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Review Article

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Review of Various Types and Routes of Administration of Chondroitinase Enzymes

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ABSTRACT

Derived from the bacterium *Proteus vulgaris*, chondroitin ABC lyase is an enzyme that can be used in treating proteoglycans that affect neural activity (communication, plasticity). Chondroitinase can be used for vision abnormalities and spinal injuries. The biological activity of chondroitinase is due to its ability to act on chondroitin sulfate proteoglycans (CSPGs), which are required for normal functioning. This study aims to examine various types and routes of administration of Chondroitinase enzymes. There is an increasing application of chondroitin sulfate proteoglycans in spinal cord injury, vitreous attachment, and the management of various carcinogenic conditions. Research must be done to create an effective chondroitinase delivery mechanism so that the pharmacological activity seen in vitro and in preclinical research may be applied in the clinic. More studies are required to widen the application of chondroitinase in therapeutics. In this review, chondroitinase ABC, B, and C are all discussed. The routes of administration, like caudal or rostral, intracerebroventricular, hydrogels, and intrathecal, have been detailed. The current review article highlights the different medical uses for chondroitinase, drug delivery methods for the enzyme, and chondroitinase dispersion across bacteria. In conclusion, this study can reduce the chance of edema by the intracerebroventricular route. However, it is not effective for people due to the gyrencephalic anatomy of brain.

Key-words: Chondroitinase, Chondroitin, Chondroitin Sulfate Proteoglycans, Spinal Injuries, Ocular Abnormalities, Proteoglycans

INTRODUCTION

Traumatic Spinal Cord Injury (SCI) is one of the most difficult neurological disorders to study because of its well-known effects, including significantly lower quality of life for patients and a significant impact on the economy [1]. SCI results from an initial event brought on by a contusive, compressive, or stretch injury, followed by a so-called "secondary injury," which is thought to be the principal driver of post-traumatic neuronal degeneration of the cord [2]. It is now widely accepted that the main goal of neuroprotection is to block the mechanisms causing this secondary damage to lessen the adverse effects that result from it [3]. Numerous obstacles prevent proper axon regeneration after the original injury, such as glial scarring that causes chemical signals to up-regulate Chondroitin Sulphate Proteoglycans (CSPGs), which limits axonal healing [4]. Fig. 1 shows the structure of chondroitin sulphate.

After spinal cord damage or injury, chondroitin sulphate proteoglycans pose a tremendous obstacle to axon regeneration. The recovery of motor, sensory, and autonomic functions is improved when experimental spinal cord injuries are treated with chondroitinase.

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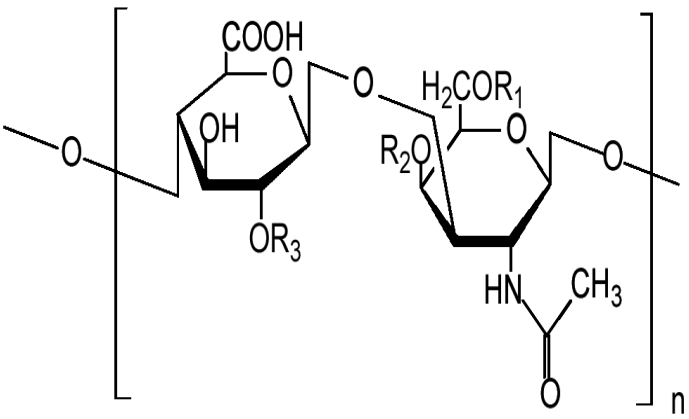

Fig. 1: 2D structure of Chondroitin Sulphate

One unit of chondroitin sulfate chain.
Chondroitin-4-sulfate: $R_1 = H; R_2 = SO_3H; R_3 = H$
Chondroitin-6-sulfate: $R_1 = SO_3H; R_2, R_3 = H$

The main proteins of chondroitin sulphate proteoglycans are split into glycosaminoglycans (GAG) by chondroitinase. In reaction to damage, CSPGs are released, and their GAG side chains provide steric hindrance that prevents axons from growing through the location of the injury [5]. Because it acts on chondroitin sulphate proteoglycans, chondroitinase has biological functions. The body needs CSPGs to function normally. CSPG levels can rise or fall, causing a variety of clinical disorders. Spinal cord damage, vitreous attachment, and cancer are examples of diseases in which there is an increasing level of CSPGs that can benefit from chondroitinase [6]. The enzyme chondroitinase can reduce the CSPG GAG chains, but because of the enzyme's brief window of activity, there are problems with its direct administration [7].

