Protocol of an investigator-initiated clinical open-label pilot trial on high-dose ibuprofen treatment after acute traumatic, motor-complete SCI. A sample of n=12 patients will be enrolled in two cohorts treated with 2400 mg/day ibuprofen for 4 or 12 weeks, respectively. The primary safety end point is an occurrence of serious adverse events, primarily gastroduodenal bleedings. Secondary end points are pharmacokinetics, feasibility and preliminary effects on neurological recovery, neuropathic pain and heterotopic ossifications. The primary safety analysis is based on the incidence of severe gastrointestinal bleedings. Additional analyses will be mainly descriptive and casuistic.

Ethics and dissemination: The clinical trial protocol was approved by the responsible German state Ethics Board, and the Federal Institute for Drugs and Medical Devices. The study complies with the Declaration of Helsinki, the principles of Good Clinical Practice and all further applicable regulations. This safety and pharmacokinetics trial informs the planning of a subsequent randomised controlled trial. Regardless of the result of the primary and secondary outcome assessments, the clinical trial will be reported as a publication in a peer-reviewed journal.

Strengths and limitations of this study

- The SCISSOR study is the first clinical trial on high-dose application of the globally approved non-steroidal anti-inflammatory drug (NSAID) ibuprofen as a 'small-molecule' Rho inhibitor after acute traumatic spinal cord injury (SCI) within a concept of drug repurposing.
- Preclinical evidence for recovery-enhancing effects of ibuprofen-mediated Rho inhibition after SCI has been corroborated by systematic review and meta-analysis.
- Limitations of this pilot study inherent to a phase I trial are small sample size, the lack of a placebo control group and a relatively wide time frame for inclusion.
- The results of the SCISSOR trial might inform an interim bed to bench-side translation and subsequent randomised controlled trials.

Trial registration number: NCT02096913; Pre-results.

INTRODUCTION

At present, the effective pharmacological treatment of acute traumatic spinal cord injury (SCI) is an unmet medical need.[1] The current opportunities for restitution of neurological function after SCI are limited to early surgical decompression, stabilisation, intensive care, rehabilitation and the prevention or therapy of SCI-specific sequelae.[2] Neuroprotective or plasticity-enhancing therapies are under investigation in preclinical studies and early-phase clinical trials. As yet, however, none of these approaches could be translated into clinical routine.[2–4]

A major reason for the poor prognosis of central nervous system (CNS) injury is the incapacity of axons to regrow within the

CNS. Molecular barriers preventing axonal regeneration after SCI are situated in the environment of the injured axon, that is, in the scar tissue and myelin or myelin debris.[5 6] The molecules such as chondroitin sulfate proteoglycans (CSPGs), Nogo-A, myelin-associated glycoprotein (MAG), oligodendrocyte-myelin glycoprotein (OMgp), ephrins and repulsive guidance molecule A (RGMa) are upregulated after CNS injury and interfere with a repertoire of cognate receptors on the axon membrane as reviewed elsewhere.[6 7] Signals from those receptors converge on the Rho pathway. The small GTPase RhoA is a key molecule in a pathway which, once activated, leads to the collapse of axonal growth cones and consequently to the failure of axonal plasticity or regeneration.[8] Furthermore, myelin debris inhibits the differentiation of oligodendrocyte precursor cells partially dependent on RhoA-associated pathways[9] and thus may prevent remyelination of spared axons.

Therefore, the Rho pathway constitutes a target for treatments aiming to overcome molecular obstacles to a restoration of neuronal connectivity and subsequent functional recovery. The inhibition of Rho or the downstream-located Rho-associated coiled kinase (ROCK) has been demonstrated to foster axonal sprouting or plasticity,[10–23] to have neuroprotective effects,[10 11 13 14 20 24 25] to promote oligodendrocyte precursor cell differentiation[9] or remyelination[25] and to enhance neurological recovery[10 11 13 16 18–20 22–24 26] after acute SCI (figure 1). These findings are backed up with evidence from other experimental CNS injury conditions as reviewed elsewhere.[7 27] The reported effects of various Rho/ROCK-blocking approaches on open-field motor recovery after experimental SCI have been reassessed by a systematic review and meta-analysis including correction for publication bias.[28] Specific Rho inhibition mediated by the *Clostridium botulinum*-derived enzyme C3 transferase, also referred to as BA-210 or Cethrin,[29] has been studied in a recently completed phase I/IIa clinical trial. The investigators concluded that topically applied BA-210 is safe and is associated with favourable neurological outcome.[30] However, a confirmatory phase III trial has not yet been conducted.

Over the last decade, upcoming evidence has assigned a subset of non-steroidal anti-inflammatory drugs (NSAIDs) to the group of unspecific Rho inhibitors. The Food and Drug Administration (FDA)-approved NSAIDs ibuprofen,[11 17 19 21 31 32] indometacin[19 31] and sulindac sulfide[31] were shown to inhibit Rho activation independently of their 'classical' mode of action as inhibitors of cyclooxygenases (COXs). It was subsequently demonstrated that ibuprofen treatment enhances axonal sprouting,[11 17 19] including that of human model neurons,[21] and improves neurological recovery.[11 19] It is noteworthy that ibuprofen-mediated Rho inhibition involves peroxisome proliferator-activated receptor γ (PPARγ) activation.[17] It remains unclear, however, what the exact mechanism for this activation is and whether cofactors are required for PPARγ-associated Rho inhibition, because other PPARγ activators such as rosiglitazone also inhibit Rho,[17 33] while other NSAIDs such as naproxen activate PPARγ strongly[34] but do not cause Rho inhibition.[11 17 31]

Importantly, ibuprofen dose regimes currently applied in the clinical setting are subtherapeutic as being likely unable to block the Rho pathway in the CNS compartment sufficiently. Moreover, NSAIDs are usually applied in a later phase after SCI. Thus, retrospective analysis studying the effect of lower dose NSAID as being applied at present cannot address the hypothesis sufficiently whether ibuprofen-mediated Rho inhibition may elicit improved neurological recovery when being applied in sufficient dosage and appropriate time frame.[35]

Other pharmacological targets of ibuprofen, namely PPARγ activation,[17 34 36] COX-1/2 inhibition[37] and NF-κb inhibition,[38] promise a concomitant limitation of secondary damage by anti-inflammatory actions, but might also modify the effects of Rho blockade (figure 1). In more detail, PPARγ activation reduces the cellular[39] and soluble inflammatory response,[40] which is suggested to alter tissue pathology after SCI as reviewed by McTigue.[41] In the context of experimental systemic inflammation, COX-1, which reveals sustained upregulation in the spinal cord after SCI,[42] promotes sickness behaviour.[43] COX-related pathways also exert immune modulation in terms of immune depression[44] and impaired host defence.[44 45] These effects might aggravate the maladaptive immune response after SCI[46 47] that is associated with increased susceptibility to infections, which are a risk factor for poorer neurological outcome after SCI.[48] Furthermore, NF-κB, which is activated after SCI,[49] contributes in neurodegenerative disease to microglia-induced loss of motor neurons.[50] Together, antiphlogistic actions of ibuprofen are likely to reduce neurodegeneration driven by CNS inflammation,[50 51] which is triggered through the COX and/or NF-κB-related systemic inflammatory response syndrome,[44 52] or infections.[44 45] Besides, NF-κB[53] and COX metabolites such as prostaglandin E_2[54] are linked to the induction of neuropathic pain. Thus, NSAIDs might be effective in preventing SCI-specific sequelae such as neuropathic pain,[53 55–58] as well as inflammation-related neurogenic heterotopic ossifications (figure 1).[59–61]

Ibuprofen is recommended primarily to improve neurological function through the enhanced plasticity conferred by its Rho-inhibiting properties. The combination of Rho inhibition with anti-inflammatory actions of ibuprofen might, however, dissolve conflicting aspects of anti-inflammatory therapies after axonal injury. It has been demonstrated that secondary axonal damage is reduced when inflammation has been limited, but this occurs at the expense of the regenerative capacity of the spared axons.[62] In this context, an increased blockade of axonal regrowth capacity as a side effect of anti-inflammatory neuroprotective therapy could be prevented by concurrent Rho inhibition (figure 1).

