Title: Histopathological Effects of the Intrathecal Chondroitinase-ABC Administration in Spinal Cord Injured Rats; A Systematic Review

Running Title: Chondroitinase-ABC effect on SCI

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Abstract

Background/Objectives: Chondroitinase ABC (ChABC) has been known as a potential treatment option for spinal cord injury (SCI). We aim to identify and evaluate the histopathological effects of intrathecal ChABC administration in SCI rat models.

Methods: We searched PubMed/MEDLINE, Scopus, Web of Science, Embase, and Cochrane Library for studies published from the inception of each database until 22 November 2022.

Results: Of 3857 screened citations, 17 studies met eligibility criteria and were entered into the qualitative analysis. Sixteen studies were of high quality and one study was of medium quality. The Four main types of rats used in studies included Sprague Dawley, Wistar, Lister hooded and Long-Evans rats, respectively. ChABC treatment phases were considered acute (within 24 hours after injury), sub-acute (five or seven days after injury), or chronic (four or six weeks after injury). Accordingly, ChABC administration in the acute phase of injury significantly reduced cyst formation and promoted tissue preservation and sensory neuron plasticity. Regardless of the treatment phase, ChABC administration significantly promoted serotonergic and corticospinal fiber plasticity. Nine of the 14 studies that reported on functional outcomes found that ChABC administration either alone or combined with other treatments including rehabilitation improved motor functions.

Conclusions: The specification of anatomical changes for ChABC treatment can be used to explain functional improvements that have been reported for ChABC use in SCI. The limited studies on more clinically relevant contusion and compression injury models warrant further studies on these injury models and alternate treatment phases.

Keywords: Chondroitinase ABC, Histopathology, Spinal Cord Injuries, Functional outcome

1. Introduction

Traumatic spinal cord injury (SCI) is a frequent condition that significantly burdens societies(Ackery et al., 2004; Ahuja et al., 2017; Singh et al.). SCI patients have numerous therapeutic challenges since neurologic recovery is limited despite rehabilitation.

There is a growing foundation of data and knowledge concerning SCI mechanisms and the resulting histopathologic effects after spinal cord injury. The secondary injury after spinal cord injury begins with the migration of inflammatory cells and marked inflammation at the injury site, resulting in cell toxicity and neuronal damage (Donnelly & Popovich, 2008; Hausmann, 2003). Myelin is one of the key inhibitory factors following SCI. Unlike Schwann cells in the peripheral nervous system (PNS), spinal cord oligodendrocytes do not clear or remove the injured axons and myelin debris following SCI. Also, oligodendrocytes do not recruit macrophages and microglia to assist in this process(Lemons et al., 1999).

In the sub-acute phase of secondary injury following SCI, astrocytes become reactive and produce intermediate filaments, through a process called astrogliosis, which leads to the formation of a glial or perilesional scar around the injury site. This perilesional scar has tremendous effects in restoring the blood-brain barrier, supporting wound contraction, minimizing leukocyte infiltration, and limiting neuronal damage and demyelination (Faulkner et al., 2004); however, it inhibits later neuronal plasticity, axonal regeneration and sprouting (Fitch & Silver, 1997; Herrmann et al., 2008; Karimi-Abdolrezaee & Billakanti, 2012). Expression of chondroitin sulfate proteoglycans (CSPG) is one of the major parts of glial scar formation (Jones et al., 2002; Morgenstern et al., 2002). Thus, Glial scar removal or clearance by targeting CSPGS in the perilesional scar may create an environment in favor of regeneration of the injured spinal cord.

Chondroitinase ABC (ChABC), a bacterial endolyase, removes glycosaminoglycan (GAG) chains from CSPG, which cause CSPG proteolysis after its formation (Fawcett, 2015; Wang et al., 2008). ChABC targeted proteolysis is safer than enzyme therapy which has been used in the past (Guth et al., 1980). Enzyme therapies (including trypsin, hyaluronidase, elastase, elastase plus trypsin, or vehicle) can result in dissolving blood vessel walls causing hemorrhage(Guth et al., 1980). Therefore, ChABC is a promising alternative therapy for promoting axonal plasticity in SCI, not only in the acute phase by preventing glial scar formation, but also in the chronic SCI phase through digesting of the glial scar and promoting regeneration and functional recovery(Filous et al., 2010; Houle et al., 2006). Bradbury et al.(Bradbury et al., 2002) for the first time, showed beneficial effects of ChABC in promoting axonal regeneration and functional improvement after SCI. Despite the vast number of preclinical studies in this area(Filous et al., 2010; Houle et al., 2006; Massey et al., 2008), the exact mechanism of action is not known. however, much of the mechanism has been identified. Here, we systematically review the literature to summarize all potential histopathological effects of intrathecal administration of ChABC in spinal cord injured rats.

2. Methods

Our systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) 2020 Checklist(Page et al., 2021).

2.1 Information sources and search strategy

We performed a comprehensive electronic search for studies published until November 22, 2021 in PubMed; Scopus; Web of Science; Embase and Cochrane library. Details of the search strategies

in different databases are presented in Appendix 1. We did not limit the search strategy regarding the study types, language or time of publication.

2.2 Eligibility Criteria, selection process and data extraction

Two independent reviewers performed the screening and any discrepancies were resolved by a third reviewer. The inclusion criteria were experimental animal studies on SCI models in adult rats that investigated intrathecal ChABC treatment. To explore the exact histopathologic effect of ChABC on SCI recovery process, we limited our inclusion criteria to just intrathecal administration of ChABC. We included a study if there were at least two intervention groups: intrathecal ChABC treatment group and the control group (i.e., sham surgery or without treatment), and the study presented details of the histopathological outcomes. According to the group's neurosurgeons' opinions, we preliminary defined five histopathologic outcome groups as the most clinically relevant outcomes of interest including:

1. Lesion breadth; also known as cavity volume, cysts' size, myelin to cavity size ratio or atrophic area.

2. Serotonergic (5HT) neurons' plasticity; defined as total 5HT fibers' number crossing the lesion or 5HT fibers' side branches in the lesion site.

3. Corticospinal tract (CST) plasticity; defined as total CST fibers' number in the lesion site, CST fibers' crossing the lesion site or CST fibers' side branches in the lesion site.

4. Sensory neurons' plasticity; defined as total sensory fibers' number in the lesion site, sensory fibers' crossing the lesion or sensory fibers' side branches in the lesion site.

5. Electrophysiological outcomes; defined by postsynaptic cord dorsum potentials (CDPs), the amplitude of action potential volleys, and neuronal conduction latency.

Studies that included: 1) Review articles, 2) Case reports and case series, 3) Conference abstracts and posters without published paper in the peer-reviewed journals were excluded. Also, we manually searched the references of the related reviewed articles to identify any additional related articles.

A pre-defined data extraction form was used and presented in Appendix 2.

Two independent reviewers performed the data extraction process into the forms and in case of any discrepancy, a spinal cord injury specialist was consulted.

2.3 Risk of bias assessment

The risk of bias of the included studies was evaluated using the assessment tool for the quality of SCI animal models(Hassannejad et al., 2016) for pathophysiological events after experiments on traumatic SCI. This assessment tool evaluates the studies regarding 15 domains: (1) animal species; (2) applying suitable tests; (3) severity of the SCI; (4) level of SCI; (5) age/weight; (6) experimental group sizes; (7) description of strain; (8) description of control groups; (9) statistical analysis details; (10) regulation of ethical issues; (11) bladder expression; (12) the blindness of measurements; (13) genetic background; (14) method of random allocation to experimental groups; (15) details of animal exclusions during the study.

If each column had no risk of bias, it was scored as positive "+ "and if the presence of risk of bias was unclear due to insufficient descriptions in the article, it showed as "– "and high risk of bias in

each column was scored as negative. Differences in assessment were discussed during a consensus meeting. A total score was computed by adding the number of positive scores, and high quality was defined as fulfilling 8 or more (more than 50%) of the 15 internal validity criteria. Finally, risk of bias was assessed for each article included in data extraction form.

