Cerebral Injury Promotes Regeneration of The Brachial Plexus

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Research

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Abstract

Background: Our previous trial of contralateral seventh cervical nerve transfer (CC7) for spastic arm paralysis suggested that regeneration of the C7 nerve seems to be faster in patients undergoing nerve transfer due to cerebral injury compared to patients receiving surgery due to brachial plexus injury (BPI). This finding needs to be further verified, and the underlying mechanism remains largely unknown. The present study compared C7 regeneration between two groups of patients and animal models. Proteomics was utilized to reveal the mechanism mediating the promotion of C7 nerve regeneration.

Methods: We assessed Tinel's sign in the arm after C7 transfer and compared the latency to the appearance of Tinel's sign to evaluate regeneration. Traumatic brain injury (TBI) and BPI were induced in C57 mice to establish injury models. C7 regeneration was assessed by electrophysiology and histology. Enrichment analysis of the differentially expressed proteins identified by proteomics suggested altered inflammation related to TBI. qPCR and histology were performed to assess the inflammatory environment in the C7 nerve. We evaluated the influence of the contributing factor serum amyloid protein A1 (SAA1) on the regenerative ability and inflammatory response of the C7 nerve by electrophysiology, qPCR and histology.

Results: Faster C7 regeneration was identified in patients, which was further confirmed in mouse models by electrophysiological recordings and histology. Altered systemic inflammation, which led to increased M2 macrophage activation, may represent an underlying mechanism of increased regeneration. In mice, SAA1 facilitated C7 regeneration and interfered with macrophage polarization in vivo.

Conclusions: Altered inflammation promoted the regenerative capacity of the C7 nerve by altering macrophage behavior, and SAA1 may be a therapeutic target to improve the recovery of injured peripheral nerves.

Background

The peripheral nervous system can be affected by different types of injuries, including blunt trauma, traction injury, freeze injury, and chemical injury, which give rise to functional losses and even disability (1). Although axons in the peripheral nervous system have a remarkable capacity for regeneration, clinical improvement consistently requires a long period of time, and functional recovery is usually incomplete (2). Even after peripheral nerve surgery, approximately 25% of patients experience reduced quality of life and remain out of the workforce for more than 1.5 years (3). In our previous study, regeneration of the seventh cervical nerve (C7) seemed to be faster was in patients subjected to contralateral C7 transfer (CC7) for the treatment of spastic arm paralysis due to cerebral injury (4) compared to those who received C7 transfer due to brachial plexus injury (BPI) (5, 6). In a rat model, craniocerebral injury was reported to promote regeneration of the sciatic nerve ganglia after nerve transection (7). Based on this interesting finding, we aimed to decipher how cerebral injury contributes to peripheral nerve regeneration.

In response to injury, myelinating Schwann cells dissociate from distal nerve segments. While degenerated nerve fragments are cleared by recruited neutrophils and macrophages, Schwann cells adopt a repair phenotype and work in concert with phagocytes. As degenerated substances are inhibitory to nerve regeneration, the timely removal of myelin and axonal debris is essential for optimal regeneration (8) . Although the inflammatory response is required for the timely removal of myelin and axonal debris, which inhibit axonal outgrowth, optimal nerve regeneration also requires good resolution of so-called neuroinflammation. During this period, macrophages in the distal segment shift from the M1 to the M2 phenotype, which is anti-inflammatory and promotes repair (9, 10).

Serum amyloid A (SAA) refers to a group of proteins consisting of four members. Induced expression of SAA1 and SAA2 by hepatocytes accounts for the majority of the increase in SAA levels in the plasma following stroke and traumatic brain injury (TBI). In contrast, SAA3 is the major form of SAA in inflammatory sites, and SAA4 is constitutively expressed and is not inducible (11). In the traditional view, SAA is a proinflammatory molecule that is induced by inflammatory cytokines, including IL-1β and IL-6, and exerts physiological activity by interacting with multiple proinflammatory receptors, such as FPR2, TLR2 and TLR4. This concept was largely challenged by the fact that SAA not only upregulates the expression of anti-inflammatory cytokines (IL-10 and IL-1rn) but also polarizes macrophages toward the M2 phenotype. To date, substantial evidence suggests that the role of SAA in inflammation is regulatory and homeostatic rather than simply proinflammatory (12-15).

