Experimental Neurology 306 (2018) 10–21

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Research Paper

The effect of myelotomy following low thoracic spinal cord compression injury in rats

Carolin Meyer[a,1], Habib Bendella[b,1], Svenja Rink[c,1], Robin Gensch[d], Robert Seitz[d], Gregor Stein[e], Marilena Manthou[f], Theodora Papamitsou[f], Makoto Nakamura[b], Bertil Bouillon[g], Mary Galea[h], Peter Batchelor[h], Sarah Dunlop[i,2], Doychin Angelov[c,*,2]

[a] Department of Orthopedics and Trauma Surgery, University of Cologne, Germany
[b] Department of Neurosurgery, University of Witten/Herdecke, Cologne Merheim Medical Center (CMMC), Cologne, Germany
[c] Department of Prosthetic Dentistry, School of Dental and Oral Medicine, University of Cologne, Cologne, Germany
[d] Department of Anatomy I, University of Cologne, Germany
[e] Department of Orthopaedics and Trauma Surgery, Helios Klinikum Siegburg, Ringstrasse 49, 53721 Siegburg, Germany
[f] Department of Histology and Embryology, Aristotle University Thessaloniki, Greece
[g] Department of Trauma and Orthopedic Surgery, University of Witten/Herdecke, Cologne Merheim Medical Center (CMMC), Cologne, Germany
[h] Department of Medicine (Royal Melbourne Hospital), The University of Melbourne, Parkville, VIC 3010, Australia
[i] School of Biological Sciences, The University of Western, Australia

ARTICLE INFO

Keywords:
Spinal cord injury
Rat
Myelotomy
Locomotor
Bladder and sensory function
Cellular markers

ABSTRACT

Myelotomy is a surgical procedure allowing removal of extravasated blood and necrotic tissue that is thought to attenuate secondary injury as well as promote recovery in experimental spinal cord injury (SCI) models and humans. Here we examined in rat whether myelotomy at 48 h after low-thoracic compressive SCI provided any benefit over a 12 week period. Compared to animals receiving SCI alone, myelotomy worsened BBB scores ($p < 0.05$) and also did not improve plantar stepping, ladder climbing, urinary bladder voiding or sensory function (thermal latency) during the 12-week period. Quantitative analyses of tissue sections at 12 weeks showed that myelotomy also did not reduce lesion volume nor alter immunohistochemical markers of axons in spared white matter bridges, microglia, astrocytes or serotinergic fibres. However, myelotomy reduced synaptophysin expression, a marker of synaptic plasticity. We conclude that further studies are required to evaluate myelotomy after SCI. (*142 words*).

1. Introduction

Traumatic spinal cord injury (SCI) is catastrophic with different grades of motor and sensory deficit (Kristinsdottir et al., 2016; Wang et al., 2016; Wyndaele and Wyndaele, 2006). Loss of function is attributed to the primary injury as well as to secondary degeneration affecting ascending and descending pathways that convey information between the brain and the spinal cord as well as to multiple organ systems (Ahuja et al., 2017; Min et al., 2015). There are currently no definitive therapies following SCI (Fouad and Pearson, 2004; Gordon et al., 2010).

Spinal cord swelling is a cardinal feature particularly for severe injuries (Saadoun et al., 2008; Werndle et al., 2014). A major approach, therefore, is to relieve pressure (decompress) during acute stages to reduce spinal cord swelling. In humans, such reduction may either be “closed” using traction (Newton et al., 2011) or “open” using surgical laminectomy, re-alignment and, when necessary fixation of the vertebral column (Fehlings et al., 2012). There is increasing clinical evidence that early decompression at < 8 - 12 h results in better functional outcomes (Jug et al., 2015; Yousefifard et al., 2017). However, both closed and open reduction involve manipulation of the vertebral column alone. A more invasive clinical approach is to include durotomy (Perkins and Deane, 1988) and a recent study in humans showed that laminectomy alone does not always relieve pressure but that durotomy