3. Results

A total of 3857 records were retrieved during databases and bibliographies search, of which 2,144 were unique after duplicates were removed. After screening by title and abstract, 1957 articles were excluded and 187 papers were selected for full-text assessment. Of the 187 articles, 156 were excluded due to lack of intervention's criteria (n=63), lack of control group (n=1), conference abstracts without peer-reviewed paper (n=38), review articles (n=2) and lack of histopathological outcome measures of interest (n=52). Seventeen studies(Barritt et al., 2006; Caggiano et al., 2005; Führmann et al., 2018; García-Alías et al., 2008; García-Alías et al., 2011; Ishikawa et al., 2015; Karimi-Abdolrezaee et al., 2010; Kim et al., 2006; Massey et al., 2008; Mountney et al., 2013; Pan et al., 2018; Shields et al., 2008; Shinozaki et al., 2016; Tom et al., 2009; Wang et al., 2011; Xia et al., 2015; Yang et al., 2009) were included in the qualitative analysis based on the following PICO: (Fig. 1) P: Adult rats, I: Intrathecal ChABC administration, C: Sham surgery or non-treated injured animals, O: As entered in the method section. All included studies had a low risk of bias except one, which had a moderate risk of bias (Table 1).

All studies used adult rats, of which 11 studies used the Sprague Dawley(Führmann et al., 2018; García-Alías et al., 2011; Ishikawa et al., 2015; Kim et al., 2006; Massey et al., 2008; Mountney et al., 2013; Shields et al., 2008; Shinozaki et al., 2016; Tom et al., 2009; Xia et al., 2015; Yang et al., 2009), 3 the Wistar (Barritt et al., 2006; Karimi-Abdolrezaee et al., 2010; Pan et al., 2018), 2

the Lister hooded(García-Alías et al., 2008; Wang et al., 2011), and 1 the Long-Evans rat(Caggiano et al., 2005), respectively. Although all studies stated the size or number of the animals treated with ChABC and controls, only seven mentioned the total number of animals sampled (a total of 440 rats) (Barritt et al., 2006; Führmann et al., 2018; Ishikawa et al., 2015; Karimi-Abdolrezaee et al., 2010; Pan et al., 2018; Shields et al., 2008; Shinozaki et al., 2016). The most common injury model in these articles was transection injury (García-Alías et al., 2008; García-Alías et al., 2011; Ishikawa et al., 2015; Kim et al., 2006; Massey et al., 2008; Pan et al., 2018; Shields et al., 2008; Tom et al., 2009; Wang et al., 2011; Xia et al., 2015). Compression injury (Caggiano et al., 2005; Führmann et al., 2018; Karimi-Abdolrezaee et al., 2010) and contusion injury(Barritt et al., 2006; Mountney et al., 2013; Shinozaki et al., 2016; Tom et al., 2009; Yang et al., 2009) were other injury methods. One study deployed both transection and contusion models(Tom et al., 2009). The size, level and severity of the injury as well as the injection time and whether uni- or bilateral injury were used are presented in Table 2

The results based on the phase of treatment and the details of methodological information, and key findings of the included studies are presented in Table 3. The treatment phase was referred to the time of ChABC administration (first dose in multiple dose regimens), and the studies were grouped as acute (within 24 hours after injury), sub-acute (five or seven days after injury) or chronic (four or six weeks after injury) treatment phases. Four studies presented results of ChABC treatment on SCI lesion breadth(Caggiano et al., 2005; Führmann et al., 2018; Pan et al., 2018; Shinozaki et al., 2016). Eight studies evaluated the effects of ChABC treatment on serotonergic fibers plasticity and regeneration after SCI through anti-serotonin (5-hydroxytryptamine (5-HT)) immunostaining(Barritt et al., 2006; Ishikawa et al., 2015; Karimi-Abdolrezaee et al., 2010; Kim et al., 2006; Mountney et al., 2013; Shinozaki et al., 2016; Tom et al., 2009; Wang et al., 2011).

CST remodeling after ChABC treatment was assessed in twelve studies(Barritt et al., 2006; García-Alías et al., 2008; García-Alías et al., 2011; Ishikawa et al., 2015; Karimi-Abdolrezaee et al., 2010; Kim et al., 2006; Massey et al., 2008; Shields et al., 2008; Shinozaki et al., 2016; Wang et al., 2014; Xia et al., 2015; Yang et al., 2009). Of the 17 studies, eight used biotinylated dextran amine (BDA)-labeling for axonal tracing (Barritt et al., 2006; García-Alías et al., 2008; García-Alías et al., 2011; Karimi-Abdolrezaee et al., 2010; Kim et al., 2006; Wang et al., 2011; Xia et al., 2015; Yang et al., 2009), one used WGA labeling (Shinozaki et al., 2016) and another used Protein kinase C-γ(Ishikawa et al., 2015). Two high-quality studies used cholera toxin B-subunit (CTB) labeling in the acute SCI model to assessed the potential effects of ChABC treatment on sensory neurons (Massey et al., 2008; Shields et al., 2008). The other study used calcitonin gene-related peptide (CGRP) immunohistochemistry(Barritt et al., 2006). Again, two other high-quality studies presented the results of electrophysiological outcome measures of our interest (García-Alías et al., 2011; Yang et al., 2009).

3.1. Acute phase

3.1.1. Lesion breadth

One study used transection injury models(Pan et al., 2018), and two used compressions(Caggiano et al., 2005; Führmann et al., 2018) to report the results of acute ChABC administration on lesion breadth. In transection and contusion models, ChABC administration significantly decreased lesion length, cyst length at the injury site, hole size, and atrophic area volume(Pan et al., 2018; Shinozaki et al., 2016). However, acute administration of ChABC did not change the lesion length in the compression injury model.

3.1.2. Plasticity of serotonergic neurons

Two studies used transection injury models(Ishikawa et al., 2015; Kim et al., 2006), two studies used contusion(Barritt et al., 2006; Mountney et al., 2013), and one study used both transection and contusion models(Tom et al., 2009) to assess the effects of acute ChABC administration on serotonergic neuron plasticity. In transection models, ChABC increased the number of serotonergic fibers at the injury site and rostral part to the injury, but it had a weak effect on reaching the caudal sites. In contusion injury models, treatment positively affected the ventral caudal horn in contrast to the caudal dorsal horn, where it did not change.

3.1.3. Plasticity of corticospinal neurons

Four studies used transection injury models (García-Alías et al., 2008; Kim et al., 2006; Wang et al., 2014; Xia et al., 2015), and two used contusion(Barritt et al., 2006; Yang et al., 2009) to assess changes in CST neuron plasticity in response to acute ChABC administration. In transection models, treatment increased the number of CST fibers and length rostrally, but the results were not consistent at the injury site and caudal regions. Axonal sprouting was also increased at the injury site. One of two contusion studies reported increased growth of CST fibers into and beyond the injury site and increased terminal arborization (Barritt et al., 2006). In contrast, the other study(Yang et al., 2009) reported no difference in the length and number of fibers. The only compression study reported increased fiber growth and sprouting at the injury site and caudal regions(Führmann et al., 2018). For more powerful conduction, more studies on contusion and compression models are warranted.

3.1.4. Plasticity of sensory neurons

One study used transection injury models(Shields et al., 2008) and another used both compression and contusion models (Barritt et al., 2006). Both studies observed that acute administration of ChABC promoted afferent fiber plasticity and growth into and beyond the injury site. However, none of the included studies evaluated electrophysiological outcomes during the acute phase.

3.2. Sub-acute phase

3.2.1. Lesion breadth

Two studies that used compressive injury models (Caggiano et al., 2005; Führmann et al., 2018) reported results regarding the effects of ChABC sub-acute administration on lesion breadth that showed weak positive effects for this treatment. Concerning the other outcome of interest, we did not find studies that reported on the evidence on the effect of ChABC administration on serotonergic, sensory and corticospinal neurons plasticity.

3.3. Chronic phase

3.3.1. Lesion breadth

Only one contusion injury study (Shinozaki et al., 2016) assessed lesion breadth changes in response to chronic administration of ChABC, and found no influence on ChABC treatment on lesion breadth changes. However, two studies reported increased tissue or axonal preservation with this treatment with treadmill rehabilitation (Shinozaki et al., 2016) or neural progenitor cells (NPCs) transplantation(Karimi-Abdolrezaee et al., 2010).