The present study aimed to verify the influence of cerebral injury on C7 regeneration in both patients and animals. Furthermore, we investigated the mechanism underlying the beneficial effect of cerebral injury on C7 regeneration. By using proteomics, ELISA and immunohistochemical staining techniques, we documented a further increase in SAA1 expression in mice subjected to TBI and BPI compared with mice subjected to BPI alone. The effect of SAA1 on C7 recovery was explored by applying a commercial recombinant SAA1 protein. Histological examination was subsequently performed to evaluate morphological recovery by measuring the regeneration of C7 nerve, while functional recovery was assessed by electrophysiological recordings. Mechanistically, the extent of the inflammatory response and macrophage phenotype were determined at various time points after BPI.

Methods

Patients

The study included two groups of 16 subjects, with 8 patients in each group. All the patients received standard rehabilitation treatment in our department after CC7 surgery. One group included patients with spastic paralysis of one upper limb caused by brain injury (such as stroke or trauma.), and the other group included patients who underwent nerve transfer due to BPI. All the participants underwent Tinel's sign examination once per month after surgery to assess the regeneration of the C7 nerve. The institutional review board of Huashan Hospital approved the study, and each participant provided written informed consent.

Experimental animals

The mouse experiments were approved by the Animal Care and Use Committee of Huashan Hospital and were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Adult mice (male, 8-10 weeks) were purchased from the Shanghai SLAC Laboratory (http://www.lascn.com/SupplyDemand/Site/Index.aspx?id=77). The mice were housed at 22°C and 50% humidity on a 12-h light/dark cycle. All experiments were conducted during the light phase, and the mice were anesthetized with sodium pentobarbital (80 mg/kg, ip).

Controlled cortical impact

The procedures were similar to those described in our previous study (16, 17). An anesthetized mouse was fixed in a stereotactic frame, the scalp was opened, and the skull was cleaned. A 3.0-mm-diameter hole was made in the skull over the motor cortex of the left hemisphere (anteroposterior = +1.0 mm to -2.0 mm, mediolateral = 0.5 mm to 3.5 mm). A head trauma contusion device (TBI-0310 Impactor, Precision Systems and Instrumentation) was equipped with a 3.0-mm-diameter impact tip. The injury was delivered with a speed of 3.5 m/s, a depth of 1.0 mm, and a dwell time of 150 ms. For the sham TBI operation, a hole was made in the skull, and then the skin was sutured without induction of cortex injury.

Brachial plexus injury

Two weeks after TBI or sham TBI surgery, brachial plexus injury was induced. An anesthetized mouse was placed in the beach-chair position, and the brachial plexus was exposed as described in our previous study (18). The C5-T1 nerves on the right side were transected, and only the C7 nerve was sutured.

Electrophysiology

An electrophysiology device (Dantec Keypoint G4, Natus Medical Inc.) was utilized for electromyography. On the indicated days, the mice were anesthetized, and the sutured C7 nerve was re-exposed. A bipolar electrode was placed on the proximal side of the C7 coaptation site to deliver the stimulation, and the recording needle electrodes were placed on the triceps brachii. Waves with an amplitude of 1.0 mA and a width of 0.04 ms were delivered, and the average amplitudes of the compound muscle action potentials (CMAPs) were recorded to assess C7 regeneration.

Histological studies

To evaluate nerve regeneration, immunofluorescence was performed to detect the expression of neurofilament (to label axonal fibers) and myelin protein zero (MPZ, to indicate myelination) in formalin-fixed, paraffin-embedded longitudinal sections of the C7 nerve. For each nerve, 3-μm-thick sections were cut, and then the sections were deparaffinized, boiled in 10 mM sodium citrate buffer for epitope retrieval and permeabilized with 0.5% Triton X-100. After incubation for two hours in blocking buffer, the sections were incubated with primary antibodies against neurofilament (Sigma, N4142, 1:100) and MPZ (Abcam, ab134439, 1:1000) at 4°C overnight. Afterwards, the sections were washed three times and then

