

Frozen Vein Wrapping for Chronic Nerve Constriction Injury Reduces Sciatic Nerve Allodynia in a Rat Model

Michiaki Mukai (✉ michiaki.mukai@gmail.com)

Chiba University: Chiba Daigaku <https://orcid.org/0000-0002-4673-4734>

Kentaro Uchida

Kitasato University School of Medicine: Kitasato Daigaku Igakubu

Naoya Hirose

Chiba Daigaku - Nishichiba Campus: Chiba Daigaku

Kenichi Murakami

Nagareyama Central Hospital: Nagareyama Chuo Byoin

Gen Inoue

Kitasato University School of Medicine: Kitasato Daigaku Igakubu

Masayuki Miyagi

Kitasato University School of Medicine: Kitasato Daigaku Igakubu

Yasuhiro Shiga

Chiba University: Chiba Daigaku

Kazuhide Inage

Chiba University: Chiba Daigaku

Hiroyuki Sekiguchi

Shonan University of Medical Sciences: Shonan Iryo Daigaku

Sumihisa Orita

Chiba University: Chiba Daigaku

Takane Suzuki

Chiba University: Chiba Daigaku

Yusuke Matsuura

Chiba University: Chiba Daigaku

Masashi Takaso

Chiba University: Chiba Daigaku

Seiji Ohtori

Chiba University: Chiba Daigaku

Keywords: peripheral nerve injuries, frozen vein wrapping, allodynia

Posted Date: June 16th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-600165/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

Autologous vein wrapping (VW) is used in the treatment of recurrent chronic constriction neuropathy and traumatic peripheral nerve injury. However, use of autologous veins is limited by the inability to obtain longer veins of sufficient length for larger sites. Frozen allograft tissue has several advantages, including its availability for large grafts, avoidance of donor-site morbidity, and shorter operation time. Here, we investigated the effect of frozen vein wrapping (FVW) in Wistar rats as a model of sciatic nerve injury.

Methods

The rats were grouped by treatment as (i) untreated after chronic constriction injury surgery (CCI; control group), (ii) treated with vein wrapping using freshly isolated vein (VW), and (iii) treated with vein wrapping using frozen vein (FVW). Mechanical allodynia was assessed with von Frey filaments on postoperative days (PODs) 1, 3, 5, 7, and 14.

Results

The response of heme oxygenase-1 gene, Hmox-1, expression to VW and FVW was assessed by RT-PCR. Both VW and FVW significantly increased withdrawal threshold levels compared to the untreated control group on POD 1, 3, and 5. Both VW and FVW also showed increased HO-1 expression compared to the CCI group.

Conclusions

Our results suggest that FVW may be a suitable therapeutic option as a source of large grafts.

Background

Neuropathic pain resulting from compressive neuropathy or traumatic peripheral nerve injury is a common and important medical problem. Even with proper surgery, the condition sometimes recurs and can become intractable. Autologous vein wrapping (VW) using freshly isolated vein has been used to improve recurrent symptoms due to nerve scarring, and has improved outcomes in neuropathic pain resulting from recurrent compressive neuropathy and traumatic peripheral nerve injury [1-8]. We also reported that VW relieved pain behavior in a rat chronic constriction injury (CCI) model. However, use of VW is limited by the inability to obtain sufficiently long veins for larger sites.

Frozen allograft tissues such as bone, tendon, or heart valve are used to repair injured tissue. Allograft tissue has several advantages, such as its availability for large graft sizes, avoidance of donor-site morbidity, and shorter operation time. Frozen vein has become a practical therapeutic option for larger grafts. Nevertheless, the effect of frozen vein wrapping (FVW) has not been fully determined.