3.3.2. Serotonergic neurons plasticity

Chronic administration of Ch-ABC significantly increased the number and length of serotonergic fibers and sprouting at the rostral epicenter and caudal side of lesion site(Karimi-Abdolrezaee et al., 2010; Shinozaki et al., 2016).

3.3.3. Corticospinal neuron plasticity

Chronic administration of ChABC significantly increased the crossing and sprouting of CST axons and regrowth length rostrally(Karimi-Abdolrezaee & Billakanti, 2012; Karimi-Abdolrezaee et al., 2010; Shinozaki et al., 2016), but in caudal regions, there was no consensus on treatment effects. Two of the included studies(Karimi-Abdolrezaee & Billakanti, 2012; Shinozaki et al., 2016) reported no effects at caudal regions. Overall, none of the included studies evaluated electrophysiological outcomes during the chronic phase.

Functional outcome

Most of the studies used the Basso, Beattie, Bresnahan (BBB) scale (for hindlimb), ladder walk test/Staircase reaching test, Grid-walking/strength test, hindlimb contact placing response/ vertical exploration (rearing) and sticker removal tests (for forelimb function), single pellet reaching task, fine touch and mechanical hyperalgesia assessment, spinal cord evoked potential (SCEP) and motor evoked potential (MEP) assessment to evaluate motor and sensory functional outcomes(Caggiano et al., 2005; Führmann et al., 2018; García-Alías et al., 2008; García-Alías et al., 2011; Ishikawa et al., 2015; Kim et al., 2006; Mountney et al., 2013; Pan et al., 2018; Shinozaki et al., 2016; Tom et al., 2009; Wang et al., 2011; Xia et al., 2015; Yang et al., 2009). Also one study used residual urine volumes to test for autonomic functions(Caggiano et al., 2005). Overall,

the functional outcome were mixed, with 3 studies showing no improvement in functional outcomes after ChABC administrations(Führmann et al., 2018; Mountney et al., 2013; Tom et al., 2009) and 9 showing that ChABC administration alone(García-Alías et al., 2008) or combined with neurotrophin NT-3 secretion and NR2D expression(García-Alías et al., 2011) or NPCs(Karimi-Abdolrezaee et al., 2010) or antisense vimentin cDNA(Xia et al., 2015) or poly(glycerol sebacate)(Pan et al., 2018) or insulin+ methylprednisolone(Yang et al., 2009) or transplant mediated axonal remodeling or rehabilitation (Ishikawa et al., 2015; Shinozaki et al., 2016; Wang et al., 2011) improved functional outcomes, particularly motor functions (Table 3).

4. Discussion

Spinal cord injury (SCI) is a devastating clinical condition that results in rapid-onset and long-term disability related to the central nervous system. Several underlying mechanisms have been identified as factors responsible for primary and secondary damage following SCI. Primary mechanisms include neural death and axonal injury, which subsequently eventuate in sensorimotor disruption(de Almeida et al., 2023). After the primary complications, secondary mechanisms are initiated, compromising inflammation, vascular changes, ion disproportion, glutamate excitotoxicity, and radical formation that result in additional complications, including progressive neural death, edema, hyperpyrexia, and paralysis(de Almeida et al., 2023; P., 2019). Inflammation and granulocyte colony stimulating factor (GCSF) production are two major components of glial scar formation that hamper the recovery of SCI(Shechter et al., 2011). A correlation between inflammation and the expression of GCSF through inflammatory cytokines has been shown in the literature(Shechter et al., 2011). The evidence suggests that inflammatory cytokines enhance the expression of GSK3β that subsequently promote demyelination and degeneration of neurons (Nagai et al., 2016; Renault-Mihara et al., 2011). Furthermore, GSK3β may prevent post-SCI

neuronal regeneration through the influence of GSCF discharge (Nagai et al., 2016). Therefore, the therapeutic agents are made to hamper the molecular mechanism of complications related to SCI.

Although several therapeutic approaches including pharmacological, stem cell-based, and enzyme-based approaches have been developed for SCI, the management of patients with SCI remain challenging in developing or even developed countries. The difficulty in treating SCI patients is thought to be related to the environmental factors presented in the injured area(de Almeida et al., 2023). However, progression in the understanding of the pathology of SCI has led to de novo research on new treatment approaches. Recent studies have been focused on the secondary mechanisms involved in SCI to prevent further damages and facilitate the neural regeneration. Kwon et al. (Kwon, Okon, Hillyer, et al., 2011) systematically reviewed the pre-clinical studies of neuroprotective agents already prescribed in humans. This study documented the potential effect of non-invasive medications for acute SCI, including erythropoietin, progesterone, estrogen, riluzole, Polyethylene glycol, atorvastatin, magnesium, minocycline, inosine, NSAID, anti-CD11, and pioglitazone. In 2010, Cadotte et al. (Cadotte & Fehlings, 2011) highlighted the effect of riluzole, anti-Rho antibody, and surgical decompression on SCI to establish the evidence for translating pre-clinical research to clinical research.

In 2011, another systematic review study by Kwon et al.(Kwon, Okon, Plunet, et al., 2011) aimed to investigate the in-vivo studies on the efficacy of intra-spinal ChABC, anti-Nogo antibody, and anti-Rho antibody strategies to provide evidence for translation of the pre-clinical studies to human studies and clinical participan. They found that Nogo receptor on neural cells prevented the neural growth, suggesting that Anti-Nogo antibody to be a promising therapeutic approach to influence neural recovery following SCI. Also, clinical trials have been conducted to investigate the

influence of Anti-Nogo on acute and chronic SCI patients. Additionally, although riluzole is a sodium glutamate antagonist agent applied in patients with amyotrophic lateral sclerosis (ALS), it was shown to be potential drug for SCI patients, due to its neuroprotective effect. A recent systematic review which was carried out on the efficacy of riluzole on SCI including animal and human studies showed that riluzole is also a promising pharmacological agent which improve behavioral, histological sparing, and had electrophysiological features following SCI and diminishes the sequela of this condition(Srinivas et al., 2019).

Several key components, such as inflammation, oxidative stress, and edema have been considered in the pathophysiology of SCI that can be directed to developing effective therapeutic modalities. For instance, oxidative stress is putative to be significantly involved in the secondary deterioration of SCI. Therefore, depletion of reactive oxidative stress could be a capable approach to prevent SCI associated secondary deterioration among patients. Medication including steroid glucocorticoids and non-glucocorticoid 21-aminosteroid tirilazad also have an anti-oxidative stress activity that significantly enhance the recovery of SCI. (Jia et al., 2012). Likewise, edema is another essential component of the pathophysiology of SCI, which is initiated rapidly within minutes following injury (Rowland et al., 2008). Leonard et al. observed that edema induced injury deterioration of and was associated with more severe complications(Leonard et al., 2015). Therefore, a systematic review conducted on the treatment option targeting edema in SCI introduced three main approaches to eliminate edema, including inhibition of aquaporin 4 (AQP4), immunosuppression, and surgery. Furthermore, trifluoperazine which prevent AQP4 localization was proposed to be the most effective treatment that significantly reduces edema within a week after injection (Masterman & Ahmed, 2021).

Lemons et al.'s (Lemons et al., 1999) investigation is the first study that reported the increase of Chondroitin Sulfate Prostaglandins (CSPGs) after SCI at the site and adjacent of injury. Moreover, they showed that astrocyte is a source of CSPG production which ensued lack of neural regeneration. Besides, they suggested that exogenous ChABC administration could digest the CSPG which manifest the role of CSPGs as inhibitory out-growth factor. These findings also unveil a potential therapeutic effect of ChABC. Further studies have documented that local injection of ChABC is associated with protein-based regeneration of neurons(Barritt et al., 2006; Ishikawa et al., 2015). Moreover, Lee et al.(Lee et al., 2010) found that ChABC administration had effects on the CSPG and inflammation in that it reduced the level of CSPG and GSK3β and accelerated neural growth(Yılmaz & Kaptanoğlu, 2015).