incubated with secondary antibody (Abcam, ab150077, 1:500 for neurofilament; Abcam, ab150176, 1:500 for MPZ) at room temperature for one hour. After multiple washing steps, the specimens were counterstained with 1 μg/ml DAPI (SouthernBio, 0100-20). To assess macrophage phenotype, staining for F4/80 (primary antibody: Abcam, ab254293, 1:100, 4°C overnight; secondary antibody: Abcam, ab150077, 1:500, 1 hour at RT) was performed to detect all macrophages, and staining for CD86 (primary antibody: Abcam, ab119857, 1:100, 4°C overnight; secondary antibody: Sigma, SAB4600086, 1:2000, 1 hour at RT) and CD206 (primary antibody: Invitrogen, MA5-16871, 1:100, 4°C overnight; secondary antibody: Invitrogen, A-11007, 1:200, 1 hour at RT) was performed to detect activated M1 and M2 macrophages, respectively. Five random images were obtained for each section, and the CD86: F4/80 and CD206: F4/80 densities were measured to assess M1 and M2 macrophage activation. Image processing and analysis were conducted with ImageJ.

For ultrastructural analysis, transmission electron microscopy was performed at the indicated weeks after BPI. The C7 nerve (2 mm distal to the anastomotic point) was dissected and fixed in a 2% glutaraldehyde solution at 4°C. Afterwards, the nerve was postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer for 2 hours at 4°C and embedded in Epon for 72 hours at 60°C. Cross-sections (70 nm thick) were cut, stained with uranyl acetate and lead citrate, and examined using a JEM-1400 transmission electron microscope (JEOL, Akishima, Tokyo, Japan).

Immunohistochemical staining was carried out to assess SAA1 localization. After being blocked in 10% goat serum for 40 min at 37°C, sections were incubated with primary antibody (Abcam, ab199030, 1:200) overnight at 4°C. After being washed, the sections were incubated with secondary antibody (Novus, NB7160, 1:400) for 1 hour at RT. Then, a DAB Peroxidase Substrate Kit (SK-4100; Vector Laboratories) was used to visualize antibody binding. ImageJ software was used for image analysis. Five random images per slide were taken to assess protein localization.

Proteomics and data analysis

Four weeks after BPI, serum was obtained from three mice from each group and prepared with the PreOmics “iST” Kit (P.O. 00001, PreOmics GmbH) according to the kit manual. After TMT labeling and high pH reverse-phase separation (ACQUITY UPLC Peptide C18 column, 2.1 mm x 150 mm, 1.7 μm), the samples were measured using an LC-MS instrument (Triple TOF 5600, AB SCIEX, USA).

Raw MS files were processed by MaxQuant version 1.5.2 (http://www.maxquant.org). The precursor mass and fragment mass were identified with initial mass tolerances of 6 ppm and 20 ppm, respectively. The minimal length was set to 7 amino acids, and the maximum length was set to two miscleavages. The “match between runs” option was selected when performing the MS runs, and a 20-second time window for retention was utilized for matching. Proteins that matched the reverse database were filtered out. The threshold for peptide and protein identification was set to FDR-corrected 0.01.

The profile was then clustered based on Euclidean distances with average linkage using the modified function Clustergram in MATLAB R2014b, and a heatmap was also constructed in MATLAB.

We used a volcano plot to identify significantly differentially expressed proteins, with the X axis indicating the log2-based fold change and the Y axis representing the negative log10 of the p-value calculated from a two-tailed t-test. The red points in the upper left panel are significantly upregulated proteins, while the blue points are significantly downregulated proteins. Cutoff values of 1.5-fold for upregulated proteins and of 0.67-fold for downregulated proteins were used to identify differentially expressed proteins between the TBI+BPI mice and BPI mice ($P < 0.05$ for the t-test).

For bioinformatics analysis, differentially expressed proteins were subjected to ingenuity pathway analysis (IPA; Qiagen, Hilden, Germany) to elucidate their biological significance. Biological information obtained from the analysis is presented as biological function and disease and network data. For function and disease analysis, the threshold value was set to FDR-corrected $P < 0.001$. The figure showing enrichment data was constructed by the R programming language. Networks with scores greater than 30 were considered significant, and the figure showing the network was constructed by IPA.