The amount of protein in CPSG was not significantly affected by chondroitinase treatment. It is also found that these CSPGs' expression changes based on the conditions. There is evidence that CSPGs used in the management of contusion injury give a similar output when they are used in the management of surgical hemisection lesions, and it is also found that the effects of chondroitinase can improve the sensory and motor function of these healing tissues, which are probably caused by the expulsion of GAG chains rather than a decrease in CSPG content. Glucosamine and chondroitin sulfate may be aspects that the body has. Chondroitin is a kind of protein syrup that is thought to assist in building and rebuild tissue [8]. Fig. 2 shows the steps through which condroitinase is clinically beneficial in nerve regeneration.

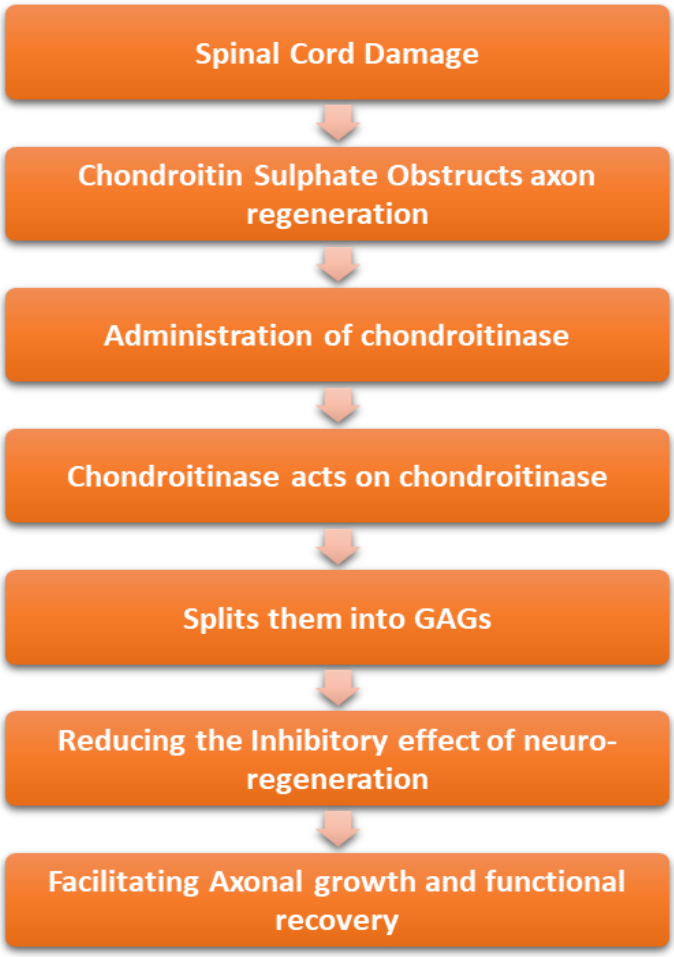

Fig. 2: Steps by which Chondroitinase is beneficial in axonal recovery

Chondroitin sulfate is a component of a massive protein chain that provides cartilage flexibility. Food supplements are offered both for glucosamine and chondroitin sulfate. People are managed to make from human organs: glucosamine emerges from the shells of crabs, lobsters, and shrimp, and chondroitin comes from the tissue of wildlife like tracheas or sharks. Research findings from the past demonstrate that some individuals with moderate to severe osteoarthritis (OA) who took either glucosamine or chondroitin noted pain medication similar to that of NSAIDs like aspirin and ibuprofen. Different research shows that probiotics might also help other people with OA slow down the deterioration of their cartilage [9]. An in-depth medical trial that the National Institute of Health is doing also must give solid answers to how these treatments function. Glucosamine chain stores are long, repeating

units of carbohydrates with glycosidic bonds, D-glucuronic acid (GlcA) and N-acetyl-D-galactosamine, that emerge and go (GalNAc). A few of these GlcA toxins can be started to turn into L-iduronic acid (IdoA). Whenever this happens, the arising glycosaminoglycan, which used to be considered chondroitin sulfate A, is called chondroitin sulfate. In addition, this Chondroitin sulfate is made from natural product lines, and the duration of the sequence and the way the sulfate gatherings are arranged can vary significantly.

The multiple methods chondroitin sulfate can also impact where it arises from. Therefore, it is feasible to discern the distinction between glucosamine from land-based activities and sea examples [10]. Each method for considering this distinction is in terms of the ratio of glycosidic units: glucosamine sulfate from land mammals is now almost entirely made up of non-sulfated (O) and monosulfated (A and C) components when in marine organisms, the share of disulfate (D, E, and B) components is higher. Furthermore, sea glucosamine chain stores are generally longer. Chondroitin sulfate from a shark has a small molecule mass of up to 70 kDa, whereas the molecular size of chondroitin sulfate from domesticated animals is normally below 45 kDa [11]. Glucosamine chains are connected to hydroxyl groups on serine residues from some proteins. Glycosylated serine almost always come after a glycine and are near citric residues, but that does not always mean that they have been glycosylated. Connection to the GAG sequence starts with four simple sugars that always go in the same order: Xyl, Gal, Gal, and GlcA. Each carbohydrate is connected by a different enzyme, which gives GAG synthesis many degrees of control. The plasma membrane is just where xylose tends to start to link up with proteins. This same Golgi apparatus is where the rest of the sugars link up [12].