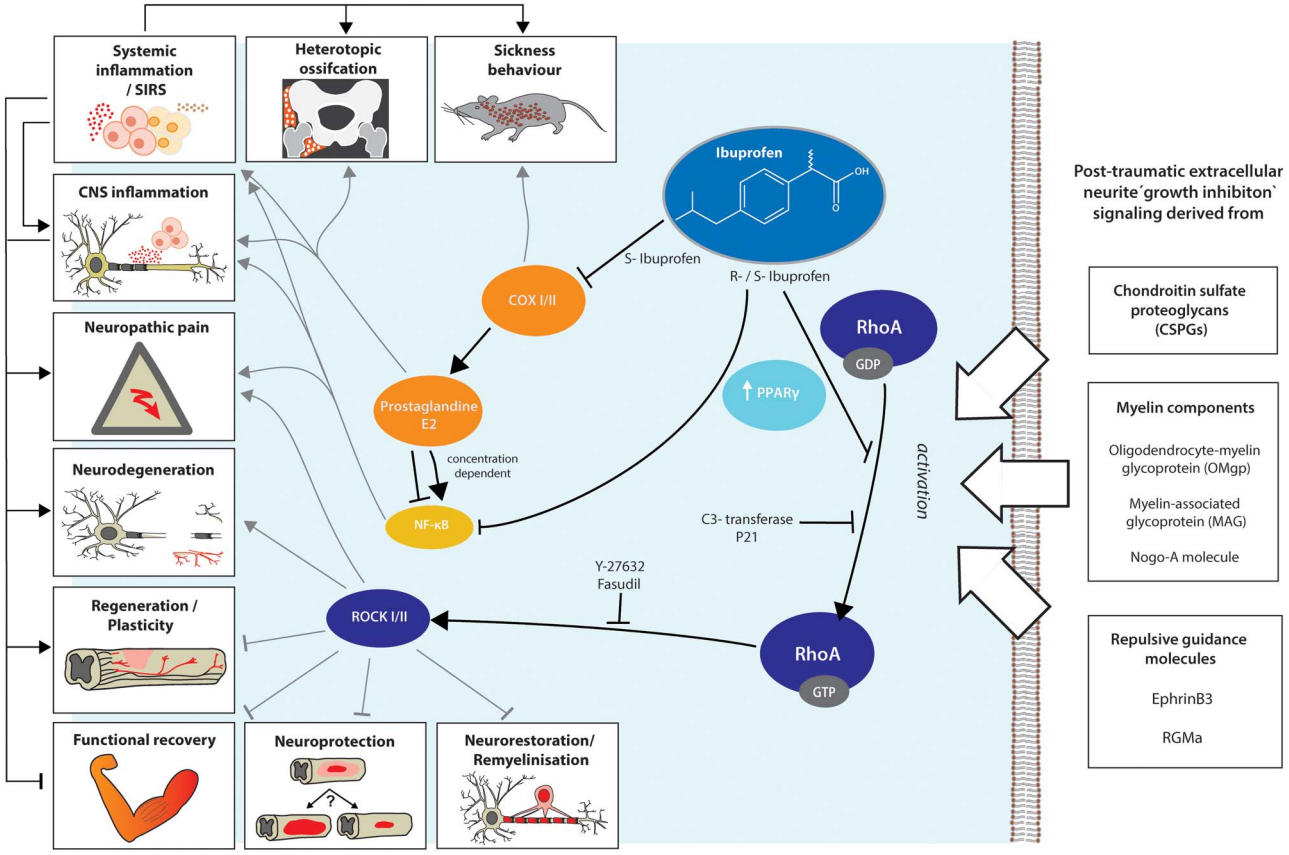

Figure 1 Pharmacological targets of ibuprofen. Intracellular signalling cascades converge at the GTPase RhoA, which is activated after SCI by myelin and scar-associated proteins (for review, see refs. 5, 7 and 29). Downstream to Rho, the activated ROCK inhibits axonal regrowth, promotes neurodegeneration, contributes to the development of neuropathic pain and tissue loss and impedes neurorestoration and functional recovery (reviewed by Watzlawick *et al*).[28] This pathway can be blocked by the ROCK inhibitors Y-27632 and fasudil or the specific Rho inhibitors P21CIP1/WAF1, C3 transferase,[28] and by the R(−) and S(−) enantiomers of ibuprofen,[11 17 19 21 31 32] as the most convincing Rho inhibitor among individual drugs from the group of NSAIDs. Ibuprofen-mediated Rho inhibition depends on the upregulation of PPARγ.[17] Treatment with PPARγ agonists was demonstrated to have anti-inflammatory effects[39 40] and to protect tissue and thereby motor function in other CNS injury conditions (reviewed by McTigue).[41] It is not yet clear whether the inhibition of NF-κB as a further target of R(−)/S(+) ibuprofen[38] is independent of PPARγ. Notably, PPARγ inhibits gene expression by antagonising the activities of the proinflammatory transcription factors NF-κB.[39] Another pathway, mainly operated by the S enantiomer of ibuprofen, is the inhibition of COX 1/2 and consequently of the prostaglandin E2 production, which activates NF-κB or counterregulates it at very high concentrations.[54] COX 1/2 and NF-κB are associated with inflammation-induced neuropathic pain,[53 54] neurodegeneration,[50] sickness behaviour[43] and the systemic inflammatory response syndrome.[44 52] Systemic inflammation contributes to neurogenic heterotopic ossifications.[59] Taken together, Rho-blocking NSAIDs have the potential to decrease the systemic and acute CNS inflammatory response by targeting at least two separate pathways, PPARγ and COX 1/2. The suspected side effect of neuroprotective anti-inflammatory therapy, that is, that it further limits the regeneration capacity of spared axons,[62] is suggested to be abrogated by Rho inhibition. CNS, central nervous system; COX, cyclooxygenase; GDP, guanosine diphosphate; GTP, guanosine triphosphate; NSAIDs, non-steroid anti-inflammatory drugs; PPARγ, peroxisome proliferator-activated receptor γ; NF-κB, nuclear factor κB; RGMa, repulsive guidance molecule A; ROCK, Rho-associated coiled kinase; SCI, spinal cord injury.

In vitro sprouting responses under ibuprofen,[11 17 19 21] or indometacin,[19] treatment in the presence of myelin or inhibitory matrix components such as CSPGs are well reproducible. However, in vivo evidence provided by some groups for promoting effects of Rho inhibition on axonal sprouting,[11 19] or on neurological recovery,[11 19 63 64] has not or has only partially been confirmed by others.[32 65] Reasons for the variability in the results could be multiple. One possible reason would be differences in the experimental design, such as in timing of the experiments, the animal model applied, the route of drug delivery and assessment tools. On the other hand, the variability could be a product of chance due to small sample sizes, which is a general problem in preclinical studies.[66] One approach to address the variability of preclinical studies is to subject them to meta-analysis.[66]

This work includes a systematic review and meta-analysis of experiments reporting the effect of Rho-inhibiting NSAIDs on neurobehavioral recovery after SCI. The published preclinical evidence and its

positive predictive value, representing the justification of the current clinical investigation, were challenged by the meta-analysis. The study protocol of the first clinical trial on high-dose ibuprofen as a Rho inhibitor after acute SCI addresses safety, feasibility and pharmacokinetics. Additionally, the study explores preliminary efficacy, including aspects of repurposing ibuprofen[67] as a compound with multiple pharmacological targets for the treatment of SCI.