A systematic review and meta-analysis conducted by Yousefifard et al.(Yousefifard et al., 2022) demonstrated that ChABC has a moderate effect on the locomotor function of animal models of SCI without differences regarding the injury severity. They also revealed that the induction model of SCI and number of ChABC injection did not influence the efficacy of ChABC on the locomotor function of SCI. In mouse models, Carter et al. showed that ChABC was neuroprotective for cortical layer V projection neurons after ICV infusion (Carter et al., 2008). ChABC also prevented cell atrophy after localized delivery to the spinal cord, suggesting a possible retrograde neuroprotective effect mediated at the injury site(Carter et al., 2008).

Additional studies designed based on the combination of other treatment options like stem cells(Jevans et al., 2021), tissue engineering approaches(Raspa et al., 2021), sucrose(Raspa et al., 2019), photobiomodulation therapy(Janzadeh et al., 2020) antisense vimentin cDNA(Xia et al., 2015), poly(glycerol sebacate)(Pan et al., 2018), insulin+ methylprednisolone(Yang et al., 2009) and rehabilitation(Ishikawa et al., 2015; Shinozaki et al., 2016; Wang et al., 2011) with ChABC

showed improvement of the efficiency of Ch-ABC in SCI and improved functional outcomes. Additionally, a combination of ChABC and delayed injection of adeno-associated virus encoding the L1 cell adhesion molecule (AAV-L1) or pluripotent stem cell-derived NSCs (PS-NSC) in mouse models showed enhanced locomotor recovery after treatment (Lee et al., 2012; Suzuki et al., 2017). Furthermore, multiple injection of ChABC in Rhesus monkeys was associated with increased corticospinal axon growth, the number of synapses formed by corticospinal terminals in gray matter caudal to the lesion and improved hand functions(Rosenzweig et al., 2019). In this review, the functional outcomes assessed were motor, sensory and autonomic such as forelimb and hindlimb functions, gait, pellet reaching task fine touch and mechanical hyperalgesia assessment and residual urine volume assessment. Although the results were heterogeneous, majority of the studies showed ChABC administration improved functional outcomes in the animal models.

This systematic review entails the histological aspects of the efficacy of intra-thecal ChABC in SCI rats. Our comprehensive search resulted in seventeen eligible studies assessing relevant histopathological outcomes of intrathecal ChABC administration following SCI in rats. When analyzing treatment results based on the treatment phase, our results showed ChABC treatment in acute phase: 1) reduced necrosis and atrophic area and increased tissue preservation, 2) increased sensory neuron plasticity and growth into and beyond the injury site. Moreover, ChABC 3) increased the number of serotonergic fibers (5-HT), 4) reduced the apoptosis of neurons, 5) increased digestion of the CSPG, differentiation of stem cells to neurons and GAP-43, NG-2, chondroitin 4 sulfate, Biotinylated dextran amines (BDA) levels.

Regardless of the treatment phase, ChABC promoted the survival of serotonergic fibers, plasticity and regrowth to and beyond the injury site, the global plasticity and survival rostrally of CST fibers. To the best of our knowledge, this is the first systematic review evaluating the

histopathological effects of the intra-thecal ChABC treatment. To make the result of the ChABC treatment more tangible, we compared our results to studies using combinational treatment involving ChABC. In the systematic review by Kwon et al. (Kwon, Okon, Plunet, et al., 2011), the putative underlying mechanisms of intra-spinal ChABC injection for acute SCI was assessed. In this regard, axonal germination/growth of fibers, specifically serotonergic fibers, and neuroprotective effect of ChABC preventing the neural atrophy were the most promising mechanism of intra-spinal ChABC. Our results indicated that CST and serotonergic fibers sprouting, terminal arborization, and crossing were significantly increased after ChABC treatment, similar to the results of ChABC treatment in combination with rehabilitation(Marsh et al., 2011), Nogo-A inhibitors(Zhao et al., 2013), and increased neurotrophin-3 levels(Massey et al., 2008). We also found that ascending fiber regeneration was significantly promoted after intrathecal ChABC administration. This finding was reproduced in three combinational treatment studies using neurotrophin-3, cell plant(Massey et al., 2008), and conditioning agents like zymosan (Harel et al., 2012; Steinmetz et al., 2005). Grimpe et al.(Grimpe et al., 2005) and Vavrek et al.(Vavrek et al., 2007) reported that a combination of cell transplant and ChABC increased CST axon regeneration rostrally and promoted their elongation through the injury site and to the caudal sites. Our included articles in this area did not present congruent results. While another study (García-Alías et al., 2008) reported increased regrowth length of CST axons into and beyond the injury site, the results of four other studies (Kim et al., 2006; Shinozaki et al., 2016; Wang et al., 2014; Yang et al., 2009) did not show significant effects on regrowth to caudal sites for ChABC treatment. It seems that when a considerable atrophic area is present after injury, a graft is necessary to provide a tissue scaffold for regenerating axons, traversing the injury site. Although it is well known that ChABC improve axonal sprouting and neural functions through digestion of

CSPGs, the exact molecular mechanism underlying this process is not fully understood. However, Hu et al.(Hu et al., 2021) explored the key function of CSPGs in the regeneration of axons and neural apoptosis. They showed that the caspases activity is significantly increased within 2 to 11 weeks after injury. Importantly, ChABC is shown to reduce the total number of functional caspase-3 which validate the anti-apoptotic activity of ChABC displayed by kwon et al. (Kwon, Okon, Plunet, et al., 2011) study. Protein tyrosine phosphatase sigma (PTPs) is an example of CSPG receptors which is involved in the SCI-induced retrograde neural apoptosis. Correspondingly, the expression of PTPs which was associated with neural apoptosis was decreased following ChABC treatment that document the exact anti-apoptotic activity of ChABC in the SCI. Regarding functional outcomes, we found that ChABC in combination with other treatment, as mentioned above, seemed to be associated with better motor functions in animal models with SCI(García-Alías et al., 2011; Karimi-Abdolrezaee et al., 2010; Pan et al., 2018; Xia et al., 2015; Yang et al., 2009).

5. Strengths and limitations

As this is the first systematic review on the histopathological effects of ChABC treatment in SCI, results can be used to: 1) better our understanding on the mechanisms underlying positive functional outcomes, 2) understand the possible adverse effects to look for, and 3) discover the pitfalls in studies that require more attention in future works. The most important limitation of this study is that the small sample size of animal studies may both increase the risk of selection bias and make most effects non-significant. We grouped studies by their phase of intervention and injury model, and in some groups, there was only one study available, leaving very low evidence to deduct some points. Furthermore, since studies using different enzyme administration regimens

are limited, we did not summarize the results according to different regimens. This limitation can affect the results and may be one source of the non-convergent results.

However, clinical studies have more priority and can more directly benefit individuals with SCI, but preclinical studies provide the fundamental base for clinically approved treatments. Our study emphasizes the indispensable role of animal studies in advancing treatments for SCI and provides insight for bridging preclinical studies to patient care. This study also provided a comprehensive review of the histopathological effects of ChABC on rat SCI models. To have a clearer vision of the roles of ChABC in SCI, we classified the reviewed studies based on the phase of SCI, which is of high importance in this matter.

Conclusion

In summary, our systematic review of the available evidence for finding the histopathological effects of intrathecal ChABC administration in spinal cord injured rats suggests that this treatment can 1) reduce necrosis and atrophic area and increase tissue preservation, 2) increase sensory neuron plasticity and growth into and beyond the injury site, 3) reduce the apoptosis of neurons, 4) promotes the survival of serotonergic fibers, plasticity and regrowth to and beyond the injury site, the global plasticity and survival rostrally of CST fibers, 5) increase digestion of the CSPG, differentiation of stem cells to neurons and the GAP-43, NG-2, chondroitin 4 sulfate, Biotinylated dextran amines (BDA) level. ChABC treatment was associated with improved functional outcome in rats, mice and primates. Although these findings are promising, further studies can provide additional evidence for final deduction on the risks and benefits of ChABC in SCI.