PCR

Prior to nerve harvesting, the mice were perfused with cold PBS to remove the blood. RNA was isolated using an RNA extraction kit (TIANGEN, DP419) according to the manufacturer's instructions. Afterwards, the RNA was transcribed into cDNA using the PrimeScript™ RT Reagent Kit (Takara, RR047A). Finally, gene expression was analyzed by real-time PCR using PrimeScript™ RT Master Mix (Takara, RR036A) in a LightCycler® 480 instrument (Roche Diagnostics GmbH, Mannheim, Germany)

ELISA analysis

The dynamic levels of SAA1, which was identified by bioinformatics analysis, were further determined by ELISA. At 0, 2, 4, and 6 weeks post-BPI, serum was obtained from 3 mice in each group (the TBI+BPI and BPI groups). Commercial ELISA kits were purchased from Life Diagnostics (West Chester, PA, USA), and the protein concentrations were measured according to the manufacturer's instructions.

SAA1 treatment

SAA1 (Peprotech, Rocky Hill, USA) was administered as previously described (14). Mice were injected subcutaneously with 120 μg SAA1 per kg body weight immediately after nerve injury and every day thereafter. PBS without SAA1 served as the vehicle control.

Statistical analysis

Statistical analysis was performed using SPSS 22.0. The Wilcoxon rank sum test (U test) was used to compare the time for the C7 nerve to regenerate to the target muscle (Tinel's sign) between the two groups of patients. Two-way ANOVA followed by the Holm-Šidák post hoc test was utilized to compare dynamic nerve recovery between the two groups of mice. A U test was performed to assess the influence of SAA1 on nerve regeneration and the differences in SAA1 localization, cytokine expression and macrophage polarization. The differences were considered statistically significant if $P < 0.05$.

Results

Subjects with cerebral injury obtained faster C7 regeneration

As shown in Figure 1A, regeneration of the C7 nerve after surgery was faster in patients with cerebral injury than in patients who underwent CC7 surgery due to BPI. Furthermore, promoted recovery of C7 injury was observed in mice with the combination of BPI and TBI compared with mice with BPI alone (Figure 1B-D), as measured by the amplitudes of triceps brachii CMAPs. Histological examinations were further performed to evaluate C7 regeneration at the structural level at various time points after C7 transection. Both immunofluorescence staining of longitudinal sections and electronic microscopy analysis of cross-sections suggested that morphological regeneration was accelerated in mice subjected to TBI+BPI compared to mice subjected to BPI alone (Figure 1E and 1F). These results confirmed the cerebral injury-induced increase in peripheral nerve regeneration capacity, but its underlying mechanisms remain largely unknown.

Serum amyloid protein A1 expression was systemically induced by traumatic brain injury

After cerebral injury, both the systemic stress response and an increase in the permeability of the blood-brain barrier result in significant changes in the systemic circulation. We next carried out proteomics analysis to evaluate the difference in systemic response between mice with TBI+BPI and those with BPI alone (Figure 2A), and a total of 135 TBI-related differentially expressed proteins were detected (Figure 2B). Both biological function and disease enrichment analysis and network analysis revealed enrichment of inflammation- and acute phase response-related pathways (Figure 2C and 2D). Furthermore, the protein SAA1, the level of which is significantly increased in mice with TBI+BPI, was identified by both analyses (Figure 2C and 2D). These analyses suggested that SAA1 is a candidate regulator underlying the cerebral injury-mediated increase in C7 regeneration capacity. Subsequently, ELISA was performed at various time points after C7. As shown in Figure 2E, the serum levels of SAA1 were increased in the TBI+BPI group compared with the BPI group; the levels peaked at 2 weeks, and the increase was sustained at 4 weeks. Consistently, an increase in the protein levels of SAA1 was also observed in the tissue around the injured nerve, as determined by immunohistochemical staining (Figure 2F and 2G). Although previous studies have demonstrated that SAA1 is primarily produced by the liver and secreted into the circulation upon stress, we cannot rule out the possibility that SAA1 may be generated in situ. The mRNA levels of SAA1 in the tissue around the injured nerve were determined by RT-PCR, and no significant difference was detected between the two groups (Figure 2H). Thus, our results suggested that cerebral injury may systemically upregulate the expression of SAAs, which infiltrate into the tissue around the injured nerve and may contribute to peripheral nerve regeneration by modulating inflammation.