Types of chondroitinase enzyme

Chondroitinase ABC- In the adult CNS, CSPGs are effective growth inhibitors. It has been discovered that CSPG inhibition can be reduced by using the enzyme chondroitinase ABC (ChABC), which has a remarkable potential for tissue repair. ChABC therapy, whether used alone or in conjunction with other approaches, can promote recovery after spinal cord injury in a variety of positive ways. These include encouraging the repair of damaged axons, enhancing the flexibility of unharmed routes, and protecting injured projection neurons from further damage [13].

In the adult mammalian central nervous system (CNS), the incapability of axons to revitalize after a spinal cord injury can result in irreversible paralysis. A glial scar containing extracellular matrix molecules, such as chondroitin sulphate proteoglycans (CSPGs), forms at the sites of CNS injury; these CSPGs inhibit axonal growth. Intrathecal administration of ChABC resulted in the breakdown of CS-GAG at the site of the damage, the upregulation of a protein linked to regeneration in injured neurons, and the promotion of the regeneration of descending corticospinal tract axons and ascending sensory projections. Additionally, after electrically stimulating corticospinal neurons, the ChABC therapy restored post-synaptic activity below the lesion and encouraged the functional recovery of proprioceptive and locomotor behaviours [14].

By focusing on axon plasticity and functional circuit connectivity, combining multiple therapies is a viable way to encourage spinal cord healing. In particular, the creation of rigorous task-specific motor rehabilitation and the breakdown of chondroitin sulphate proteoglycans at the site of damage by the activity of the bacterial enzyme ChABC have shown synergistic effects to increase behavioural recovery [15].

Studies have evaluated the results of combining chondroitinase with novel methods and other tactics known to increase recovery after spinal cord damage. There is mounting evidence that combining chondroitinase with cell transplants is one of the most effective combinations. There is a discussion of the specific advantages of each type of cell used in these transplant experiments. Improvements are also made when chondroitinase and therapy are combined. Gene therapies effectively address the problem of the enzyme's thermo-instability and deliver enzymes to the wounded spinal cord. However, the results of these trials imply that greater modification is needed for these methods of distribution to achieve similar levels of efficacy to those produced by a biological system. Other means of delivery, such as through nanoparticles or synthetic scaffolds, have shown promise. Chondroitinase is also effective in treating various disorders such as peripheral nerve damage, stroke, coronary reperfusion, Parkinson's disease, and several forms of cancer, according to pre-clinical models. The extensive spectrum

of ailments for which the advantages of chondroitinase therapy have been proven shows the intricate roles that chondroitin sulphate proteoglycans, the enzyme's substrate, play in health and disease and justifies the enzyme's continued research as a treatment [16].

In the peripheral nervous system, chondroitin sulphate proteoglycans (CSPGs) are strong inhibitors of neural regeneration. Inhibitory CSPGs build up in the endoneurium and Schwann cell basal lamina of the distal nerve stump after nerve damage. Utilizing chondroitinase ABC (chABC) has significantly improved the capacity of damaged axons to regenerate through openings in the extracellular matrix that is rich in CSPG. ChABC can break down the CSPGs that obstruct neurite outgrowth. The characteristics of CSPGs, their increase after peripheral nerve damage, and possible processes underlying their proliferation and inhibition are discussed in this article. The literature to date suggests that the addition of ChABC to the digestion of inhibitory CSPGs may aid in peripheral nerve regeneration [17].

Chondroitinase AC- Flavobacterium heparin produces and exports 2chondroitinases, chondroitinase AC and chondroitinase B, into the periplasmic space when the medium is supplied with chondroitin sulphate. While only dermatan sulphate is degraded by chondroitinase B, chondroitinase AC selectively depolymerizes chondroitin sulphates A and C (chondroitin sulphate B). From + *F. heparinum*, the genes for both enzymes were extracted and given the names cslA (chondroitinase AC) and cslB (chondroitinase B) [18]. On the chromosome of *F. heparinum*, they were discovered to be transcribed in the same direction and separated from other heparinase genes by 5.5 kb.