Previous studies proposed the mechanism by which VW relieves pain [9-11]. Heme oxygenase-1 (HO-1) is a rate-limiting enzyme which catalyzes oxidative degeneration of heme into biliverdin, carbon monoxide, and iron. Over-expression or induction of HO-1 is associated with potent anti-inflammatory and antinociceptive effects, both in vitro and in mice [12-15], and also ameliorates neuropathic pain induced by sciatic nerve injury [13, 16-19]. We previously reported that VW promotes *HO-1 expression* [9]. However, the question of whether FVW also promotes HO-1 remains unanswered.

Here, we investigated the effect of frozen vein wrapping on mechanical allodynia and HO-1 expression in a rat CCI model.

Methods

Animals

Eight-week-old male Wistar rats (240–260 g; n=115) were housed under controlled conditions in a semi-barrier housing system (12-h light/dark cycle, 21–23°C, 45–65% humidity) and kept on a standard rodent chow diet (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan).

Preparation of graft materials

The abdominal portion of the vena cava was harvested from 20 donor rats, immediately immersed in phosphate-buffered saline (PBS), opened by sectioning along the longitudinal axis, and then cut into 8-mm lengths. A total of 60 vein graft materials were prepared from the vena cavae of 20 donor rats. Thirty vein sections from 10 rats were immediately applied to the CCI model. For frozen VW, the remaining 30 vein sections from 10 rats were stored in a freezer at -80°C for 1 week, then thawed at 37°C for 1 hour before use in the vein wrapping procedure.

Cell viability of frozen vein

The freezing and thawing process leads to cell death in tissues via membrane damage, osmotic shock, and ice crystal formation [20, 21]. We investigated the cell viability of frozen vein, because viable cells could exhibit immunogenicity. Fresh vein and frozen (n=6, each) vein which was thawed in culture medium at 37°C were digested in 0.1% collagenase for 1 h at 37°C. Vein-derived cells were cultured in a minimal essential media supplemented with fetal bovine serum for 7 days. After 7 days, attached cells in the culture dish were detached with a 0.25% trypsin/EDTA solution, counted using an automated cell counter (Countess™; Invitrogen Life Technologies, Carlsbad, CA, USA) and stained with trypan blue to measure cell viability.

Creation of CCI model

90 rats were randomly assigned to three treatment groups (n =30 each): a control group, a group undergoing vein wrapping with freshly isolated vein (VW group), and a group undergoing vein wrapping with frozen vein (FVW group). The control group rats underwent surgery to induce CCI (=CCI group). CCI

of the sciatic nerve was induced under anesthesia with 100 mg/kg ketamine hydrochloride and 10 mg/kg xylazine hydrochloride according to a previously described method [22]. In the VW group, rats were treated with the vein wrapping procedure after the CCI surgery. Veins were then used in wrapping the ligated sciatic nerve, with the endothelial surface positioned adjacent to the epineurium of the nerve.

von Frey Test

Rats (n = 5 per group) were subjected to the von Frey test according to our previous report [9-11, 23] on postoperative days (PODs) 1, 3, 5, 7, and 14. Following randomization and 1-h acclimation to the test cage, the von Frey test was conducted by applying a von Frey filament (Mono-filament Kit; Smith & Nephew, Germantown, WI) to the hind paw perpendicular to the plantar surface. Stimulus strength was slowly increased or decreased to evaluate the withdrawal threshold. Baseline thresholds were measured 3 days prior to surgery. The Dixon nonparametric test [24] was used to analyze the data in accordance with a previous report [25].

HO-1 Gene (Hmox1) Expression Analysis

mRNA expression of Hmox1 in the sciatic nerve was examined by quantitative polymerase chain reaction (qPCR). In these experiments, an additional group of rats (n = 5) which did not receive CCI surgery or treatment before sciatic nerve resection (normal group) were used to evaluate relative gene expression levels following CCI. The rats (n = 5 per group) were anesthetized and the right sciatic nerve was resected prior to (normal group), and 1, 3, 5, 7, and 14 days after wrapping, and scar tissue, the veins, and ligatures were removed from the resected nerves. After RNA extraction and complementary DNA (cDNA) synthesis, we performed qPCR with PCR primers (Table 1) using the *sybr* green method under the following settings: initial denaturation at 95°C for 1 min, 40 cycles of 95°C for 5 s, and 60°C for 30 s. Gene expressions were calculated by the delta-delta-method. Hmox1 mRNA expression was normalized to glyceraldehyde dehydrogenase (Gapdh) levels and values in the three treatment groups were compared.