METHODS AND ANALYSIS

Ibuprofen is a drug that has been FDA approved and is available worldwide for decades. However, in the context of traumatic injuries, its use is generally restricted to short-term low-dose administration as an analgesic. The mid-term high-dose application of ibuprofen, as a Rho-inhibiting and anti-inflammatory treatment after SCI, is not an approved indication and information on its tolerability is not available for the population of patients with acute SCI. This is relevant because critically injured patients with SCI require treatment in an intensive care unit, which is a risk factor for gastric ulcers.[68] Particularly patients with cervical and high thoracic SCI might be at risk for damage to the gastric mucosa due to a disturbance of autonomous innervation.[69] Furthermore, pharmacological data on CNS permeability are available for non-trauma patients, but little is known about pharmacokinetics of orally administered ibuprofen after SCI. Therefore, the SCISSOR study primarily addresses safety, feasibility and pharmacokinetics under the clinical condition of acute traumatic SCI. Secondary objectives are neurological recovery and SCI-specific complications.

Assessment of underlying evidence

In order to reassess the preclinical evidence regarding Rho-inhibiting NSAIDs and to justify the risks and efforts of the clinical trial, a systematic review was performed. Six publications,[11 19 32 63–65] containing 11 single experiments with a total of n=255 animals (table 1), were included for meta-analysis after stepwise study selection (figure 2).

Preclinical study characteristics were extracted for each publication and functional outcome was measured for each experiment in order to perform the meta-analysis. The method and statistical approach is described in greater detail elsewhere.[28 70] In brief, we used a random-effects weighted mean difference meta-analysis to calculate an overall estimate of effect size between treated and untreated (control) animals based on the final time point of the assessment of functional recovery. A random-effects metaregression was used to determine how much heterogeneity can be explained by study design characteristics using STATA13 with a significance level of p<0.05. We checked for possible publication bias using trim and fill method for funnel plots and Egger regression in STATA13.

Table 1 Preclinical study characteristics

ID	Publication	Drug	Species	n	Dose (duration)	Motor score	Injury level	Follow-up	Type of injury	Application
1	Redondo-Castro, E	Ibu	Rats	16	60 mg/kg/day (42 days)	BBB	T8	42 days	Contusion	Subcutaneous
2	Sharp, K	Ibu	Rats	73	60 mg/kg/day (28 days)	BBB	T6/7	42 days	Hemisection	Subcutaneous
3	Wang, X	Ibu	Mice	46	35–70 mg/kg/day (28 days)	BMS	T8	35 days	Transection	Subcutaneous
4	Guth, L	Indo	Rats	12	0.2 mg/day (21 days)	Tarlov	T8	21 days	Compression	Intraperitoneal
5	Wang, X	Ibu	Rats	47	70 mg/kg/day (28 days)	BBB	T7	49 days	Contusion	Subcutaneous
6	Fu, Q	Ibu	Rats	19	60 mg/kg/day (28 days)	BBB	T6/7	42 days	Hemisection	Subcutaneous
7				12	60 mg/kg/day (28 days)				Contusion	
8	Pantovic, R	Indo	Rabbits	6	0.1 mg/kg/day (9 days)	Tarlov	L2	9 days	Contusion	Intravenous
9				6	0.3 mg/kg/day (9 days)					
10				6	1.0 mg/kg/day (9 days)					
11				6	3.0 mg/kg/day (9 days)					
				6	*vehicle*					

BBB, Basso, Beattie and Bresnahan; BMS, Basso Mouse Scale; Ibu, ibuprofen; Indo, indometacin; n, number of animals.

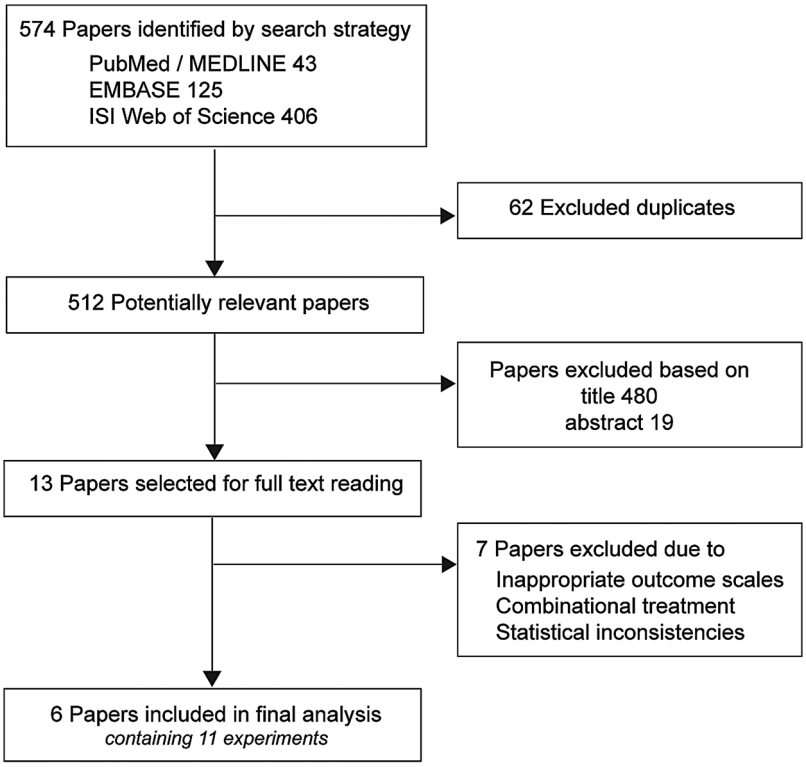

Figure 2 Systematic review preclinical study selection chart. To identify animal studies reporting the effect of ibuprofen or indometacin treatment for neurobehavioral recovery after SCI, the following search term was used for PubMed, EMBASE and ISI Web of science (search conducted on 18 May 2015): (Ibuprofen OR Indometacin OR NSAID OR nonsteroidal anti-inflammatory drugs) AND (SCI OR hemisection OR contusion OR dorsal column injury OR transection OR corticospinal tract injury OR compression OR spinal cord lesion). Search results were limited to animals. Studies were included if they reported the effects of ibuprofen or indometacin in animal models after various types of SCIs. We included SCI experiments comparing functional motor outcome between a group of animals receiving treatment and a control group receiving no treatment (sham group). Non-traumatic models of SCI were excluded, as well as studies reporting only combined treatments. Studies had to report the number of animals for each group, the mean effect size and its variance. Studies were excluded due to inappropriate outcome scales, combination of treatments and statistical inconsistencies. SCI, spinal cord injury.

The effect size in the open-field motor testing of treatments with ibuprofen or indometacin after experimental SCI was 20.2% (95% CI 10.8% to 29.6%) in the overall analysis (figure 3) and varied in the single experiments from −33.2% (−79.2% to 12.8%) to 44.9% (19.5% to 70.4%). Metaregression analyses to identify subgroup effects regarding the administered drug, the behavioural assessment tool, the SCI model, the route of drug delivery or the study quality revealed no statistically significant proportion of between-study heterogeneity for any of the stratifications. Likewise, the tests to detect possible publication bias implied no missing experiment, although statistical significance should not be expected given the study's small overall size.[71]

Nevertheless, the design of the studies on ibuprofen was different from those on indometacin treatment in terms of the neurobehavioral scales, the animal models and the route of drug delivery (table 1). The ibuprofen-treated animals had all been assessed with the Basso, Beattie and Bresnahan (BBB) score,[72] or the Basso Mouse Scale (BMS),[73] whereas modifications of the outdated Tarlov score[74] were applied for the indometacin-treated animals. Furthermore, the ibuprofen-treated groups underwent contusion, transection and hemisection models in contrast with the exclusive use of compression or contusion experiments in the indometacin-treated groups, which received the drug intravenously or intraperitoneally compared with subcutaneous administration in the ibuprofen groups. Therefore, differences in effect size between the two investigated compounds require careful interpretation and do not allow conclusions on differences in their potential therapeutic efficacy.