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Ethical approval

The Ethics Committee of Sina Trauma and Surgery Research Center, Tehran University of Medical Sciences, approved the study, and the reference number is IR.TUMS.MEDICINE.REC.1398.886.

Conflict of interests

The authors declare that they have no competing interests except Alexander R. Vaccaro (Appendix 3).

Data availability

All data underling the results are available as part of the article, or in supplementary information files.

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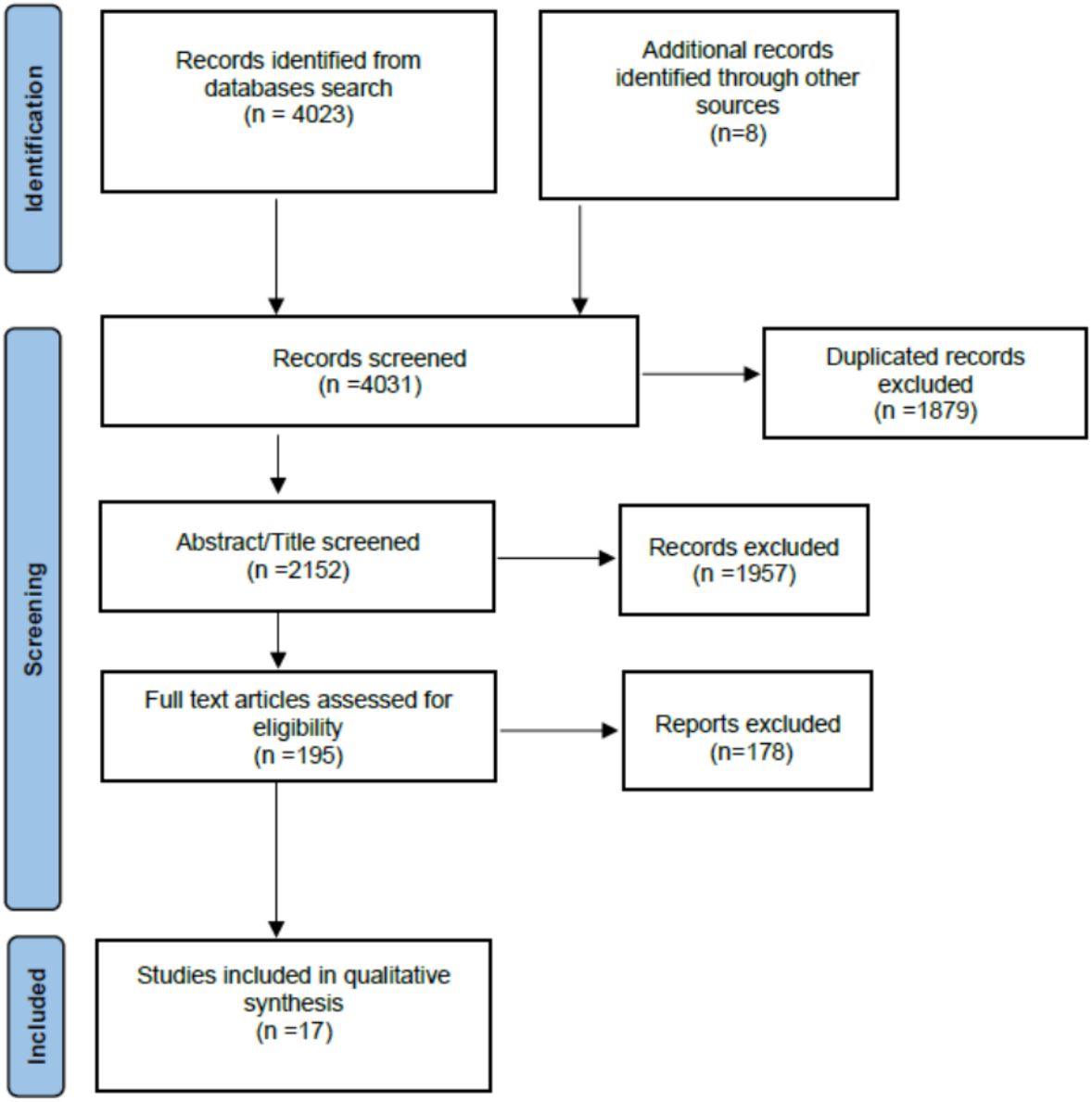

Figure 1. PRISMA flowchart diagram of the study process

Figure 1. PRISMA flowchart diagram of the study process

Table 1. The risk of bias assessment of included studies

Authors, year	Species	Age/weight of animal mentioned	Designation of strain	Number of samples/per groups	Level of injury	Measures Severity of injury	Consideration of genetic background	Method of allocation to intervention	Bladder expression	Description of the reasons to exclude animals from the experiment during the study	Regulation and ethics	Definition of control group	Using appropriate tests for evaluation of outcome	Blindness of assessor	Description of statistical analysis	Quality
Caggiano, 2005 [22]	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	H(14)
Barritt, 2006 [24]	+	+	+	+	+	+	+	+	-	-	+	+	+	-	+	H(12)
Kim, 2006 [26]	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	H(13)
Massey, 2006 [19]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	H(15)
García-Alías, 2008 [33]	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	H(14)
Shields, 2008 [29]	+	+	+	+	+	+	+	-	-	+	+	+	+	-	+	H(12)
Tom, 2009 [32]	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	H(13)
Yang, 2009 [23]	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	H(13)
Karimi-Abdolrezaee et al [37]	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	H(14)
Wang, 2011 [35]	+	-	+	+	+	+	+	+	-	-	+	+	+	-	+	H(11)
García-Alías, 2011 [31]	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	H(13)
Mountney, 2013 [25]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	H(15)
Xia, 2015 [30]	+	-	+	+	+	+	+	+	-	+	+	-	-	-	-	M(8)
Ishikawa, 2015 [27]	+	+	+	-	+	+	+	-	-	+	+	+	+	-	+	H(11)
Shinozaki, 2016 [34]	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	H(14)
Führmann, 2017 [36]	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	H(13)
Qi pan, 2017 [28]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	H(11)

No risk of bias: (+); the presence of risk of bias is unclear due to insufficient descriptions in the article or high risk of bias: (-); H: high quality; M: medium quality.

Table 2. Study Characteristics

First Author, year	Animal strain	No. of Rats	Gender/ age/ weight	Sample Size	Injury model/treatment phase group	Injury model	Injection time(S)	Injury level	Uni-or bi-lateral injury	Size of injury	Injury severity
Caggiano, 2005 [22]	Long-Evans rats	N.S	Female - -	ChABC I (Severe:10 Mod:19 Mild:15) Penicillinase control (Severe:10 Mod:19 Mild:15)	Acute Compression	Compression	Immediately after SCI + every other day for 2 weeks	T9-T10	Bilateral	0.9-1.3 mm	Mild Moderate Severe
Barritt 2006 [24]	Wistar rats	82	Male adult 200-250 gr	ChABC (N=16) Vehicle control (N=16)	Acute Contusion	Contusion	Immediately after SCI and subsequent injections on days 2, 4, 6, 8 and 10	C4	Bilateral	2 mm	Moderate
Kim 2006 [26]	Sprague Dawley rats	N.S	Female 2-3 months 200-250 g	ChABC (N=7) Penicillinase (N=7) Control (N=5)	Acute Transection	Hemi-transection	Immediately after SCI + 3, 7, and 11 days after SCI	Cervical	Uni-lateral: The entire right side of the cord plus the left dorsal column and adjacent gray matter	N.S	Destruction of more than two-thirds of the transverse area of the cervical spinal cord

Massey 2006 [19]	Sprague Dawley rats	N.S	Male Adult 250–300 g	ChABC (N=6) Penicillinase control (N=5)	Acute Transection	Transection	Immediately after SCI	C6–C7	Uni-lateral: from the lateral portion of the right side of the dorsal spinal cord, through the right dorsal horn, and into the left gracile fasciculus	1.5 mm	A 1.5-mm deep dorsal-to-ventral laceration
García-Alías 2008 [33]	Lister Hooded rats	N.S	- - 250–300 g	ChABC 6 (acutely after the spinal cord injury) ChABC 6 (2 days (G2) after the injury) ChABC 6 (4 days (G4) after the injury) ChABC 6 (7 days (G7) after the injury) Penicillinase control 6 (acutely after the spinal cord injury)	Acute Transection Sub-Acute Transection	Hemi-transection	Refer to column D	C4	Bilateral	2 mm	Complete cut 2 mm in depth into the spinal cord parenchyma