Cerebral injury modulated inflammation and macrophage phenotypes in injured peripheral nerves

To explore the mechanism of improved peripheral nerve regeneration triggered by cerebral injury, we assessed the expression of inflammatory mediators and macrophage phenotypes. Total RNA was isolated from tissue segments around the C7 nerve from the indicated groups at different time points

after BPI. The expression of inflammatory cytokines, including IL-1β, IL-6, MCP-1, and Fizz1, was strongly induced by BPI (Figure 3A). Specifically, the mRNA levels of IL-1β and IL-6 reached a maximum at 2 weeks after BPI and subsequently dropped. The expression of MCP-1, a chemoattractant for macrophages, was slightly elevated at 1 and 2 weeks and then returned to baseline soon after. The induction of Fizz1 expression, an M2 macrophage-associated gene, was increased at 2 weeks and gradually reduced to basal levels at 4 weeks after BPI. Compared with BPI, TBI had no influence on the maximum expression of IL-1β and IL-6 at 2 weeks after BPI but accelerated reductions in the levels of these cytokines at 4 weeks. No significant difference in the level of MCP-1 was detected between the two groups. Strikingly, the BPI-mediated increase in Fizz1 levels was further enhanced by TBI (Figure 3A). Immunofluorescence staining was performed to determine the phenotype of the macrophages. M1 activation (CD86: F4/80) peaked at 1 week after BPI and gradually decreased subsequently. Although no significant difference in M1 activation was detected between the groups at any time point, a decrease in M1 macrophage activation seemed to be promoted by TBI (Figure 3B and 3C). More importantly, TBI significantly promoted M2 activation of macrophages around the injured C7 nerve (Figure 3D and 3E). Therefore, TBI may contribute to peripheral nerve regeneration by fine tuning of the proinflammatory response and induction of the macrophage M2 phenotype.

SAA1 treatment promoted peripheral nerve regeneration

To date, it is unclear whether SAA can increase peripheral nerve regeneration capacity. To answer this question, we set up a six-week treatment protocol using recombinant human SAA (Figure 4a). The control group received an equivalent volume of PBS. Electrophysiological recordings and histological examination were performed to evaluate peripheral nerve recovery at the functional and morphological levels. As expected, SAA1 treatment promoted C7 regeneration after BPI (Figure 4B, 4C and 4D). Collectively, the results indicate that upregulation of SAA1 expression may be responsible for the beneficial role of TBI in peripheral nerve regeneration. The effects of SAA1 administration on the inflammatory response and macrophage phenotype in peripheral nerves subjected to BPI were further determined. SAA1 had no influence on the maximum expression of IL-1β, IL-6 and MCP-1 after BPI (Figure 4E). The nerve injury-induced M1 macrophage phenotype was also comparable between these two groups (data not shown). In contrast, the resolution of the proinflammatory response was accelerated by SAA1 administration, as evidenced by decreased levels of IL-1β and IL-6 (Figure 4E). Moreover, SAA1 treatment resulted in higher mRNA levels of Fizz1 and M2 activation than the vehicle control (Figure 4E, 4F and 4G). Collectively, these findings suggest that the effects of SAA1 on the nerve injury-induced immune response and macrophage phenotype are similar to those of TBI, providing further evidence that TBI may promote peripheral nerve regeneration via an SAA1-dependent mechanism.

Discussion

The beneficial effect of cerebral injury on peripheral nerve regeneration was reported in a previous study, but the underlying mechanisms remain unknown (7). In the present study, a faster C7 regeneration related to cerebral injury was identified in patients receiving CC7 treatment, and TBI enhanced morphological

regeneration and functional recovery of the brachial plexus nerve in mouse models. Intriguingly, the increase in SAA1 expression in both the plasma and local tissue was more obvious in the BPI+TBI group than in the BPI group. After BPI, SAA1 administration contributed to the regeneration of the brachial plexus nerve. Mechanistically, the beneficial effect of SAA1 on peripheral nerve regeneration may mainly depend on proper regulation of the inflammatory reaction and the phenotype of M1/M2 macrophages in the lesioned nerve.