Among the 'small-molecule' Rho inhibitors, ibuprofen is the most feasible for clinical investigation in the indication of acute traumatic SCI due to its greater quantity and the higher quality of its preclinical data. All studies on ibuprofen revealed Rho inhibition in vivo within the spinal cord after systemic drug administration[11 19 32] and comprise experimental models applicable for translational research,[75] as well as recent behavioural scores.[72 73]

Study design

The SCISSOR study is designed as a prospective, non-randomised, open-label phase I study, as this is a well-

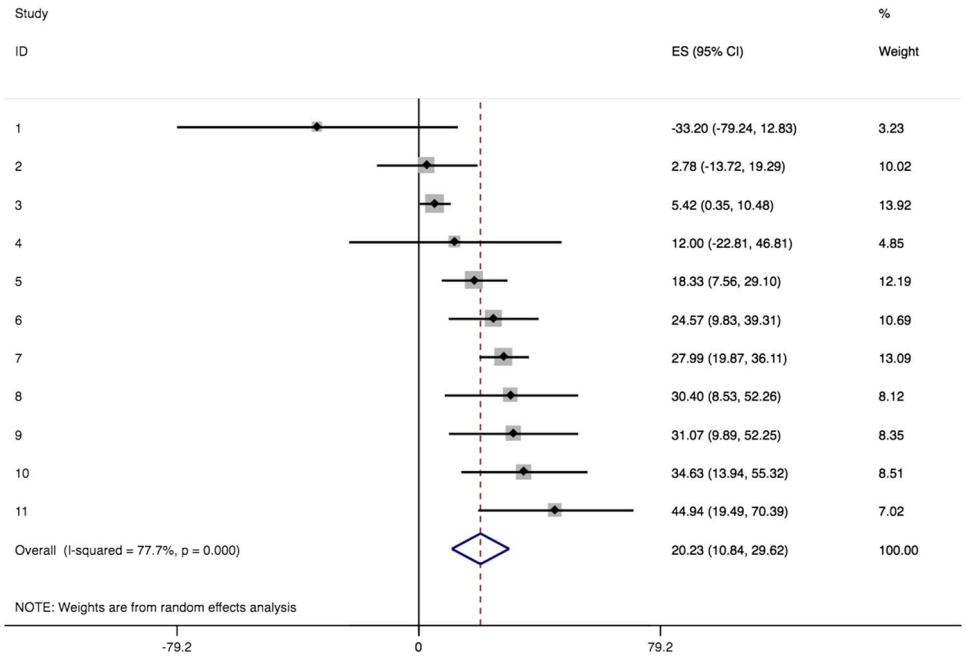

Figure 3 Meta-analysis of preclinical effects on motor recovery. Improvement in neurobehavioral score is ranked by effect size (ES). The overall number of included animals was n=255 (median n=12, range 8–73). Black dots represent studies using ibuprofen and white dots show indometacin studies. The horizontal bar represents the 95% CI of the ES. Details on the design of the included studies are summarised in table 1.

established design for tolerability and pharmacokinetic investigations.[76 77] Study participants are enrolled consecutively in two treatment cohorts characterised by the duration of therapy as further detailed below and illustrated in figure 4.

Setting

The initiating sponsor and coordinator of the trial is the Department of Experimental Neurology, Clinical and Experimental Spinal Cord Injury Research (Neuroparaplegiology) at Charité University Hospital, Campus Mitte, Berlin, Germany, represented by Professor Jan Schwab. For contact information, see correspondence address. Data management and statistics are performed by the Department of Clinical Epidemiology and Applied Biostatistics at Eberhard Karls Universität Tübingen, Germany. The recruiting trial centre is the Treatment Center for Spinal Cord Injuries at the Trauma Hospital Berlin, Germany. The study investigators are physicians trained and experienced in the management and assessment of patients with acute and chronic SCI.

The reference centre for laboratory safety parameters is the Central Laboratory at the trial centre, Trauma Hospital Berlin, Germany. The central laboratory is regularly certified for clinical diagnostics. The Department of Pharmaceutical and Medicinal Chemistry, Institute of Pharmacy, Eberhard Karls Universität Tübingen, Germany, will perform the measurement of ibuprofen concentrations in plasma and cerebrospinal fluid (CSF) using high-performance liquid chromatography-mass spectrometry (HPLC-MS). The *Labor Berlin—Charité Vivantes GmbH*, a certified laboratory for clinical and research diagnostics, will run the nephelometric protein measurements in serum and CSF for quantification of post-SCI blood–spinal cord barrier breakdown.

Intervention

The study medication is ibuprofen in the galenic preparation of water-soluble lysine salt. Ibuprofen lysine salt is absorbed faster, leading to earlier peaks of plasma concentrations compared to the free acid.[78] The brand name of the study medication is Dolormin extra. Ibuprofen is applied as tablets administered orally for 4 weeks in cohort I or 12 weeks in cohort II (figure 4). The daily dose of 2400 mg is administered as three single doses of 800 mg. In the case of swallowing disorders, which occur in 16% of tetraplegic patients with acute SCI,[79] it is recommended that the tablets be disaggregated in water and the medication administered via stomach tube.

The proton pump inhibitor pantoprazole is used as a concomitant medication in a dosage 40 mg/day. This reduces the risk of damage to the gastrointestinal mucosa. After 4 weeks of treatment and individual risk–benefit assessment, the dosage of pantoprazole may be reduced to 20 mg/day during the following weeks of treatment (applicable to cohort II).

Dose estimation

Ibuprofen doses of 60–70 mg/kg/day have been used in preclinical trials.[11 19 32 65] To estimate the pharmacologically active dose (PAD) in humans, we applied a conversion model which is feasible for systemically administered