Additionally, it appeared that both enzymes' synthesis was coregulated. The open reading frames for peptides with 700 and 506 amino acid residues, respectively, were found to be 2,103 and 1,521 base pairs in length in the cslA and cslB DNA sequences. While chondroitinase B has a signal sequence made up of 25 residues, chondroitinase AC has a signal sequence made up of 22 residues. The mature versions of chondroitinases AC and B have calculated molecular weights of 77,169 and 53,563 Da, respectively, and are made up of 678 and 481 amino acid residues, respectively. In the cytosol of *E. coli* active, mature chondroitinase has been produced by truncated cslA and cslB genes. Recombinant chondroitinase B and AC have undergone partial purification and display particular activities that are comparable to those of chondroitinases B and AC from *F. heparinum* [18].

The majority of the extracellular matrix is made up of glycosaminoglycans (GAGs), highly sulfated polymers composed of hexosamine-uronic acid disaccharide units. They interact with a wide range of proteins, including those involved in the blood coagulation cascade. In mammalian systems, GAG hydrolases are responsible for GAG degradation. The hexosamine-uronic acid bond is broken by the several GAG-degrading lyases that are expressed by bacteria, forming an unsaturated sugar ring. The GAG lyases that *F. heparinum* generates have at least five distinct specificities. When it comes to chondroitin-6 sulphate and chondroitin 4-sulfate, chondroitin AC lyase is quite active [19].

Chondroitinase B- The family of acidic heteropolysaccharides known as GAGs includes substances including chondroitin sulphate, dermatan sulphate, heparin, and keratan sulphate. Hydrolases and lyases can both cleave the O-glycosidic link within GAGs, resulting in the production of oligosaccharide and disaccharide products. Chondroitinase B, a glycosaminoglycan lyase from *F. heparinum*, and its complex with a dermatan sulphate disaccharide product have both had their crystal structures established [20].

From *F. heparinum*, a chondroitinase that selectively breaks down chondroitin sulphate B was recovered, and it was distinguished from a constitutive chondroitinase AC that was also found in *F. heparinum* extracts. In addition to oligo- and tetra-saccharides and an unsaturated 4-sulphated disaccharide, the enzyme only reacts with chondroitin sulphate B. (deltaDi-4S). The oligosaccharide fraction is chondroitinase AC susceptible and primarily produces deltaDi-4S. Chondroitinase B differs from chondroitinase AC in several ways, including how certain metal ions affect it, what temperature it needs to function at its best, and how sensitive it is to rising salt concentrations. All chondroitin sulphates, as well as the disaccharides made from chondroitins, trigger the enzyme in *F. heparinum* [21].

Chondroitinase C- From *F. heparinum*, a chondroitinase that works on chondroitin sulphate C and hyaluronic acid was identified. The extracts of *F. heparinum* that had

previously been cultivated in the presence of chondroitin sulphates A, B, or C contained this enzyme, which was distinguished from the fundamental chondroitinase AC and an induced chondroitinase B. The enzyme reacts with both chondroitin sulphate C and hyaluronic acid to produce an unsaturated nonsulfated disaccharide and a tetrasaccharide plus an unsaturated 6-sulfated disaccharide (delta Di-6S) (delta Di-OS). Delta Di-4S is not produced during the breakdown of chondroitin sulphate A, only oligosaccharides and delta Di-6S. Chondroitinase C differs from chondroitinases AC and B in several ways, including how ions affect it, what temperature it needs to be active, and how sensitive it is to rising salt concentrations [22].

Molecular Insights from *In Silico* based chondroitinase enzyme engineering- Solving the conformational stability problem of the chondroitinase enzyme is a challenge thrown by the enzyme to the experimental and computational scientific communities. There are reported computational alanine screening studies to improve the stability of the helix located at the N and C-terminal domains of proteins by incorporating mutations to manipulate and rigidify the flexible sites to enhance their therapeutic applications [23]. However, the issue of conformational stability of enzymes remains the same and continues to challenge biotechnology and *In Silico* scientists to find a plausible solution. Computationally constructed point mutations by selecting hotspot residues in the flexible regions of enzymes displayed improved structural features & activity, whereas the construction of double point mutations may help scientists further improve catalytic efficiency, as very few studies are reported to highlight the catalytic efficacy enhancement upon double point mutation of hotspot residues [24]. Replacing non-aromatic amino acids located at the N- and C-terminal domains of enzymes improved their stability, as aromatic interaction components contribute to the stability of enzymes [25]. A few research groups discovered residues with a higher B-factor value, indicating that they are more flexible and unstable. The enzyme's flexible residues in the catalytic, C, and N-terminal domains had been stiffened with proline amino acid [26]. To solve the conformational stability and other kinetic issues of chondroitinase, promising X-ray crystal structures have been resolved with <2 Å resolution, which can potentially serve as a starting point to address the structure-function issues meticulously (Fig. 3). To improve the enzyme's biomedical applications by rebuilding the damaged portion of the spinal cord, atomic and electronic level computational enzymatic investigations are required, according to our understanding of the chondrotinase.