A growing amount of evidence has demonstrated that mutual regulation exists between the central and peripheral nervous systems upon injury. After peripheral nerve injury, neuronal plasticity, glial hypertrophy and even adult neurogenesis can take place in the adult central nervous system (19-21). However, limited studies have investigated the regulatory effect of the central nervous system on the injured peripheral nervous system. Our previous study implied a faster regeneration of peripheral nerves in patients who underwent CC7 to treat spastic arm paralysis due to cerebral injury. In addition, rats with combined sciatic and craniocerebral injury exhibited better functional and morphological recovery of the sciatic nerve than controls subjected to sciatic nerve injury alone (7). While these findings support the concept that cerebral injury contributes to regeneration after peripheral nerve injury, the precise mechanisms underlying this phenomenon remain unclear. Numerous studies have documented that TBI facilitates fracture healing by increasing the levels of various cytokines and growth factors (22, 23). Thus, we presumed that cerebral injury may contribute to peripheral nerve recovery in a similar manner.

Early activation of the inflammatory response after peripheral nerve injury, called neuroinflammation, may result in both beneficial and detrimental effects (24). Following nerve injury, neuroinflammation is essential for degeneration and clearance of the distal nerve segment, which is termed Wallerian degeneration. However, a prolonged and excessive inflammatory response exacerbates peripheral nerve injury and inhibits neurite outgrowth. Both resident macrophages and infiltrating macrophages play important roles in neuroinflammation of the peripheral nervous system. In the early phase, the majority macrophages exhibit an M1 phenotype, which is associated with proinflammatory functions, neurodegeneration and myelin phagocytosis (8). In addition, downregulation of the expression of M1-related factors by pharmacological or genetic approaches compromises Wallerian degeneration and subsequent regeneration. In the present study, the total number of macrophages in injured peripheral nervous system was comparable between mice subjected to TBI and controls. Furthermore, neither the M1-activatied macrophages nor M1-associated gene expression was altered by TBI prior to 2 weeks after BPI. According to these results, TBI does not participate in the proliferation of resident macrophages, the infiltration of circulating macrophages or macrophage phenotype in the early phase. Thus, we hypothesize that TBI may have no influence on Wallerian degeneration after peripheral nervous injury.

In contrast to the role of M1 macrophages in Wallerian degeneration, M2 macrophages are involved in the nerve regenerative process. Typically, M1 macrophages are associated with proinflammatory and neurodegenerative characteristics, while M2 macrophages are associated with anti-inflammatory and reparative functions in the late phase of inflammation (8). In our study, TBI induced an increase in the M2 activation of macrophages 2 weeks after BPI. Considering that the total number of macrophages was not

significantly altered by TBI, it is reasonable to speculate that BPI does not influence the proliferation of resident macrophages or the infiltration of circulating macrophages but contributes to the phenotypic switch of M1 to M2 macrophages. Due to the neurotoxic effect of M1 macrophages on cultured neuronal cells, M2 macrophages have been reported to promote neurite outgrowth in vitro (8, 25). An elevated number of M2 macrophages is also related to accelerated structural and functional recovery after peripheral nerve injury in vivo (26). Thus, our data suggest that cerebral injury promotes peripheral nerve regeneration by facilitating the M1-to-M2 transition of macrophages.

To explore the molecular mechanism by which cerebral injury regulates the macrophage phenotype in the peripheral nervous system, proteomics assays combined with bioinformatics analyses were performed to identify the underlying molecules. Among the diverse molecules in the circulation that were significantly altered by TBI, SAA1, the protein expression of which was upregulated in the serum and tissue around the injured nerve, stands out as a promising candidate. However, the mRNA levels of SAA1 were not influenced by TBI. These findings are in agreement with the classic notion that SAA1 is primarily produced by the liver and secreted into the circulation upon stress. In a previous study, SAA1 was shown to have a potential effect on the induction of the M2 macrophage phenotype both in vitro and in vivo (27, 28). By using recombinant human SAA1, we provided further evidence that SAA1 administration contributes to the resolution of the proinflammatory response, facilitates the M1-to-M2 transition of macrophages and promotes peripheral nerve regeneration. As documented by previous investigations, neurotrophic factors released by macrophages may underlie the promotion of nerve regeneration (29, 30). Cattin and colleagues recently found that peripheral nerve regeneration is a multicellular process initiated by hypoxia. Among multiple cell types, only macrophages can sense hypoxic stimuli and induce neovascularization by stabilizing HIF-1a and secreting VEGF-A. The newly formed blood vessels provide a scaffold for Schwann cell-mediated peripheral nerve regeneration (9). Considering that the proangiogenic effect of M2 macrophages is well documented in other tissues and under pathological conditions, we reason that M2 macrophages may also play a similar role in the regeneration of peripheral nerves (31). However, further studies are needed to verify our hypothesis.