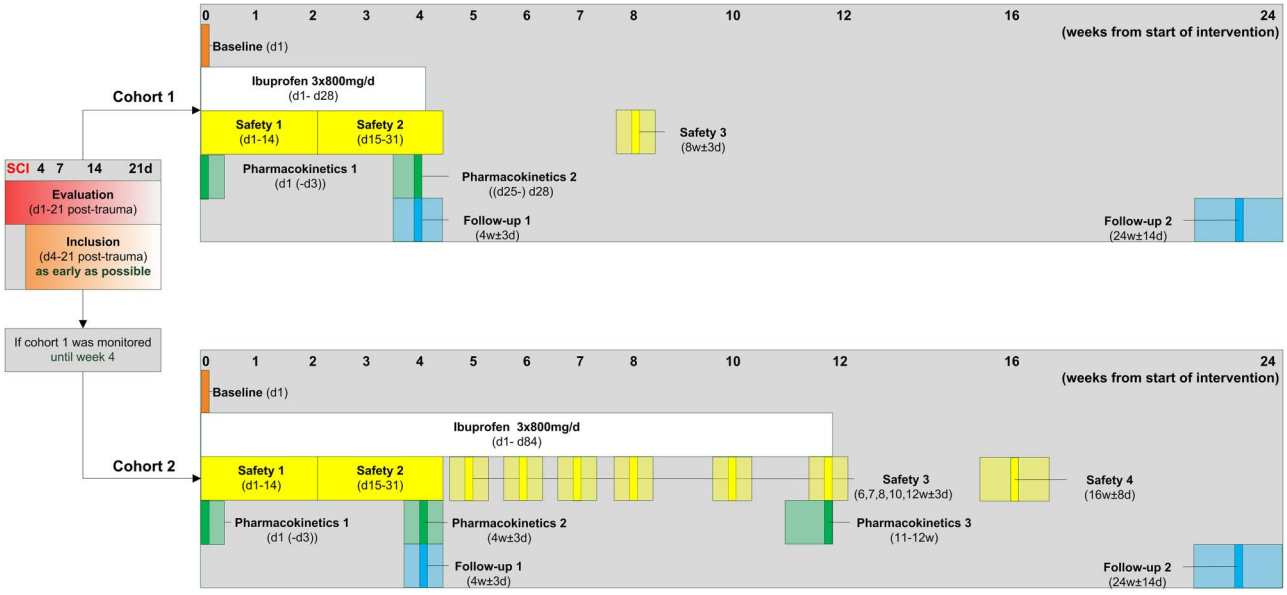

Figure 4 Longitudinal clinical trial design. Diagram of frequency and scope of trial procedures. The evaluation for eligibility should start as early as possible after acute SCI. The baseline will be obtained at the day of the inclusion from day 4 and latest at day 21 post trauma, in any case as early as possible. Start of the study medication is directly after the baseline assessment. The duration of the intervention is 4 weeks for cohort I and 12 weeks for cohort II. Frequent safety laboratory measurements are performed. Samples for pharmacokinetic measurements are collected two times in cohort I and three times in cohort II. The follow-up visits for the determination of secondary end points are performed at week 4 (±3 days) and after the end of the intervention at week 24 (±14 days). Final safety laboratory measurements will be performed 4 weeks after the end of the study medication. SCI, spinal cord injury.

active substances of a small molecular size, provided that further pharmacological properties of the compound have been taken into account.[80] The human equivalent dose (HED), converted from the PAD in rats, is about 11.3 mg/kg/day (rat PAD of 70 mg/kg/day/6.2=HED 11.3 mg/kg/day). The binding capacity for ibuprofen in vitro is higher in human albumin than it is in rat albumin. At identical concentrations, the free bioactive ibuprofen fraction in human albumin solution is lower by a factor of about 3.[81 82] We therefore multiplied the HED by that factor to achieve an estimate of comparable bioactive concentrations. Assuming an average body weight of 70 kg, the estimated PAD in humans is 34 mg/kg regardless of individual body weight. The daily dose of ibuprofen in this trial was therefore set at 2400 mg/day. This is within the FDA-approved range of up to 3200 mg/day for adults.

Outcome measures

The primary end point of the study is the safety of high-dose ibuprofen application after SCI as measured by the occurrence of serious adverse events (SAEs) related to the study medication. In particular, severe gastroduodenal bleeding attributable to the study medication is the primary safety parameter (table 2). SAE definitions are in accordance with the International Council for Harmonisation (ICH) guidelines.[83] All other adverse events (AEs) that do not fulfil these definitions are documented on AE documentation sheets, and type, severity, relatedness, treatment and outcome are recorded.

Secondary end points are all further AEs including SAE and suspected unexpected serious adverse reactions (SUSARs). Clinical, laboratory and technical safety examinations facilitate the detection of AEs that can be expected as well as the assessment of their causality (table 2). In addition, the sensitive measurement of neuropathic pain and spasticity is also relevant for safety reasons, since the course of those very frequent SCI-specific sequelae might be altered by plasticity-enhancing therapies. The Neuropathic Pain Scale[84 85] and the Modified Ashworth Scale[86] are therefore applied for assessment of pain and muscle tone, respectively. The pharmacological laboratory end points are ibuprofen levels in plasma and CSF as measured at the time of expected peak levels.[87] The neurological examination is performed according to the International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) definitions of 2011.[88] The ISNCSCI comprises the American Spinal Injury Association (ASIA) Impairment Scale (AIS) as a measure for completeness and severity of SCI, the ASIA motor scores for upper and lower extremity motor function, the ASIA sensory scores for residual pin prick and light touch sensation, the motor and sensory neurological level, as well as the zone of partial preservation, if applicable. Optional elements such as non-key muscles for determination of the AIS are not applied in this study.[88] Neurogenic heterotopic ossifications constitute a further clinical end point. These will be identified with an ultrasound screening of the hip

Table 2 Clinical trial outcome measures

Parameter	Assessments/measures	Timing (see also figure 4)	Safety issue
Primary end point			
Gastroduodenal bleeding	SAE report	Continuous observation	Yes
Secondary end points			
Adverse events	Adverse event monitoring, SAE/SUSAR report	Continuous observation	Yes
Spasticity	Modified Ashworth Scale, antispastic medication	Follow-up 1 and 2	Yes
Neuropathic pain	Neuropathic Pain Scale, pain medication	Baseline, follow-up 1 and 2	Yes
Severity of SCI	ASIA Impairment Scale	Baseline, follow-up 1 and 2	No
Motor function	Upper and lower extremity motor score	Baseline, follow-up 1 and 2	No
Sensory function	Pin prick, light touch	Baseline, follow-up 1 and 2	No
Lesion height	Motor and sensory level, zone of partial preservation, if applicable	Baseline, follow-up 1 and 2	No
Ibuprofen levels	Blood and CSF collection	Pharmacokinetics 1, 2 and *3**	No
Serum/CSF protein levels	Blood and CSF collection	Pharmacokinetics 1, 2 and *3**	No
Heterotopic ossifications	Ultrasound of the hip joints, MRI, if applicable	Baseline, follow-up 1 and 2	No
Other end points			
Laboratory abnormalities	Blood and urine collection	Safety 1, 2, 3 and 4*	Yes
Cardiac arrhythmia	ECG	Baseline, follow-up 1 and 2	Yes
Deep vein thrombosis	Ultrasound of pelvic and lower extremity veins	Baseline, follow-up 1 and 2	Yes
Circulatory disturbance	Blood pressure and heart rate	Baseline, safety 1, 2	Yes
Clinical observation	Epigastric pain/pain projected to the shoulder tip	Baseline, safety 1, 2 and 3*	Yes
Feasibility of recruitment	Screening protocol	Screening	No

Differences between the cohorts are based on the course of an extended intervention. In cohort II, additional pharmacokinetic and safety assessments are scheduled (indicated by asterisks).
ASIA, American Spinal Injury Association; CSF, cerebrospinal fluid; SAE, serious adverse event; SCI, spinal cord injury; SUSAR, serious unexpected suspected adverse reaction.

joints,[89] followed by MRI if heterotopic ossifications are suspected (table 2).

Data on adverse effects of perioperative NSAIDs on bone healing in terms of pseudoarthrosis after spinal fusion have been discussed in the past.[90] These data, however, are based on different types of NSAIDs and from retrospective cohort studies, the results of which are sometimes conflicting.[91 92] In this study, all spinal surgeries during the follow-up period will be documented. In combination with data from routinely performed spinal imaging procedures, relevant impairment of bone healing can be detected and would be documented as SAE.

Enrolment

In the study centre, we expect to be screening about 40–60 SCI admitted patients per year, about 6–8 of whom are estimated to meet eligibility criteria. The investigators will evaluate patient eligibility as soon as possible after admission to the trial centre. The investigators will conduct an interview with each patient to verify the inclusion and exclusion criteria as related to individual medical history as well as to inform the patient about the trial and its potential risks and benefits. Prior to inclusion, written informed consent will be obtained from the patient. If the patient is willing to consent but is unable to sign, a witness independent from the trial team must confirm the verbal informed consent by providing his/her signature. A written announcement of recruitment will be sent out to the sponsor by the investigators.