Shields 2008 [29]	Sprague Dawley rats	22	Female adult 200-225 g	ChABC low dose (N=8) ChABC High dose (N=9) Penicillinase control (N=5)	Acute Transection	Hemi-transection	Immediately after SCI + alternate days for a total of 5 injections (days 0,2, 4, 6, and 8)	C3	Uni-lateral	3.6 mm (blade) × 1.5 mm deep	Complete cut 1.5 mm deep
Tom 2009 [32]	Sprague Dawley rats	N.S	Female Adult 250-225 g	ChABC (hemisection, rostral tx) (N=8) ChABC (hemisection, caudal tx) (N=8) ChABC (hemicontusion) (N= 8) PBS controls (N=22)	Acute Transection Acute Contusion	Hemi-transection Hemi-contusion	Immediately after SCI	C5	Uni-lateral	2-3mm	1-Complete transection 2- 200 kdyne force with a displacement of tissue to a depth of 1600–1800 mm
Yang 2009 [23]	Sprague Dawley rats	N.S	Male and Female Adult 180 - 250 g	ChABC (N=36) Saline control (N=36)	Acute Contusion	Contusion	30 minutes after SCI	T8 – T10	N.S	3 mm	A 10 g rod, 3 mm in diameter and 100 mm in length was dropped onto the exposed spinal cord surface
Karimi-Abdolrezaee [37]	Wistar rats	165	Female Adult 250g	ChABC (number not specified) Penicillinase control (number not specified)	Chronic compression	Compression	6 weeks after SCI	T7	N.S	N.S	23 g clip (Walsh) compression injury for 1 min

Wang 2011 [35]	Lister hooded rats	N.S	Male - 150–200 g	Ch-ABC (N=10) Penicillinase control (N=10)	Chronic Transection	Transection	One month after the SCI, a total of five injections once every 2 d following surgery	C4	Bilateral	2mm	Complete transection
García-Alías 2011 [31]	Sprague Dawley rats	N.S	Female Adult 200g	ChABC 22 uninjured Control 6 sham control 6	Acute Transection	hemi-transection	immediately after SCI	T8	Uni-lateral (left hemicord was transected completely)	N/A (left hemicord transection)	Complete transection
Mountney 2013 [25]	Sprague Dawley rats	N.S	Female Adult 250–375 g	ChABC (N=10) Carrier control (N=12)	Acute Contusion	Contusion	Immediately after injury	T9	Bilateral	1.2 – 0.1 mm	Moderate (200 kdyne)
Xia 2015 [30]	Sprague Dawley rats	N.S	Female - 250-300	ChABC (N=6) Saline control (N=6) Sham (N=6)	Acute Transection	Transection	Immediately after injury	T9-T10	Uni-lateral	1.8 mm	Severe
Ishikawa 2015 [27]	Sprague Dawley rats	58	Female Adult 200-250 g	ChABC (N=5) Uninjured Control (N=3) Sham control (N=5)	Acute Transection	Transection	Immediately after SCI	C3-C4	Uni-lateral	2 mm	Complete cut 2 mm in depth in the spinal cord.
Shinozaki 2016 [34]	Sprague Dawley rats	61	Female Adult 200–220 g	ChABC (5HT, cavity) (N=7) Vehicle control (N=8) Ch-ABC (CST) (N=3) Vehicle control (N=5)	Chronic Contusion	Contusion	T10	Six weeks after SCI	N.S	N.S	Severe (250 kdyn)
Führmann 2017 [36]	Sprague Dawley rats	60	Female Adult 300 g	Ch-ABC (N=11) Vehicle	Sub-Acute Compression	Compression	One week after SCI	T1-2	Bilateral	Not mentioned exactly.	21 g modified aneurysm clip for 1

				control (N=11)						Approximately ~1 mm	min (moderate)
Qi Pan 2017 [28]	Wistar rats	50	Female average 10 weeks average 250 g	ChABC (N=12) Sham control (N=6) NaCl/Saline control (N=8)	Acute Transection	Transection	Immediately after SCI	T10	Uni-lateral	2 mm	Complete transaction

N.S; Not specified, ChABC; Chondroitinase ABC, SCI; spinal cord injury

Table 3. Chondroitinase ABC treatment phase, the details of methodological information, histopathological and functional outcomes

First Author, year	Tracing of CST axons	Injection dose	Injection details	Injection site + device	Histopathological Findings	Functional outcomes
Caggiano 2005 [22]	N.S	0.06 Units (defined as the quantity of the enzyme that catalyzes the formation of 1 mole of unsaturated disaccharide from chondroitin-6-sulfate per minute at 37°C, pH 8.0.) per rat per dose	5 µL of ChABC-XMC+ Seven days following clip compression injury and ChABC-XMC, 5 µL of ChABC-XMC (or XMC or buffer) was intrathecally injected through a 30 gauge angled blunt-tipped needle	Into the intrathecal space through a 30 G angled blunt-tipped needle	1-No obvious qualitative effect on injury length and breadth in different compressive injury severities for chondroitinase treatment	ChABC I treatment demonstrated improvements in both somatic motor and autonomic function in that animals treated with ChABC I scored higher on the BBB scale (>8), indicating consistent sweeping of the legs or paw placement and also some showed some degree of weight support. Regarding autonomic functions, residual urine volumes from the severely injured control group returned to approximately 4–6 mL per day, while residual volumes in the ChABC-treated rats declined to approximately 2 mL per day (Fig. 8). This finding was similar for ChABC-treated rats with moderate injury; ChABC treated rats and controls with mild injury showed no significant difference regarding residual urine volume.
Barritt 2006 [24]	Biotinylated dextran amine (BDA; 10,000 MW; Invitrogen, Eugene, OR)	10 U/ml. 6 µl	0.06 Units (defined as the quantity of the enzyme that catalyzes the formation of 1 mole of unsaturated disaccharide from chondroitin-6-sulfate per minute at 37°C, pH 8.0.) per rat per dose, in artificial cerebrospinal fluid (aCSF: cat. no. 58-7316, lot no. 111261; Harvard Apparatus, Holliston, MA)	Intrathecally (i.t.) + A 32-gauge catheter (cat. no. CS132G, lot no. 20422; ReCathCo, LLC, Allison Park, PA) with Chondroitinase ABC I (cat. no. 100332, lot no. E02201; Seikagaku, Associates of Cape Cod, Falmouth, MA) was inserted through the dural incision and fed rostrally to lay immediately caudal to the T9/T10 laminectomy	1. Chondroitinase ABC promotes the sprouting of serotonergic fibers ventral and caudal to a spinal cord injury 2. Chondroitinase treatment confirmed a significant increase in CST fibers' density compared to the vehicle group. 3. Chondroitinase ABC promotes sprouting of the corticospinal tract rostral, within, and caudal to a spinal cord injury 4. Chondroitinase treatment significantly promotes CGRP+ afferents plasticity caudally to a lesioned spinal cord. 5-Treatment with ChABC robust sprouting also occurred ventral to the injury site, with intense immunoreactivity and numerous sprouting fibers apparent in the ventral columns 6-Lesioned animals treated with ChABC had significantly increased intensity of PKC immunoreactivity	N.S
Kim 2006 [26]	The number of axons that are labeled by BDA	0.2 U oCh-ABC in 20 L vehicle solution (0.1% bovine serum albumin in saline) was applied with gel foam	10 µl of 100 U/ml	N.S	1-Remodeling of corticospinal axons is not affected by the degradation of CSPGs. 2-Raphespinal axon remodeling is promoted by ChABCase treatment following transplantation 3-Increase of serotonin immunoreactive fibers in rats treated with ChABC 4- ChABC greatly decreased the CS-56 immunoreactivity 5-ChABC increase in 2B6 immunoreactivity	Chondroitinase ABC in combination with transplantation improved fine control of the distal forelimb and skilled motor functions such as sticker removal and grid walking.
Massey 2006 [19]	5–10 µl of 1% CTB (Sigma-Aldrich, St. Louis, MO), dissolved in 0.25	1 µl of 50 U/ml	1.96 × 106 TU/µL/ 0.5 µL each injection	3 injection sites: at the lesion epicenter, 0.5 mm rostral, and 0.5 mm caudal to the	1-When the brainstems of ChABC-treated injured rats were examined, the CTB-immunoreactive forepaw terminal area was significantly greater compared with the P-ase-	N.S