In the present study, we found that TBI enhances morphological regeneration and functional recovery of the C7 nerve. In addition, the beneficial effect of cerebral injury on peripheral nerve regeneration is at least partly mediated by upregulation of SAA1 expression, which contributes to the resolution of the proinflammatory response and facilitates the M1-to-M2 transition of macrophages. These findings imply that SAA1 represents a potential therapeutic target for the promotion of peripheral nerve regeneration.

Conclusions

CC7 surgery provides an opportunity to gain insight into the interaction between the brain and peripheral nervous system. The circulation is one system that connects them and altered neuroinflammation at least partly accounts for the influence of cerebral injury on peripheral nerve regeneration. Modulation of neuroinflammation represents an approach to enhance nerve repair, and SAA1 may be a promising therapeutic target.

Abbreviations

CC7, contralateral seventh cervical nerve transfer; BPI, brachial plexus injury; TBI, traumatic brain injury; SAA1, serum amyloid protein A1; C7, seventh cervical nerve; CMAPs, compound muscle action potentials; MPZ, myelin protein zero; IPA, ingenuity pathway analysis.

Declarations

Ethics approval and consent to participate

The institutional review board of Huashan Hospital approved the study on patients, and each participant provided written informed consent. The mouse experiments were approved by the Animal Care and Use Committee of Huashan Hospital and were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Wendong Xu contributed to the concept of this study and revised the manuscript. Fan Su and Guobao Wang carried out the study in mouse models, and Fan Su analyzed the data and drafted the manuscript. Tie Li followed up the patients to obtain the data regarding the Tinel’s sign.

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None.

References

1. Martyn CN, Hughes RA. Epidemiology of peripheral neuropathy. J Neurol Neurosurg Psychiatry. 1997;62(4):310-8.
2. Deumens R, Bozkurt A, Meek MF, Marcus MA, Joosten EA, Weis J, et al. Repairing injured peripheral nerves: Bridging the gap. Prog Neurobiol. 2010;92(3):245-76.
3. Novak CB, Anastakis DJ, Beaton DE, Katz J. Patient-reported outcome after peripheral nerve injury. J Hand Surg Am. 2009;34(2):281-7.
4. Zheng MX, Hua XY, Feng JT, Li T, Lu YC, Shen YD, et al. Trial of Contralateral Seventh Cervical Nerve Transfer for Spastic Arm Paralysis. N Engl J Med. 2018;378(1):22-34.
5. Spinner RJ, Shin AY, Bishop AT. Rewiring to Regain Function in Patients with Spastic Hemiplegia. N Engl J Med. 2018;378(1):83-4.
6. Feng JT, Xu WD. Contralateral Cervical Nerve Transfer for Arm Paralysis. N Engl J Med. 2018;378(15):1460-1.
7. Wang W, Gao J, Na L, Jiang H, Xue J, Yang Z, et al. Craniocerebral injury promotes the repair of peripheral nerve injury. Neural Regen Res. 2014;9(18):1703-8.
8. Zigmond RE, Echevarria FD. Macrophage biology in the peripheral nervous system after injury. Prog Neurobiol. 2019;173:102-21.
9. Cattin AL, Burden JJ, Van Emmenis L, Mackenzie FE, Hoving JJ, Garcia Calavia N, et al. Macrophage-Induced Blood Vessels Guide Schwann Cell-Mediated Regeneration of Peripheral Nerves. Cell. 2015;162(5):1127-39.
10. Mokarram N, Merchant A, Mukhatyar V, Patel G, Bellamkonda RV. Effect of modulating macrophage phenotype on peripheral nerve repair. Biomaterials. 2012;33(34):8793-801.
11. Uhlar CM, Burgess CJ, Sharp PM, Whitehead AS. Evolution of the serum amyloid A (SAA) protein superfamily. Genomics. 1994;19(2):228-35.
12. Urieli-Shoval S, Linke RP, Matzner Y. Expression and function of serum amyloid A, a major acute-phase protein, in normal and disease states. Curr Opin Hematol. 2000;7(1):64-9.
13. He RL, Zhou J, Hanson CZ, Chen J, Cheng N, Ye RD. Serum amyloid A induces G-CSF expression and neutrophilia via Toll-like receptor 2. Blood. 2009;113(2):429-37.
14. De Santo C, Arscott R, Booth S, Karydis I, Jones M, Asher R, et al. Invariant NKT cells modulate the suppressive activity of IL-10-secreting neutrophils differentiated with serum amyloid A. Nat Immunol. 2010;11(11):1039-46.
15. Sack GH, Jr. Serum Amyloid A (SAA) Proteins. Subcell Biochem. 2020;94:421-36.
16. Wang M, Li ZY, Xu WD, Hua XY, Xu JG, Gu YD. Sensory restoration in cortical level after a contralateral C7 nerve transfer to an injured arm in rats. Neurosurgery. 2010;67(1):136-43; discussion 43.