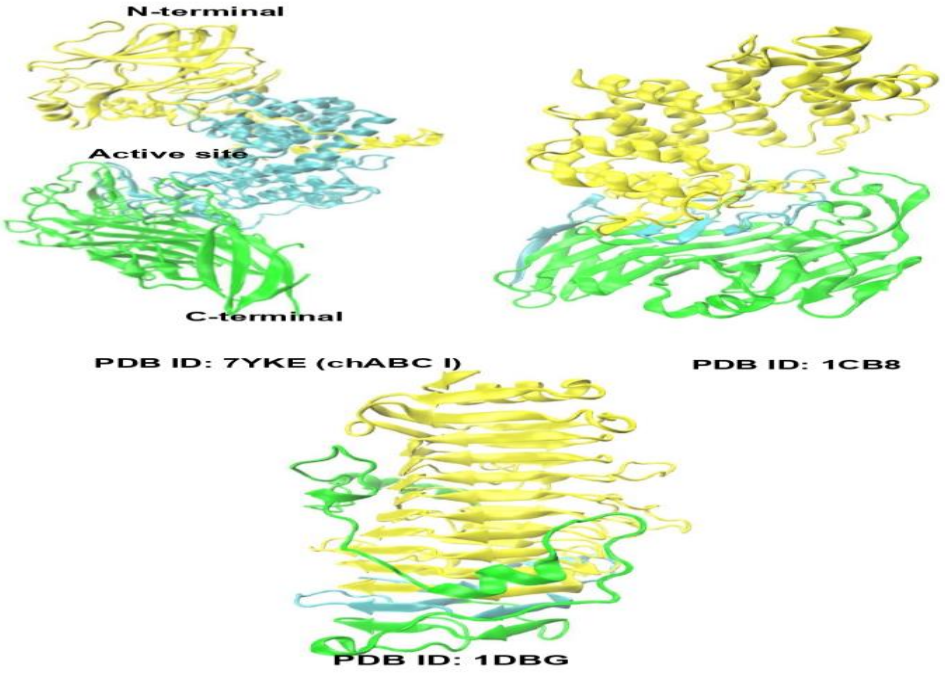

Fig. 3: 3D structures of chondroitinase representing N-terminal (Yellow), Active site (Cyan), and C-terminal (Green) domain

Routes of administering chondroitinase- Traumatic spinal cord injury (SCI) is a terrible condition that leaves the majority of patients permanently disabled [27]. Molecular medical advancements based on a better knowledge of the pathophysiology of injury have shown encouraging improvements, even though there is no known treatment for SCI. These chemicals work to either prevent the degeneration of remaining tissue or to regain lost function. When the inflammatory process and other secondary pathways of injury are at their greatest, neuroprotective medications are frequently administered in the hours to days following an injury [28]. Contrarily, neuro regenerative therapies are frequently administered for a few days to weeks, which corresponds to the amount of time needed to encourage repair [29]. The blood-spinal cord barrier (BSCB) and the physical inaccessibility of the spinal cord make it difficult to provide both neuroprotective and neurodegenerative substances. The only delivery options left is local or, intrathecal, or epidural distribution by either indwelling catheter/minipump or bolus injection because most therapeutic molecules cannot penetrate the BSCB. Other techniques involve directly injecting substances like stem cells, genetically modified cell grafts, or intraspinal medication eluting implants into the spinal cord. There is currently no cure for significant SCI in humans that can restore function [30]. Fig. 4 shows the routes available for administration of chondroitinase.