	M Tris–HCl, pH 7.4,			lesion epicenter, with 2 injection depths: 1.2 and 0.6 mm from the dorsal surface	treated injured rats 2- ChABC reduced the CSPG 3- Significantly increased number of neurons	
García-Alías 2008 [33]	1 µl of 10% BDA solution in 0.01M phosphate buffer	6 µl. 100U/ml per injection	Every animal received a total of six injections, of chondroitinase (6 µl, 100U/ml per injection) or penicillinase (6 µl, 100U/ml per injection), one every two days following the first injection.	For drug delivery, the animals were gently immobilized and the drug was manually injected with a Hamilton 10 µl syringe attached to a 31-gauge internal cannula, which extended 0.6 mm beyond the guide cannula tip into the right ventricle.	1-In general, ChABC animals had less CST axon withdrawal from the injury and more axons growing further than the control group 2-Increase the chondroitin-4-sulfate and CSPG digestion 3-Increase the BDA labeling of the corticospinal tract	Forty-two days after the injury 5 out of 6 animals of every The ChABC-treated group regained forelimb placing response. Also, forelimb stride length improved in the ChABC-treated animals but not in the penicillinase-treated animals at 42 days ($p<0.05$)
Shields 2008 [29]	CTB (1% in distilled water; List Biological Laboratories Inc, Campbell, CA)	0.18 units per 6 µL (high dose). low-dose 0.06 units/6 µL	6 ml ChABC (10Uml21) was injected followed by a 6-ml saline flush (n ˆ 17). A further group received the spinal cord lesion with either saline or control enzyme treatment (Penicillinase, Sigma; same mg protein delivered, n ˆ 21).	Intrathecally inserted to lie just rostral to the lesion site, and externalized to deliver bolus injections of high-purity, protease-free Chondroitinase ABC (ChABC; Seikagaku Corporation)., 6 ml ChABC (10Uml21) was injected followed by a 6-ml saline flush (n =17). A further group received the spinal cord lesion with either saline or control enzyme treatment (Penicillinase, Sigma; same mg protein delivered, n = 21). ChABC or control solution + a silastic tube	1-Chondroitinase treatment caused a significant dose-dependent increase in sensory neurons' axonal number (2.1-fold low dose, 3.1-fold high dose vs control group) and axonal length (8.3-fold low dose, 9.9-fold high dose vs control group) 2-increase the number of axons growing into the scar 3-CSPG, NG-2 and CS-56 expression were decreased 4-2-B-6 expression was increased 5-ChABC infusion did not influence laminin and GFAP	N.S
Tom 2009 [32]	N.S	10 µl. 50U/mL	For infusion, an enzyme aliquot (0.33mg/ml (5 units/ml)) (200µl) was loaded into the reservoir of an Alzet 2004 osmotic minipump	After stereotaxic implantation of the infusion tip through the T9 vertebra, 1.3 mm into the thoracic cord, and 0.5 mm from the distal interface, the infusate was delivered at 0.5 µl/h from the loaded mini pump	1-Chondroitinase treatment leads to significantly more serotonergic fibers' preservation rostral to hemisection injury site, but not caudally. 2-Chondroitinase treatment leads to significantly more serotonergic fibers' preservation in the ventral horn of the caudal site of the hemi-contusion injury site 3-Increased 5HT+ fiber sprouting	No functional improvement. The study concluded that although administering ChABC rostral or caudal to a unilateral spinal cord injury is sufficient to promote the plasticity of supraspinal fiber populations; however, this plasticity does not appear to have functional ramifications. Their data demonstrated that sprouting rostral or caudal to a spinal cord injury does not always translate to recovery and indicates that other mechanisms may be responsible for ChABC-mediated functional recovery.
Yang 2009 [23]	1 µl biotinylated	6 µl ChABC. 10U/ml	0.05 U/200 µl	A thin silicone tube with an osmotic mini-pump (Model 2006; ALZET,	1-More nerve fibers and longer axons were found in rats treated with insulin, insulin plus ChABC or methylprednisolone than in rats of	At 4 weeks after spinal cord injury, the rats in the groups treated with insulin, insulin plus ChABC or methylprednisolone could walk normally, but the control rats and rats treated with ChABC alone could not.

	dextran amine (BDA) (BDA-3000, Molecular Probes, Eugene, OR, USA) for 20 min.			Cupertino, CA; 200 µl of the solution, 0.5 µl/h, 14 d delivery) into the subarachnoid cavity, and set the tube tip at the C3 level under a surgical microscope	the other two groups 2-Lower apoptosis in rats treated with Ch-ABC 3-More GAP-43 positive cells in rats treated with ChABC	From 0 to 2 weeks after injury, SCEP in rats treated with insulin, insulin plus ChABC, or methylprednisolone gradually recovered in latency and amplitude. From 1 to 2 weeks after injury, MEP latency and amplitude at the proximal position gradually recovered in the rats treated with insulin or insulin plus ChABC or methylprednisolone.
Karimi-Abdolrezaee [37]	BDA (10%, 10,000 MW; Invitrogen) was injected unilaterally into the left sensorimotor cortex at eight sites (0.5 µl per site)	5 U/ml of ChABC in saline plus 0.1% rat serum albumin	Seikagaku, 5 U/ml in saline plus 0.1% rat serum albumin) was administered intrathecally using a catheter [Alzet, Rat IT, 0007741, 0.36 mm outer diameter (OD); 0.18 mm inner diameter (ID)] connected to an osmotic mini-pump (Alzet pump model No. 1007D, 0.5 l/h) for 7 d. The animals underwent intraspinal bilateral injections of NPCs or vehicle with 8 µl of cell suspension, containing 4x 10^5 live cells, was intraspinally injected into the dorsolateral spinal cord, next to the midline	The catheter was inserted in the subarachnoid space around the injured area. One week after ChABC or vehicle treatment (7 weeks after SCI), the animals underwent intraspinal bilateral injections of NPCs or vehicle. Four intraspinal injections were bilaterally made at a point 2 mm rostral and 2 mm caudal to the injury site.	1-ChABC treatment at 6 weeks after injury for 1 week resulted in a pronounced reduction in CSPGs in the perilesional areas compared to the vehicle treatment. 2-At 3, 6, and 9 weeks after transplantation, we found a striking increase in the number of surviving YFP-NPCs in the ChABC-treated group compared to the vehicle-treated group. 3-ChABC treatment induced CSPG degradation resulted in promoting the survival and integration of engrafted NPCs 4-ChABC and NPC transplantation promote axonal preservation and sprouting of the CST axons rostral to the lesion. 5-ChABC treatment and NPC transplantation promote plasticity of serotonergic fibers by increase in 5-HT expression in the spinal cord. 6-ChABC, GF, or NPC showed no significant changes in the injury-induced pattern of sprouting in CGRP afferents in laminae III–V after treatments	ChABC treatment and NPC transplantation improves locomotor function after chronic SCI with particular emphasis on rats treated with the combination of ChABC/GFs+NPCs, who regained their pretreatment BBB scores at 2 weeks after NPC transplantation and then showed progressive improvement in their scores over time. Also, statistically significant greater frequency of consistent weight-supported plantar steps and evidence of forelimb– hindlimb coordination in the ChABC/GF+NPC group than in other groups from weeks 5 to 8 after transplantation was observed. Regarding neuropathic pain, no increased sensitivity to thermal stimulation was detected among any of the experimental groups after treatments.
Wang 2011 [35]	BDA (10% w/v, MW 10,000, Invitrogen)	1 µl . 100 U/ml	Chondroitinase ABC I (Seikagaku), 0.06 U/dose (240 µg per mL), i.t. (intrathecally) in 3 µL aCSF.	Animals were treated intrathecally (i.t.) with Chondroitinase ABC I	1- More CST fibers' crossing and sprouting. 2-More serotonergic fibers sprouting. 3-No effect on sensory neurons. 4- Increase in the number of 5-HT axons, ChABC had significantly more Biotinylated dextran amine, vGlut1 synaptic puncta	ChABC induced functional recovery in chronic SCI when paired with task-specific rehabilitation. 1. Animal groups that received Ch-ABC and rehabilitation achieved six pellets eaten on average (±1.52), compared with the other three groups, which reached 0.5± 0.40 (Pen alone, p <0.001), 0.56 ± 0.29 (Ch-ABC alone, p< 0.001), and 2.3 ± 0.58 (Pen rehab, p 0.001) pellets eaten (Fig. 4a). 2. Animals in the Ch-ABC rehab group also showed greater accuracy on the staircase apparatus compared with other groups (Fig. 4b)
García-Alías 2011 [31]	1 µl of 10% BDA in the gigantocellular nucleus of the reticular formation under sterile conditions	1 µl . 100 U/ml	ChABC (Seikagaku, 5 U/ml in saline plus 0.1% rat serum albumin	23 g clip, (Walsh) compression injury for 1 min at the level of T7 of the spinal cord	1-Animals receiving chondroitinase have significantly more sprouting and more axon crossing than the penicillinase group. 2-Chondroitinase in contrast to the penicillinase group lead to a small EPSP (amplitude 0.82 ± 0.35 mV; latency 5.4 ± 0.5 ms), in contralateral stimulation. No EPSP was seen in ipsilateral stimulation.	ChABC-treated animals in all groups showed substantial improvements with most of the gait measures returning almost to normal as compared to the PEN animals