17. Jiang S, Li ZY, Hua XY, Xu WD, Xu JG, Gu YD. Reorganization in motor cortex after brachial plexus avulsion injury and repair with the contralateral C7 root transfer in rats. Microsurgery. 2010;30(4):314-20.
18. Zheng H, Zhang Z, Jiang S, Yan B, Shi X, Xie Y, et al. A shape-memory and spiral light-emitting device for precise multisite stimulation of nerve bundles. Nat Commun. 2019;10(1):2790.
19. Liu Y, Zhou LJ, Wang J, Li D, Ren WJ, Peng J, et al. TNF-alpha Differentially Regulates Synaptic Plasticity in the Hippocampus and Spinal Cord by Microglia-Dependent Mechanisms after Peripheral Nerve Injury. J Neurosci. 2017;37(4):871-81.
20. Davis KD, Taylor KS, Anastakis DJ. Nerve injury triggers changes in the brain. Neuroscientist. 2011;17(4):407-22.
21. Rusanescu G, Mao J. Peripheral nerve injury induces adult brain neurogenesis and remodelling. J Cell Mol Med. 2017;21(2):299-314.
22. Mollahosseini M, Ahmadirad H, Goujani R, Khorramdelazad H. The Association Between Traumatic Brain Injury and Accelerated Fracture Healing: A Study on the Effects of Growth Factors and Cytokines. J Mol Neurosci. 2020.
23. Hofman M, Koopmans G, Kobbe P, Poeze M, Andruszkow H, Brink PR, et al. Improved fracture healing in patients with concomitant traumatic brain injury: proven or not? Mediators Inflamm. 2015;2015:204842.
24. Jessen KR, Mirsky R. The repair Schwann cell and its function in regenerating nerves. J Physiol. 2016;594(13):3521-31.
25. Kigerl KA, Gensel JC, Ankeny DP, Alexander JK, Donnelly DJ, Popovich PG. Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord. J Neurosci. 2009;29(43):13435-44.
26. Lv D, Zhou L, Zheng X, Hu Y. Sustained release of collagen VI potentiates sciatic nerve regeneration by modulating macrophage phenotype. Eur J Neurosci. 2017;45(10):1258-67.
27. Li Y, Cai L, Wang H, Wu P, Gu W, Chen Y, et al. Pleiotropic regulation of macrophage polarization and tumorigenesis by formyl peptide receptor-2. Oncogene. 2011;30(36):3887-99.
28. Sun L, Zhou H, Zhu Z, Yan Q, Wang L, Liang Q, et al. Ex vivo and in vitro effect of serum amyloid a in the induction of macrophage M2 markers and efferocytosis of apoptotic neutrophils. J Immunol. 2015;194(10):4891-900.
29. Hikawa N, Takenaka T. Myelin-stimulated macrophages release neurotrophic factors for adult dorsal root ganglion neurons in culture. Cell Mol Neurobiol. 1996;16(4):517-28.
30. Yin Y, Cui Q, Gilbert HY, Yang Y, Yang Z, Berlinicke C, et al. Oncomodulin links inflammation to optic nerve regeneration. Proc Natl Acad Sci U S A. 2009;106(46):19587-92.
31. He L, Marneros AG. Doxycycline inhibits polarization of macrophages to the proangiogenic M2-type and subsequent neovascularization. J Biol Chem. 2014;289(12):8019-28.