Measurement of bFGF protein level in freeze vein

We previously reported that bFGF stimulated HO-1 expression in sciatic nerve-derived cells [9]. To investigate the possible mechanism of *Hmox1* induction following FVW, bFGF protein levels in FVW were measured. Vein and sciatic nerve were homogenized in RIPA buffer with proteinase inhibitors. After centrifugation, supernatant was collected to measure the total protein and bFGF concentration. Total protein concentration was evaluated with the bicinchoninic acid assay. Samples having a protein concentration of 500 µg/ml were prepared and bFGF concentration was measured using a commercial bFGF ELISA kit (Biolegend).

Statistical analyses

All statistical comparisons were conducted using SPSS (version 19.0; SPSS Inc., Chicago, IL). Tukey's multiple comparisons test was used to compare Hmox1 mRNA and protein levels among the control, VW,

and FVW groups. The t-test was used to compare bFGF protein levels between vein and sciatic nerve. $P < 0.05$ was considered statistically significant.

Table 1. Sequences of primers used in this study.

Gene	Direction	Primer Sequence (5′-3′)	Product Size (bp)
<i>Hmox1</i>	Sense	GAG CGA AAC AAG CAG AAC CC	167
	Antisense	ACC TCG TGG AGA CGC TTT AC	
<i>Gapdh</i>	Sense	TGC CAC TCA GAA GAC TGT GG	129
	Antisense	TTC AGC TCT GGG ATG ACC TT	

Results

Cell viability in frozen vein

Seven days after culture, $4.3 \pm 1.2 \times 10^5$ cells were isolated from fresh vein. In contrast, no adherent cells were observed in frozen vein.

von Frey tests

Mechanical allodynia was seen in rats in the CCI group on POD 1, and continually observed in the first 2 weeks post-surgery. Withdrawal threshold was significantly higher in the VW and FVW groups than in the CCI groups from POD 1 ($p < 0.05$; Fig. 1), POD 3 ($p < 0.05$; Fig. 1) and POD 5 ($p < 0.05$; Fig. 1). On POD 7 and 14, withdrawal threshold was higher in the VW and FVW groups than in the CCI groups, albeit that the difference was not significant. There was no significant difference between the VW and FVW groups.

Hmox1 mRNA expression

Hmox1 mRNA expression was significantly higher in the VW and FVW groups than in the control groups on POD 1 ($p < 0.05$; Fig. 2), POD 3 ($p < 0.05$, respectively; Fig. 2), and POD 5 ($P < 0.05$, respectively; Fig. 2). There was no significant difference between the VW and FVW groups.

bFGF concentration in vein

To investigate the possible mechanism of *Hmox1* induction following FVW, bFGF concentration was measured, and shown to be 10.5-fold higher in vein than in sciatic nerve (Fig. 3).

Discussion

In this study, we investigated the therapeutic effects of frozen vein wrapping for recurrent compressive neuropathy and traumatic peripheral nerve injury. Frozen vein wrapping increased the withdraw threshold level similarly to fresh vein wrapping. In addition, qPCR revealed that both fresh and frozen vein wrapping promoted *Hmox1* expression following CCI. Together, these findings suggest that frozen vein may become an alternative source of large grafts as a practical therapeutic option.