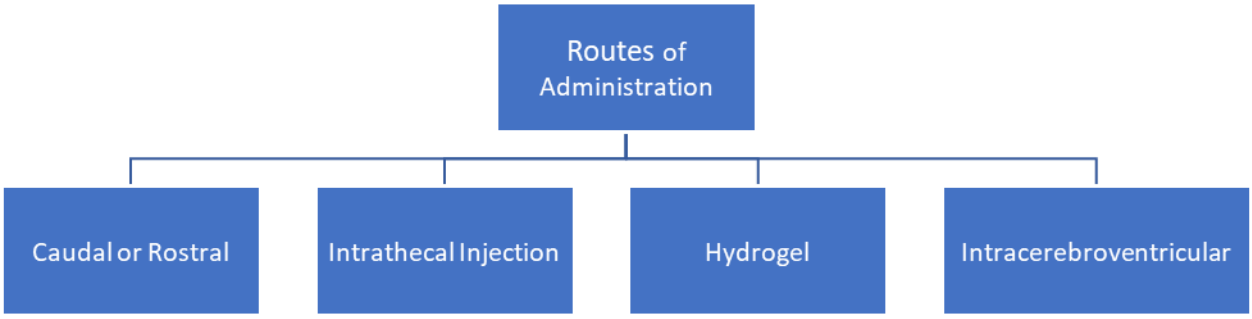

Fig. 4: Main routes of administering chondroitinase

To be used in clinical settings, an optimal drug delivery platform needs to provide targeted and sustained release as well as a favorable risk/benefit ratio. Therefore, creating a safe delivery method is a crucial step in getting a medication candidate ready for clinical testing. Given that it is doubtful that a single therapeutic drug will be able to treat SCI, a delivery approach that enables the sustained distribution of many molecules at various rates is also preferred. Past works have shown that intrathecal drug delivery can deliver one or more medications straight into the spinal cord for up to 28 days [31]. The ideal method would have the minimally invasive single-dose injection, local and continuous release (to maximise effect), be biodegradable, and not cause CNS inflammation (to minimise the risk). Previous studies have shown that hydrogels are secure at the injection site, safe, and capable of delivering neuroprotective chemicals temporarily. The clip compressive model of SCI, which closely reflects the most common kind of clinical SCI has been used to validate the surgical process for delivering drug-loaded hydrogels into the intrathecal region. Additionally, when paired with surgical decompression, the intrathecal infusion is minimally invasive [32].

Chondroitinase ABC (ChABC), a bacterial enzyme that can break down chondroitin sulphate glycosaminoglycans (CS-GAGs) of CSPGs, has been shown in vivo studies to promote axonal regeneration and sprouting, promote plasticity of uninjured pathways, and, most importantly, support functional recovery in a variety of animal models. However, due to its biodistribution and inactivation, ChABC delivery is highly constrained. Additionally, conventional intrathecal deliveries necessitate frequent administrations and are thus susceptible to recurring infections. Smart drug delivery devices that offer local sustained release can lessen systemic side effects of medications while also enhancing therapeutic efficacy to help overcome these disadvantages.

Among these systems, hydrogels appear to be particularly well suited for SCI treatments because of their distinctive qualities: High biocompatibility, the capacity to provide precisely controlled release rates, the

ability to retain water, and the simulation of live tissues [33].

After spinal cord injury (SCI), chondroitin sulphate proteoglycans (CSPGs), a significant family of axon development inhibitors, are up-regulated and play a role in regeneration failure. By breaking down the glycosaminoglycan chains on CSPGs, ChABC can override CSPG-mediated inhibition [34]. However, above 37°C, ChABC rapidly loses its enzymatic activity, necessitating the administration of multiple doses or local infusions for a few days to a few weeks. These infusion devices are intrusive, prone to infection, and have clinical issues. Many studies have shown that there are devices for the sustained local distribution of chABC in vivo to overcome this constraint and eliminate the necessity for chronically embedded catheters and pumps. For up to 4 weeks, thermostabilized ChABC was still functional *in vitro* at 37°C[35]. When thermostabilized ChABC was administered using a hydrogel-microtube scaffold technology, CSPG levels remained low in vivo for up to 6 weeks after SCI. Significant changes in CSPG digestion were shown in the axonal development and functional recovery following the prolonged local release of thermostabilized ChABC vs a single treatment of un-stabilized ChABC. The locomotor function of animals given thermostabilized ChABC together with prolonged neurotrophin-3 infusion was improved. Cholera toxin B subunit-positive sensory axon development and serotonergic fibre sprouting were also significantly boosted. Therefore, increasing ChABC thermostability makes it possible to give ChABC locally and sustainably with the least invasiveness, which may be useful in overcoming CSPG-mediated regeneration failure. After SCI, combining thermostabilized ChABC with neurotrophic agents improves axonal sprouting, regeneration, and functional recovery [36].

ChABC has the potential to be used as a treatment in people with CNS injuries because it frequently promotes axonal sprouting and improves functional recovery. When concentrated solution fibrin gel containing ChABC was implanted next to a spinal cord injury, bioactive ChABC was detected in the spinal cord for at least 3 weeks, and CSPG levels remained low in vivo up to six weeks post-SCI. When the fibrin delivery mechanism was employed instead of injecting ChABC directly into the spinal cord three weeks after the lesion, nearly six times as much bioactive ChABC was found there. In addition, 3 weeks after the damage, the delivery system-treated spinal cord's level of inhibitory GAG was 35% lower than the spinal cord treatment with an injection of ChABC. In contrast to using an intraspinal injection of ChABC, the delivery technique allowed 25% of the initial ChABC dose to be still found in the lesion after 3 weeks [37].