* Corresponding author at: Institut 1 für Anatomie der Universität zu Köln, Joseph-Stelzmann-Strasse 20, D-50931 Köln, Germany.
E-mail addresses: habib@bendella.de (H. Bendella), gregor.stein@uk-koeln.de (G. Stein), paptheo@med.auth.gr (T. Papamitsou), NakamuraM@kliniken-koeln.de (M. Nakamura), BouillonB@kliniken-koeln.de (B. Bouillon), m.galea@unimelb.edu.au (M. Galea), peter.batch@unimelb.edu.au (P. Batchelor), sarah.dunlop@uwa.edu.au (S. Dunlop), angelov.anatomie@uni-koeln.de (D. Angelov).
[1] These authors contributed equally and share first authorship.
[2] These authors contributed equally and share senior authorship.

https://doi.org/10.1016/j.expneurol.2018.04.011
Received 2 March 2018; Accepted 17 April 2018
Available online 19 April 2018
0014-4886/

is also required (Phang et al., 2015).

The above procedures do not involve the spinal cord itself. A yet more aggressive approach is acute myelotomy whereby both the dura and then the spinal cord are cut longitudinally at the injury site allowing removal of necrotic tissue and extravasated blood. (Koyanagi et al., 1989; Mccormick and Anson, 2012; Tachibana et al., 1984; Wagner Jr and Rawe, 1976). The first findings, over a century ago, involved individual case studies in dogs and humans (Allen, 1911, 1914) reporting functional improvement in dogs ($n = 5$) and in one of the three human cases. Subsequent individual case studies in humans ($n = 4$) also reported some improvement in function in one case (Koyanagi et al., 1989). A quasi-experimental study in dogs and rat (Freeman and Wright, 1953) and a more recent controlled experimental study in rat, suggest that myelotomy results in improved function and some tissue preservation (Rivlin and Tator, 1979; Yang et al., 2013). Two other studies have also shown improved function by combining myelotomy with treadmill training in rat (Kalderon et al., 2007) or with exercising the paralysed limbs in human (Zhu et al., 2008).

There are few pre-clinical experimental studies examining myelotomy alone and the technique employed was variable. Thus, myelotomy was performed at various intervals following SCI ranging from immediately (Freeman and Wright, 1953), 15 min, i.e. prior to anaesthetic recovery (Rivlin and Tator, 1979) or 8, 24 or 48 h after SCI and involving re-anaesthesia (Yang et al., 2013). The technique itself also involved half- or full-depth incision (Freeman and Wright, 1953; Rivlin and Tator, 1979) or blunt dissection and irrigation (Yang et al., 2013). Furthermore, functional outcome measures were limited to locomotion (Freeman and Wright, 1953), inclined plane (Rivlin and Tator, 1979) or both (Yang et al., 2013). Tissue analysis was either not undertaken (Freeman and Wright, 1953) or structural (luxol fast blue, electron microscopy; Yang et al., 2013). We have therefore built on these findings and examined whether myelotomy after SCI influences functional measures, namely locomotor, bladder and sensory function, as well as tissue preservation and markers of cellular reactivity and synaptic plasticity.

2. Materials and methods

2.1. Animal groups and overview

Twenty adult (175–200 g) female Wistar rats (strain HsdCpb:WU, Harlan, An Venray, The Netherlands) were used. All animals were subjected to SCI. Our initial experimental design was to provdie aninmals with daily whole body vibration (Wirth et al., 2013). However, during the course of the experiment, it became evident that whole body vibration did not provide any benefit and, for clarity, this is not considered further.

One group ($n = 10$) was not subjected to any treatment after surgery (SCI). The other group (n = 10) underwent dorsal myelotomy at 48 h following SCI (SCI + Myelotomy). Three rats from the SCI- and 2 rats from the SCI + Myelotomy group died during the 12 weeks postoperative survival period. Data from our own earlier experiments (Wirth et al., 2013) served as basis for our biometric analysis for which we used G*Power Version 3.1.4 (Franz Faul, University of Kiel, Germany). The rump-height index (RHI) that we measured 2 months after spinal cord injury (SCI) was 2.82 ± 0.13 points (Wirth et al., 2013) and we predicted that, after myelotomy, RHI would reach 4.5 points, corresponding to an effect strength of 3.3. We expected that the minimal relevant effect that we would be able to detect would be 0.5 points, which corresponds to an effect strength of 1.4 (no myelotomy vs myelotomy). For this comparison, a *t*-test requires 7–9 rats per group. In this case significant differences would be detected with a power of 80% and a type 1 error of 5%.