Eligibility criteria

The inclusion and exclusion criteria (box 1) were chosen with regard to scientific, ethical and practical considerations specific to SCI.[93] Inclusion in the trial is possible from day 4 up to day 21 postinjury, but should be performed as soon as possible, mainly dependent on the patient's ability to give his/her informed consent. Key inclusion criteria are acute traumatic motor-complete SCI, classified as AIS A or AIS B, and a neurological level of Th4–C4. Only in this group of patients is a realistic assessment of neurogenic gastrointestinal bleedings possible, because this classification is most likely to be associated with an autonomic complete lesion,[94] which in the acute stage can cause damage to the gastroduodenal mucosa.[69] The imbalance between the altered sympathetic outflow through the splanchnic nerve and the intact parasympathetic innervation through the vagus nerve[69] may increase the 'baseline' risk posed by the general post-traumatic and ventilation-triggered stress response.[68]

Box 1 Clinical trial eligibility criteria

Inclusion criteria
- Acute spinal cord injury (SCI) of the cervical spine due to trauma
- Time frame of 4–21 days post trauma
- Motor-complete injury AIS (American Spinal Injury Association (ASIA) Impairment Scale) A and B
- Neurological level of the lesion C4–Th4
- No participation in a different clinical trial according to German Medicinal Products Act 1 month before and during participation in the current trial
- The patient has been informed and his/her written consent has been obtained
- Age: 18–65 years
- For women of reproductive age: negative pregnancy test and highly effective contraception (defined as Pearl Index <1) or sexual abstinence during participation in the trial

Exclusion criteria
- Multifocal lesions of the spinal cord
- Penetrating SCI
- Accompanying traumatic brain injury (TBI) with visible structural lesions including intracranial haemorrhage on diagnostic images
- Significant accompanying injury to the peripheral nervous system, particularly plexus lesions
- Acute or chronic systemic diseases accompanied by neurological deficits or that have caused permanent neurological deficits which may overlay or hinder the registration of sensorimotor functions (eg, multiple sclerosis, Guillain-Barré syndrome, HIV infection, Lues, etc)
- Malignant neoplasms, except if these are in complete remission
- Mental diseases or dementia which, in the investigator's opinion, limit the patient's cooperation in respect of the intake of the study medication and/or significantly hinder the registration of follow-up parameters
- Haemophilia
- History of myocardial infarction or stroke
- Current and persistent misuse of illegal drugs or alcohol
- Hypothermia below 35°C
- Pregnancy and lactation
- All further contraindications to the study medication, including other ingredients of the pharmaceutical form according to the Summary of Product Characteristics
  - known hypersensitivity to the active substance ibuprofen or one of the ingredients of the drug
  - known reactions by way of bronchospasm, asthma, rhinitis or urticaria after the intake of acetylsalicylic acid or other non-steroidal anti-inflammatory drugs (NSAIDs) in the past
  - unexplained haematopoietic disorders,
  - peptic ulcers or haemorrhagia: either at the present time or occurred repetitively in the past (at least two different episodes of proven ulceration or haemorrhage)
  - gastrointestinal haemorrhage or perforation in the patient's medical history in connection with previous treatment with NSAIDs
  - cerebrovascular or other active haemorrhage
  - severe disturbance of liver function (with coagulation disorder due to reduced protein synthesis)
  - severe renal function disorder (defined as chronic renal insufficiency, including post-kidney transplantation or acute renal failure, defined as elevated creatinine values and/or oliguria for several days with a limited glomerular filtration rate (GFR))
  - severe myocardial insufficiency (New York Heart Association (NYHA) grades III–IV)
  - severe dehydration (caused by vomitus, diarrhoea or insufficient volume resuscitation).
- Known hypersensitivity to the active substance contained in the concomitant medication pantoprazole or one of the components of the drug
- Intake of ibuprofen or intake of other active substances from the group of NSAIDs (eg, diclofenac, indometacin) or the intake of NSAIDs in maximum recommended daily doses for more than 1 week prior to enrolment in the trial
- Simultaneous intake of salicylates, particularly acetylsalicylic acid
- Simultaneous intake of oral anticoagulants, or heparinisation in therapeutic dosage
- Simultaneous intake of systemic glucocorticoids
- Unwilling to consent to storage and transfer of pseudonymised medical data for the purpose of the clinical trial
- Admitted to an institution by a court or official order

In order to limit risk to patients, the exclusion criteria comprise all absolute contraindications of the study medication according to the summary of product characteristics. The exclusion criteria also include drug interactions or other conditions mandating precaution. To ensure reliable assessment of safety and preliminary efficacy, patients with concomitant injury to the CNS, pre-existing neurological diseases or severe psychiatric disorders are excluded from the trial. Other exclusion criteria assure the adherence to legal requirements (box 1).

Individual timeline

Patient evaluation and inclusion will be performed within 21 days after SCI. The Case Report Form (CRF) at baseline comprises the eligibility criteria, the

assessment of injury date and time, medical history, concomitant injury and surgical interventions. Furthermore, the clinical, laboratory and technical safety parameters as well as the ISNCSCI are assessed at baseline (figure 4, table 2).

Since the intervention in cohort II is of longer duration, more frequent safety and pharmacokinetic assessments will be performed in this arm during the intervention and follow-up (figure 4). In addition to the continuous monitoring of AEs, safety data comprise laboratory measures, and clinical observations will be collected in tightly scheduled safety assessments up to 24 weeks after inclusion. This time frame seems reasonable for the recognition of the major safety end points. Further safety issues such as spasticity and neuropathic pain are part of the follow-up documentation (figure 4) that also includes the neurological end points and possible confounders such as comedications or infections.[48]

Overall duration

A recruitment period of 24 months is scheduled. Each patient will be followed up to 24 weeks post trauma. After completion of recruitment and follow-up, a further 6-month period is planned for clearing the database, the statistical evaluation and preparation of the trial report. The trial was activated in June 2013 but was not recruiting. After completion of trial registration and instruction of the recruiting center, enrolment started in April 2014. Expected enrolment completion date is the second quarter of 2016. Publication of the trial report is scheduled for the year 2017.

Sample size estimation

The sample size of 12 patients and the analysis strategy are justified by the fact that—given that the number of gastrointestinal ulcerations/bleedings after SCI is 3.5% in the first month as reported by Kewalramani[69]—the probability of the occurrence of more than one event is 6.1%. Consequently, observation of more than one event provides evidence of safety problems of ibuprofen in the indication of acute SCI and probably limits its use in subsequent phases of the clinical trial. The occurrence of further bleedings in months 2 and 3 or during follow-up calls for the same consequences. Nevertheless, based on the above-mentioned frequency of gastrointestinal ulcerations, the probability of the occurrence of an event is low (0.7% probability) in a sample of 12 patients with SCI.[69] However, the upper bound of the CI for the probability of an event is 38.5%; for zero events, it is 26.5%. This mandates implementing additional safety criteria if subsequent study phases are considered, and a placebo control should be taken into consideration. In our pilot study, a comparison with patients receiving placebo would have a clear lack of statistical power, so no placebo group is scheduled.

Data management

All study documents including personal identifiers are stored at the recruiting trial centre in locked file cabinets in a room with restricted access. Data are collected on a paper CRF (pCRF) and pCRFs and all patient data are managed with a six-digit pseudonym. At the sponsor's study office, the trial coordinators check the pCRFs for completeness and consistency. Implausible or missing data may be corrected or added after consulting the investigator at the trial site through the sponsor (Queries). The corrected documents will be archived together with the completed pCRFs. Data are entered twice to allow double check for correctness and are stored electronically in a database (Oracle). Access to the database is restricted, and regular data backups are performed. The principal investigator/sponsor and the trial statistician will have full access to the data set.

Sample handling

Peripheral blood and urine samples collected for laboratory safety measures are analysed immediately after sample collection at the central laboratory of the trial centre, and the results are available for the study investigators at once. This facilitates the timely recognition of AEs.

Blood and CSF samples for pharmacological and protein analyses are collected under sterile conditions. The samples are labelled with the six-digit pseudonym, and any personal information of the participants is removed. All samples are processed for storage as soon as possible, at the latest within 8 hours of withdrawal by centrifugation at 3000 g for 10 min. Serum, heparin plasma and CSF supernatants are stored at the sponsor's institution at −80°C, with central temperature control up to subsequent batch analysis.