Caudal or Rostral- By breaking down CSPGs with chondroitinase ABC (ChABC), axonal regeneration outside of a lesion site is encouraged, leading to functional improvement. It has also been demonstrated that ChABC encourages the sprouting of fibres that have been spared. However, it is unclear if this plasticity leads to functional recovery. ChABC or a vehicle was injected through a rostral or caudal route to a unilateral C5 injury to achieve functional recovery. ChABC significantly increased the sprouting of 5HT fibres into the dorsal and ventral horns when injected rostral to a hemisection. No further sprouting was seen when ChABC was injected into tissue caudal to a hemisection. ChABC increased the sprouting of 5HT+ fibres into the ventral horn when injected caudal to a hemicontusion lesion but not in the dorsal horn [38].

Perineuronal nets are digested by microinjecting ChABC into healthy tissue close to a spinal cord lesion site. ChABC at a 20 U/mL dose was observed to digest perineuronal nets partially. As a result, we changed to a greater concentration that was effective in breaking down nets inside the brainstem. Wisteria floribunda agglutinin (WFA) staining around motoneurons was eliminated ten days after ChABC therapy, but there was significant 2B6 immunoreactivity, showing that ChABC microinjecting successfully digested CSPGs within perineuronal nets [39].

The removal of some CSPG-mediated inhibition of outgrowth to increase axonal sprouting of fibres and reopening of the window for synaptic plasticity may be the two effects of CSPG digestion by ChABC within perineuronal nets. A hemisection rostral or caudal ChABC therapy encourages the sprouting of serotonergic fibres [40].

There were many past experiments to observe different findings, if ChABC was injected caudally to a more clinically meaningful hemicontusion injury where more spared fibres remain ipsilateral and caudal to the lesions because the hemisection lesions disrupted all fibres ipsilateral to the lesion. To enhance the long-lasting plasticity of serotonergic fibres after a unilateral

contusion, ChABC therapy of tissue caudal to the damaged site is used [41].

Using chondroitinase ABC (ChABC) to break down the glial scar and stromal cell-derived factor 1 to attract endogenous neural progenitor cells (NPCs), tissue and functional repair could be accomplished in an impact-compression SCI (SDF) [42]. Treatment with ChABC led to behavioural improvement more quickly and consistently over time than other groups, whether ChABC was used alone or in conjunction with SDF. Improved locomotor performance may be related to the considerably decreased chondroitin sulphate proteoglycan levels and increased dispersion of NPCs throughout the spinal cord tissue with ChABC administration, both alone and in conjunction with SDF. Neither the amount nor distribution of NPCs under treatment with SDF alone nor the synergistic effects of ChABC administration appeared to be impacted [43].

Intrathecal injection- Because of its great selectivity for the nucleus pulposus matrix and potential for high efficacy in disc tissue dissolution with minimal risk of adverse effects on other tissues, ChABC has been proposed for chemonucleolysis. The chondroitinase ABC was administered intrathecally, and investigations have shown that there are no negative effects on nerve tissue or blood vessels from ChABC. It is most likely the result of surgical damage sustained while freeing the nerve roots from the intrathecal fibrous adhesions that the small decrease in conduction velocity observed following intrathecal injection of Ch ABC or its carrier. The laminectomy itself may cause these adhesions and is most likely unimportant in terms of pathophysiology [44].

Hydrogels- Following their capacity to transport cells and medications, hydrogels made from agarose and carbomer 974P macromerswere chosen for their prospective use in SCI repair techniques. One of the most crucial problems in drug delivery applications is how well hydrogels can distribute loaded pharmaceuticals with precise control and a consistent degradation kinetic. However, the vast corpus of literature frequently ignores the effects of solutes on drug delivery systems in favour of only characterising unloaded matrices. In many experimental studies, hydrogels have been loaded with a chromophoric salt that can mimic many of the steroids frequently used in SCI repair in terms of steric hindrance. The effects of the salt were examined from a structural and rheological point of view, considering the material's sensitivity to pH.

Additionally, mass loss and infrared bond response (FT-IR) were used to evaluate the chemistry of degradation [45]. Two injectable agar-carbomer-based hydrogels that were loaded with sodium fluorescein-a safe fluorophore with hydrophobic interactions similar to many small medicines, such as steroids and other neuroprotective agents were examined. It was possible to infer the self-diffusion coefficient (D) of loaded sodium fluorescein from straightforward, conventional, and affordable release tests. High-resolution magic angle spinning (HRMAS) diffusion-ordered spectroscopy NMR was also used to quantify this characteristic within the gel matrix directly. The HRMAS-NMR spectroscopy method might be seen as a quick and easy substitute for complex analytical techniques because of the estimated values and those measured by Tkalec *et al.* [46].