Animals were fed standard laboratory food (Ssniff, Soest, Germany), provided tap water ad libitum and kept at 23 °C on a 12-hour artificial light-dark cycle. All experiments were conducted in accordance with German Law on the Protection of Animals. Procedures were approved by the local Animal Care Committee (approval number 84-02.04.2014.A348).

2.2. Pre-SCI conditioning

All rats underwent pre-SCI conditioning by being trained to walk on a wooden beam and to climb a ladder daily for 2 weeks.

Before SCI, we also collected baseline data about thermal sensitivity. It is well known that at least some of the tail flick or foot withdrawal response is under spinal control so that any changes might reflect impaired function of spinal neurons rather than impaired communication between periphery and brain. Rats were trained to stay still in a transparent chamber. The chamber was placed on a plastic table below which a movable device was positioned such that, when activated, it emitted a laser beam (2 mm diameter, temperature of 55 °C). The beam was directed over the left and right hindlimbs and the tail. The device also contained sensors such that, once the laser was activated, time was measured in seconds (Ugo Basile Thermal Plantar™ Analgesia Instrument, Stoelting Europe, Dublin, Ireland). Any movement of the hindlimbs or tail away from the laser was also detected enabling us to determine the withdrawal latency; i.e. how long the beam was directed at the rat's hind limbs or tail before the animal reacted and moved away. Left and right hind limbs, as well as the tail, were stimulated three times, and values averaged (Carlton et al., 2009; Chew et al., 2013; Hargreaves et al., 1988; Takahashi et al., 2003).

All animals became rapidly (within 2–3 days) accustomed to sensitivity measurement conditions and did not show any signs of stress such as freezing or trying to bite, weight loss or lack of grooming.

2.3. Spinal cord injury

We used a technique for transient compression of the spinal cord (Curtis et al., 1993) adopted by our laboratory previously (Wirth et al., 2013; Manthou et al., 2017). Rats were anesthetized (1.8 Vol% isofluorane: Forene, Abbott, Germany, 0.6 l/min O_2: Conoxia, Linde, Germany and 1.2 l/min N_2O: Niontix, Linde, Germany) and laminectomy performed at thoracic vertebra 8. The exposed 10th thoracic segment of the spinal cord (at the level of the 8th thoracic vertebra) (Waibl, 1973) was compressed at a velocity of 100 mm/s for 1 s using electromagnetically controlled watchmaker forceps (Dumont #5, Fine Science Tool, Heidelberg, Germany) with closure being 50% of spinal cord diameter. Rats were kept at 37 °C for 12 h to prevent hypothermia and were then housed individually in standard cages. Bladders were voided manually two times a day (at about 7:00 and at about 19:00 o'clock) until the end of the experiment, i.e. 12 weeks after surgery for SCI or SCI + myelotomy.

2.4. Myelotomy

Myelotomy was performed at 48 h after SCI to allow for tissue liquefaction and enable clear identification of the lesion site anatomically, therefore allowing easy drainage. Forty-eight hours was also chosen so as to minimize complications that might arise if a second surgical procedure was performed too soon after the SCI. The decision to undertake the second surgery (i.e. myelotomy) at 48 h was therefore pragmatic and conformed to Institutional ethical requirements.

We adopted the procedure of incision followed by blunt dissection and irrigation (Yang et al., 2013). Rats were anesthetized (1.8 Vol% isofluorane: Forene, Abbott, Germany, 0.6 l/min O_2: Conoxia, Linde, Germany and 1.2 l/min N_2O: Niontix, Linde, Germany) and following disinfection, the skin and muscle sutures were removed and the back muscles retracted. Under a surgical microscope, fine granulation tissue was resected and the dark purple non-pulsating dura mater spinalis carefully inspected. The largest hemorrhage was punctured with a 27G cannula followed by a longitudinal durotomy (about 3.0 mm) through