Mountney 2013 [25]	N.S	2 U/mL, 6 µl	An injection of 6 µL of only ChABC (10 U/mL)	Injection into the subarachnoid cavity under the section (was injected once into the subarachnoid cavity under the section according to Barritt et al.30	1-Ch-ABC treatment did not increase 5-HT/serotonin axon immunostaining compared with controls 2-ChABC-treated rats did not have increased GM1 and GT1b staining	ChABC alone and sialidase/ChABC combination-treated animals did not show enhanced improvement in hindlimb motor and sensorimotor functions over the final 2 weeks of the testing compared with control-treated rats
Xia 2015 [30]	2 µl 10% solution of BDA (10,000 molecular weight, lysine fixable; Molecular Probes, Eugene, OR) into the three sites in the motor cortex	6 µl . 10 U/ml	6 µl of cABC (10 U/m)		1-Chondroitinase treatment caused some CST axons regrowth into and beyond the injury site caudally	Most ChABC-treated animals (5/6) regained hind limb placing response. Also, combined-treatment rats had higher BBB scores than single-treated rats.
Ishikawa 2015 [27]	Protein kinase C-γ (1:500)	200 µl. 0.05 U/ml	Chondroitinase ABC was obtained from Sigma-Aldrich (St. Louis, MO) (C3667) and was pure, protease-free, and supplied in vials (0.5 mg = 5.15 units). Each injection of Ch-ABC was freshly prepared on the day of administration. Saline (167 µL) was added to each vial, creating a solution of 0.18 units per 6 µL (high dose). The low-dose solution was formulated by diluting this to 0.06 units per 6 µL. High-dose Ch-ABC (0.18 units/treatment, n = 9) and low-dose Ch-ABC (0.06 units/treatment, n = 8) was injected	An IT injection through the catheter was placed through a dural opening at C4-5 (Fig. 1A) and threaded rostrally in the SAS to reach the C3 spinal cord hemisection + A polyethylene (PE-10) catheter (Becton Dickinson, Sparks, MD) The PE-10 tubing was heated and tapered to obtain a 100-µm OD at its tip. The entire catheter (80 mm long) contained a volume of 3 µL.	1-Chondroitinase treatment caused a trend toward an increase in serotonergic fibers' regrowth but it was not significant 2-Ch-ABC significantly increased the Gap-43 and 5-HT positive fibers	The combination of rehabilitation and KS- or CS-digestion as a result of ChABC treatment tended to achieve better recovery than that of rehabilitation and saline, but the difference was not significant.
Shinozaki 2016 [34]	CST fibers were labeled with Alexa Fluor 488-conjugated wheat germ agglutinin (WGA) (1.0% in saline, 4.0 µl/cortex; Invitrogen)	40 U/200 µl, 1µl	In the ChABC group: 6 µl ChABC and 6 µl of saline were given on each of the subsequent 6 days.	The tail was connected to a microinjector tube after spinal cord injury	1-ChABC treatment increased serotonergic fibers significantly rostrally, and at the lesion epicenter, and cause some fibers to traverse the lesion and reach the caudal site (rostral-1.6 mm, rostral-0.4 mm, epicenter. caudal-0.4 mm, caudal-0.8 mm, caudal-2 mm.) 2-More WGA-labeled CST fibers reached the lesion epicenter in the ChABC group vs vehicle group but no one reached caudal sites. (Rostral-5.0 mm, rostral-2.0 mm, rostral-1.0 mm, epicenter) 3- Significantly lower CSPG content in the ChABC group	The ChABC group demonstrated a third recovery phase at 12–14 weeks after SCI and showed significant differences in final BBB scores compared with the no-treatment control group ($F(2, 23) = 3.81$, $n=24$, $P=0.039$). This signifies that combinatorial ChABC/rehabilitation therapy delivered an improved functional outcome compared to rehabilitation alone, with significantly increased locomotor scores (i.e. improved motor functions)

					4-The RT-97-positive area caudal was significantly increased 5-Increase the GAP-43 positive area 6- The fibers reached more closely to the epicenter in the ChABC group	
Führmann 2017 [36]	N.S	5 µL. 100U/ml	1 µl of 50 U/ml P-ase or Ch-ABC was injected	In animals receiving enzyme treatments after SCI, the right medulla was exposed immediately after the injury, and 1 µl of 50 U/ml P-ase or ChABC was injected at 0.5– 0.3 mm below the dorsal pial surface and just lateral to the cuneate nucleus.	1-ChABC tends to decrease cystic cavitation and lesion volume but it was not significant. 2-ChABC significantly decreased the expression of CSPG 3-Increase the differentiation of neural stem cells to differentiated neurons	Treatments did not influence the behavioral outcome at 9 weeks post-injury in that, compared to uninjured animals, animals that underwent compression injury showed a drop in animal motor function[ǂ]
Qi Pan 2017 [28]	N.S	6 µL. 10 U/mL	1 microl injection of ChABC (100 U/ml, protease-free, Seikagaku), The animals received a total of five injections of ChABC through the cannula (3 l, 100 U/ml per injection, Acorda Therapeutics) or Pen (3 microl, 100 U/ml per injection), once every 2-d following surgery.	Intrathecally insertion through the opening of the cisterna magna, with the tip lying on top of the injury. of ChABC through the cannula (3 l, 100 U/ml per injection, Acorda Therapeutics) or Pen (3 l, 100 U/ml per injection) + A 32-gauge catheter (ReCathCo)	1-Big holes in the transection region of the spinal cords were seen in rats of the control group; small holes, breaks, and cysts were observed in rats of the ChABC group 2- GAP-43 and NF-200 expressions were significantly increased. Elevated expression of GAP-43 and NF-200 indicate that PGS/ChABC promotes healing by triggering even greater augmentation of axon growth and neuron sprouting	Combined treatment with PGS and ChABC resulted in the highest BBB scores, implying that PGS/ChABC further improved the recovery of motor function.

[ǂ]Motor functions assessed by the; Basso, Beattie, and Bresnahan (BBB) scale, N.S; Not specified, BDA; Biotinylated Dextran Amine, SCEP; Spinal cord evoked potential, MEP; Motor evoked potential; CTB; Cholera toxin B subunit, ChABC; Chondroitinase ABC, CSPG; Chondroitin Sulfate Prostaglandins, NPCs; neural stem/progenitor cells, GFs; growth factors