The development of SCI is a multifaceted pathological condition, and this is likely the key issue preventing the development of effective therapy strategies. The employment of innovative multidrug delivery methods allowing for the local controlled release of medicinal medicines is therefore suggested by fairly recent highlights. In this study, a biocompatible hydrogel-based system for multimodal medication delivery was created with SCI repair procedures in mind. The gel was loaded with a variety of low and high steric hindrance compounds to provide multiple release characteristics. Studies on the release of molecules *in vitro, in vivo,* and *ex vivo* revealed a distinct combination of quick diffusion-controlled kinetics for smaller molecules and slow diffusion-controlled kinetics for larger ones. It was consistently possible to maintain the functionality of the loaded compounds, proving that there were no chemically stable connections between the loaded molecules and the gel matrix. Directed delivery of the discharged molecules in the spinal cord tract caudally to the gel site is demonstrated, suggesting a more effective gel placing rostral to the lesion. This study also disclosed for the first time the relevant effect of cerebrospinal fluid flux dynamics on drug diffusion in the spinal cord tissue [47].

A stochastic block polycondensation among agarose and Carbomer 974P produces hydrogel, a chemical gel. ChABC is loaded and hence becomes physically confined

within the three-dimensional polymer system of the gel well before the sol/gel transition occurs. The principal cross-linking sites in carbomer 974 P are carboxylic groups that react with hydroxyl groups from agarose to create the three-dimensional matrix. Because of these polymers' well-known biocompatibility with the central nervous system, they were chosen (CNS). While carbomer was selected because of its anti-inflammatory qualities and its biocompatibility, agarose has been commonly employed in biomaterials for SCI restoration techniques [48]. Due to various advantageous characteristics that have been successfully established in prior investigations, including biocompatibility, high structural plasticity, thixotropic nature, and potential as a controlled delivery technique, the resulting hydrogel has tremendous promise [49]. Due to their distinct biocompatibility, adaptable synthesis techniques, variety of ingredients, and acceptable physical properties, hydrogels have been the preferred material for various applications in regenerative medicine. They can act as barriers or adhesives between tissue and material surfaces, act as scaffolds that give structural integrity to tissue constructs, and regulate how drugs and proteins are delivered to tissues and cultures. The characteristics of hydrogels that are crucial for tissue engineering applications, as well as the difficulties and limitations of the inherent material design, are examined [50].

Intracerebroventricular administration- Because it combined clinical convenience with efficacy, the ICV route of administration was chosen. ChABC intraparenchymal injection only affects the immediate vicinity of the injection site, making it potentially ineffective for treating more extensive edoema. Delivery intravenously is not feasible because ChABC is too big to pass through an undamaged BBB. As a first-line treatment for edoema and ICP control in patients with severe TBI, a ventricular shunt is frequently implanted to drain CSF. Therapeutic chemicals may be given out through this shunt. ICV administration of Idursulfase, an enzyme with a similar substrate, successfully penetrates brain tissue when given to monkeys, indicating that ChABC would do the same. The mouse brain is considerably smaller than the human brain, and compared to the gyrencephalic anatomy of the human brain, the lissencephalic anatomy may make it simpler to access the swelling cortex. ICV delivery may, therefore, be less successful in humans [5]. The routes of each type of chondroitinase are listed as given in Table 1.

Table 1: Routes of administration of each type of chondroitinase as it is available in the market

Type of Chondroitinase	Available form/route of administration
Chondroitinase ABC	Oral Capsule: 600 mg, most common
Chondroitinase B	lyophilized powder
Chondroitinase C	lyophilized powder; equal or more than 200 units/mg solid
Chondroitinase AC	Supplied as an aqueous solution containing 0.02M Trizma-HCl pH 7.2 and 2% glycerol

CONCLUSIONS

It has been demonstrated that ChABC can successfully digest CSPGs and is clinically beneficial in spinal cord recovery. The primary barrier to axonal regrowth in the spinal cord, the glial scar, must be eliminated before thinking about spinal cord repair methods. Chondroitin ABC lyase is capable of targeting proteoglycans that influence neural activity, including communication and plasticity. This breakthrough treatment presents exciting possibilities, particularly in the areas of vision abnormalities and spinal injuries. Chondroitinase's biological activity stems from its unique ability to act on CSPG. Their dysregulation can lead to various clinical disorders. This makes chondroitinase an invaluable tool for modulating CSPG levels in therapeutic contexts. Furthermore, hydrogel loading did not result in any stable bonding with ChABC or ChABC denaturation; instead, efficient digestion of molecules that resemble scars, like decorin, was seen for the whole anticipated delivery period. Thus, the possibility of the agar-carbomer hydrogel as a ChABC delivery method was confirmed.

CONTRIBUTION OF AUTHORS

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