Statistical analysis

The analysis will be based on the safety population, as this is a pilot study for safety and feasibility designed to enable planning of a subsequent study. The primary analysis is based on the incidence of severe gastrointestinal bleedings. If more than one event is observed in the study population (n=12), the principal investigator/sponsor on recommendation of the independent Data and Safety Monitoring Board (DSMB) will perform a new risk–benefit assessment and will decide the interruption or early termination of the trial. Additional safety analyses, mainly descriptive and casuistic, will be performed. The descriptive analysis will be according to the scale and distribution of the data, using frequencies and means, medians, quartiles and ranges. Linear regression will be used as appropriate.

Quality assurance

Adherence to (1) the recruitment rate, (2) the selection criteria (3) the treatment in accordance with the protocol and (4) the investigation time points is regarded as a quality indicator for the course of the trial. An

independent monitor is responsible for reviewing study progress, verifying adherence to the protocol, compliance with ICH/GCP and national regulations, and furthermore for handling any problems that arise. The monitor will visit the clinical study sites on a regular basis, first after the start of enrolment, then after completion of recruitment into cohort I and finally at study completion.

Key study data will be checked in all patients. This pertains to patients' demographic data, signed informed consent, adherence to inclusion and exclusion criteria, documentation on primary objectives and AEs. Source data verification will be performed for ~25% of the data. Any unclear and/or incomplete data will elicit increased in-depth monitoring.

Data and Safety Monitoring Board

An independent DSMB addresses patient safety and performs risk–benefit assessments to ensure that for the patients there is no unavoidable risk or harm. All DSMB members reviewed the trial protocol prior to study activation in order to ensure the implementation of safety end points and procedures necessary to fulfil the DSMB's assignment. In accordance with its operating procedures, the DSMB reviews accumulating data from the trial to fulfil the safety monitoring. Additionally, the DSMB will assess trial progress, study integrity and design aspects. The DSMB provides the sponsor with recommendations regarding study modification, continuation or termination. The DSMB consists of three members: a biostatistician, a neurologist and an internist, all of whom have practical experience in the work of a DSMB. The DSMB will perform an interim review for safety reasons when the entire cohort I has completed week 4 follow-up and after the completion of enrolment and, if necessary, on request of the sponsor and/or principal investigator.

Stopping rules

The discontinuation criteria defined for premature dropout of a patient from the trial include cases of emergency or circumstances associated with increased risk for the participant, as well as a patient's individual wish (box 2). Patients who have dropped out of the trial prematurely should be examined from the time of discontinuation of treatment according to the scheduled programme, provided the patient has given his/her consent to such examination. At least the final examination should be performed as far as possible.

Decisions on the discontinuation of the entire trial will be taken if the risk–benefit assessment demonstrates unjustifiable risks and toxicities, or new scientific conclusions during the clinical trial could compromise the safety of the study participants. The decision-making body consists of the sponsor and principal investigator and acts, if appropriate, also on recommendation of the DSMB.

Box 2 Clinical trial stopping rules

Premature dropout of a patient

- Gastrointestinal ulceration with or without haemorrhage and/or perforation
- A drop in haemoglobin levels below 5 mmol/L consistent after receiving more than eight red blood cell concentrates
- Acute renal failure, defined as an increase in creatinine levels by more than 50% of the baseline value and/or oliguria (urine volume <500 mL/day) persisting for several days after exclusion of extra renal causes
- Any hypersensitivity reaction that the investigator attributes to the trial medication
- Neurological progression of SCI with ascending paralysis with a loss of more than two motor levels
- Cerebrovascular haemorrhage
- Myocardial infarction or stroke
- Any new injury to the spine affecting the spinal cord
- The additional intake of more than 1200 mg/day ibuprofen for more than 1 week or the intake of maximal daily doses of other non-steroid anti-inflammatory drugs (NSAIDs) for more than 2 weeks during the intervention
- The patient's personal wish
- Any other situation which, according to the investigator, would be such that further participation in the clinical trial not be in the best interests of the patient
- The onset of pregnancy
- Later occurrence of exclusion criteria

ETHICS AND DISSEMINATION

The study protocol (V.1.2, date 06 May 2013) was approved by the Ethics Board of the *Landesamt für Gesundheit und Soziales (LaGeSo)*, Berlin, Germany (13/0127-EK13), and the Federal Institute for Drugs and Medical Devices *(BfArM)*. The protocol amendment (V.2.0, date 12 August 2015) was related to changes of the Summary of Product Characteristics of the study medication ibuprofen and on a recent advice of the European Medicines Agency (EMA).[95] Two further exclusion criteria were added to the protocol: (1) severe dehydration and (2) history of myocardial infarction or stroke. The above-mentioned regulatory authorities approved the amendment.

Participants will be informed about the trial and its anticipated risks and benefits, orally and in written form, using patient information sheets. Patients' written informed consent will be obtained prior to inclusion. This study complies with the Declaration of Helsinki, the principles of Good Clinical Practice (GCP), the German Medicinal Products Act (AMG) and the Personal Data Protection Act. The study with the full official title 'The Rho-Inhibitor Ibuprofen for the Treatment of Acute Spinal Cord Injury: Investigation of Safety, Feasibility and Pharmacokinetics' has been registered in the ClinicalTrials.gov database (NCT02096913). The registration data are summarised in table 3.

Risk–benefit assessment

In a large number of patients, traumatic SCI signifies a severe lifelong physical disability. A standard treatment

Table 3 Trial registration overview

Data category	Information
Primary registry and trial identifying number	ClinicalTrials.gov NCT02096913
Date of registration in primary registry	24 March 2014
Secondary identifying numbers	2011-000584-28
Sources of monetary or material support	Charité Universitätsmedizin Berlin, Else Kröner-Fresenius Foundation
Primary sponsor	Charitè Universitätsmedizin Berlin, Professor Jan M. Schwab MD, PhD
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Public title	Safety Study of Ibuprofen to Treat Acute Traumatic Spinal Cord Injury
Scientific title	The Rho-Inhibitor Ibuprofen for the Treatment of Acute Spinal Cord Injury: Investigation of Safety, Feasibility and Pharmacokinetics
Countries of recruitment	Germany
Health conditions or problem studied	Spinal cord injury
Interventions	Ibuprofen (Dolormin extra), 2400 mg/day (400 mg 2–2–2) applied orally for 4 weeks (arm I; n=6) or 12 weeks (arm II, n=6)
Key inclusion criteria	Acute traumatic SCI; neurologic level C4–Th4; AIS A or B; inclusion at day 4–21 post injury; no participation in another clinical trial; written consent; age 18–65 years; no pregnancy of female participants during trial conduction
Key exclusion criteria	Multifocal lesions; penetrating injury; TBI with visible structural lesions; accompanying injury to the peripheral nervous system (plexus lesions); acute or chronic diseases causing/including neurological deficits; malignant neoplasms; significant mental disease or dementia; haemophilia; history of myocardial infarction/stroke; drug abuse; hypothermia below 35°C; pregnancy/lactation; contraindications/hypersensitivity to study medication; current intake of ibuprofen or other NSAIDs or previous intake of maximum doses during 1 week prior to enrolment; intake of salicylates, systemic glucocorticoids, oral anticoagulants or therapeutic heparinisation; no consent to storage and transfer of trial-based data; admittance to institution by court or official order
Study type	Interventional; phase I; open label
Study activation	20 June 2013
First patient in	07 April 2014
Target sample size	12
Recruitment status	Recruiting
Primary outcomes	Severe gastroduodenal bleedings
Key secondary outcomes	Spasticity; neuropathic pain; AIS; ISNCSCI/ASIA motor and sensory score; documentation of adverse events; plasma and cerebrospinal fluid ibuprofen level; heterotopic ossifications

AIS, ASIA Impairment Scale; ASIA, American Spinal Injury Association; ISNCSCI, International Standards for Neurological Classification of Spinal Cord Injury; NSAID, non-steroidal anti-inflammatory drug; SCI, spinal cord injury; TBI, traumatic brain injury.

to promote neuronal plasticity after SCI is not yet available. Based on preclinical investigations in established animal models, a better recovery of neurological function in cases of acute SCI is anticipated from making use of 'small-molecule' Rho inhibition. The systematic review of preclinical data revealed 11 eligible studies on effects of Rho-inhibiting NSAIDs with motor function as behavioural end point. These studies were conducted in six laboratories and used four different SCI models in three rodent species. The meta-analysis demonstrated an overall effect size of 20.2%. This is backed up by pervious analyses including studies on specific Rho/ROCK inhibitors that have demonstrated overall effect sizes of 21% or 15% after correction for publication bias, respectively.[28] Ibuprofen is an established, globally approved drug available for clinical investigation of its ability to improve neurological function by Rho inhibition. Furthermore, preventive treatments for inflammation-triggered SCI-specific complications in terms of neuropathic pain[53–58] and neurogenic heterotopic ossifications after SCI[59–61] are not well established. Favourable effects on these threatening sequelae can be anticipated from ibuprofen treatment by the reduction of COX-mediated and NF-κB-mediated inflammation in the CNS and the peripheral soft tissue.

The appraised benefits of the intervention have to be weighed against its potential risks, some of which may be serious. Gastrointestinal ulcers accompanied by

haemorrhage or by perforation are the most prominent side effect of NSAIDs. According to FDA estimates from 1987, gastrointestinal haemorrhage due to peptic ulcers or perforation occurred in 1–2% of patients under sustained 3-month intake of NSAIDs.[96] The factors that increase the risk of gastrointestinal haemorrhage are as follows: advanced age, high daily doses, a medical history of ulcers, simultaneous intake of systemic corticosteroids and the intake of anticoagulants.[97] Within the group of NSAIDs, ibuprofen has a comparatively low gastrointestinal toxicity.[97] A Cochrane database review summarised results from recent clinical trials on long-term high-dose ibuprofen administered to reduce respiratory complications in cystic fibrosis. The studies showed an overall positive benefit–risk profile.[98] However, a clinical database analysis comparing 1365 ibuprofen-treated patients with 8960 controls demonstrated a low overall risk but a higher annual incidence of gastrointestinal bleeding in the ibuprofen group of 0.37% vs 0.14%.[99] In the acute phase, acute injury to the cervical and upper thoracic segments of the spinal cord is probably an additional risk factor for gastroduodenal ulceration,[69] which is why the gastrointestinal safety of ibuprofen treatment in the context of SCI is the primary end point of this trial.

Under normal conditions, acute renal failure due to NSAIDs is a rare but serious adverse reaction. The risk for acute renal failure increases in critically ill patients with a volume deficiency, myocardial insufficiency or pre-existing renal insufficiency; the same holds true for simultaneous administration of other nephrotoxic substances such as aminoglycosides, angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists.[100] Acute renal failure caused by NSAIDs such as ibuprofen, a substance with a short half-life and rapid achievement of effective levels, commonly manifests within a few days. After early diagnosis and discontinuation of the treatment, renal function usually returns to normal within 1 week. Only if renal failure is not diagnosed in time may the condition progress rapidly to dependence on dialysis. Compared with other NSAIDs, an intermediate level of nephrotoxicity is reported for ibuprofen.[101] Acute SCI is generally not associated with a disturbance of renal function. However, due to the traumatic aetiology of paraplegia, renal function may be transiently limited in some cases due to a volume deficiency or rhabdomyolysis. In those cases, renal side effects of ibuprofen might be observed more frequently.

In order to limit the anticipated risks in the Ibuprofen-Spinal Cord Injury (SCI)-Safety trial, its exclusion criteria comprise known risk factors such as age >65 years, relevant comorbidities, history of critical events, particularly peptic ulcerations, as well as drug interactions. In addition, the trial will be conducted under in-hospital conditions of acute care and rehabilitation. In-hospital monitoring and carefully scheduled laboratory investigations allow for early awareness of AEs and their immediate medical treatment. In case that a patient suffers harm from his trial participation, compensation will be covered by a clinical trial-specific insurance of the sponsor's institution. After completion of the trial, the patients will receive further treatment according to the general principles of long-term rehabilitation of SCI and therapy of related secondary complications.

Limitations

Limitations of the clinical trial protocol are its small sample size, the lack of a placebo control group and a relatively wide time frame for inclusion. This design, chosen with regard to the primary safety end point and feasibility of the pharmacokinetic issues, restricts efficacy evaluation. The time frame of inclusion extended until day 21 after SCI was incorporated for ethical reasons in order to enable the patients to give informed consent before the start of the intervention. However, a late start of the intervention might diminish therapeutic efficacy because recovery-promoting effects of Rho inhibiton,[23] as well as anti-inflammatory effects of ibuprofen,[102] depend on the timing of the intervention, and an early start of the treatment seems favourable.

The meta-analysis of published preclinical experiments is limited by the relatively low number of studies specific to ibuprofen/indometacin-mediated Rho inhibition; thus, they hardly enable metaregression or adjustment for publication bias. Yet, our analysis is in line with a larger previous meta-analysis that also includes studies on specific Rho/ROCK inhibitors that demonstrated relevant effect sizes after correction for publication bias.[28] A limitation of the single in vivo experiments on ibuprofen is that they lack dose–response curves, and all research groups have applied the drug in comparable dosages. Administration of even higher doses would still be within FDA-approved range for application in humans and might have larger effects. Confirmative preclinical analyses should therefore also consider dose–response curves to show functional recovery.

Possible consequences

The explorative safety evaluation, feasibility aspects of recruitment and treatment regime in the acute phase after SCI will inform the planning of a subsequent randomised controlled trial (RCT) in a larger sample. Of particular relevance in the clinical trial are the treatment timing and the CNS availability of the systemically delivered compound behind the blood–spinal cord barrier after acute SCI. An interim bed to bench-side translation based on the clinical pharmacological data and preliminary efficacy end points could be valuable for adjustment of the treatment schedule before embarking on an RCT.

Improved neurological recovery anticipated after SCI, which is proposed as the main objective of a subsequent RCT, might lead to an improvement of aspects of daily living, even if the recovery has affected only two segments of the spinal cord. For example, regaining more than one neurological motor level can be considered as

a notable difference with influence on physical independence[103] and long-term survival.[104] Prevention of SCI-related complications might contribute additionally to improved quality of life.

Regardless of the result of the primary and secondary outcome assessments, the clinical trial will be reported as a publication in a peer-reviewed journal, compliant with reporting and authorship criteria according to the principles of Good Scientific Practice.

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Contributors MAK, TL, PM, SMS and JMS designed the trial protocol. TL, RW, PM, SL, CB, RS, GJJ, SK, MK, AE, UD and AN reviewed the trial protocol. MAK and RW conducted the systematic review and wrote the manuscript. RW performed the meta-analysis. All authors critically revised the manuscript for important intellectual content. All authors have read and approved the final version of the manuscript.

Funding The project has received funding from the Wings for Life Spinal Cord Research Foundation (grant number WFL-CB-017/08) and is funded by the Else Kröner-Fresenius-Stiftung Foundation (grant number EKFS 2012_A32). JMS is supported by the W.E Hunt and C.M. Miller Endowment.

Competing interests None declared.

Ethics approval The German state Ethics Board located at the Landesamt für Gesundheit und Soziales (LaGeSo), Berlin, Germany (13/0127-EK13), and the Federal Institute for Drugs and Medical Devices (BfArM).

Provenance and peer review Not commissioned; externally peer reviewed.

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BMJ Open 2016 6:
doi: 10.1136/bmjopen-2015-010651