Autologous vein is generally selected for use in VW to treat recurrent chronic constriction neuropathy. However, some studies reported that glutaraldehyde-preserved allogenic vein seemed to induce a marked inflammatory response [26, 27], and that epineural scarring and adherence to the underlying nerve also had an adverse effect compare to autografting [27]. A recent study indicated that glutaraldehyde stimulated peripheral nerve scar formation and resulted in functional deficiencies [28]. In our present study, frozen VW relieved the mechanical allodynia following CCI similar to fresh VW in a rat CCI model. The freeze-thaw process markedly reduces the number of viable cells and immunogenicity in tendon cells [21]. In our study, no viable cells were observed in frozen vein. Therefore, frozen vein allograft may be a treatment option for larger sites requiring long veins.

Previous studies proposed the mechanism that autologous vein wrapping exerted its therapeutic effect through vein-derived trophic factors [9, 10, 23]. We previously reported that *Hmox1* following fresh VW and recombinant bFGF stimulate *Hmox1* expression in sciatic nerve cells in vitro [9]. In our present study, even in the absence of viable cells, FVW promoted *Hmox1* expression, similarly to fresh VW. Extracellular matrix (ECM) contains growth factors which are released by proteolytic cleavage [29, 30]. bFGF is bound to heparan sulfate in the ECM and is released and activated for angiogenesis [29]. Here, frozen vein contained a higher amount of bFGF than sciatic nerve. We previously reported that bFGF stimulated rat sciatic nerve in vitro and that bFGF-absorbed collagen sheet wrapping promoted *Hmox1* expression and improved mechanical allodynia [23]. Our present observation suggests that ECM-derived bFGF in vein may induce *Hmox1* expression.

Conclusions

Frozen vein wrapping increased withdrawal threshold levels following CCI in rats. Frozen vein stimulated *Hmox1* expression, and its expression may be induced by vein-derived bFGF. Frozen VW may become an alternative source of large grafts as a practical therapeutic option.

Abbreviations

VW: vein wrapping; FVW: frozen vein wrapping; CCI: chronic constriction injury; HO-1: heme oxygenase-1; PBS: phosphate buffered saline; qPCR: quantitative polymerase chain reaction;

Declarations

Ethical approval and consent to participate

The all animal studies were approved and conducted according to the ethics committees of Chiba University (approval number: 1-468).

Consent for publication

Not applicable

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article. The raw data can be requested from the corresponding author.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was funded by a Grant-in-Aid for Scientific Research (C) (grant number 20K9415)

Author's contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Naoya Hirose, Kenichi Murakami, Gen Inoue, Masayuki Miyagi, Yasuhiro Shiga, Hiroyuki Sekiguchi, Kazuhide Inage, Sumihisa Orita, Yusuke Matsuura, Masashi Takaso, and Seiji Ohtori. The first draft of the manuscript was written by Michiaki Mukai and Kentaro Uchida, and all authors commented on previous versions of the manuscript prior to finalization. All authors read and approved the final manuscript.

Acknowledgements

We thank DMC Corp for editing drafts of this manuscript.

References

1. Campbell JT, Schon LC, Burkhardt LD. Histopathologic findings in autogenous saphenous vein graft wrapping for recurrent tarsal tunnel syndrome: a case report. *Foot Ankle Int.* 1998;19:766–9.
2. Easley ME, Schon LC. Peripheral nerve vein wrapping for intractable lower extremity pain. *Foot Ankle Int.* 2000;21:492–500.
3. Kokkalis ZT, Jain S, Sotereanos DG. Vein wrapping at cubital tunnel for ulnar nerve problems. *J Shoulder Elbow Surg.* 2010;19:91–7.

4. Mulgrew S, Kanabar GP, Papanastasiou S. Further evidence for treatment of recalcitrant neuropathy of the upper limb with autologous vein wrapping. *Ann Plast Surg.* 2012;69:288–91.
5. Sadek AF, Fouly EH, Hamdy M. Functional and electrophysiological outcome after autogenous vein wrapping of primary repaired ulnar nerves. *Microsurgery.* 2014;34:361–6.
6. Schon LC, Anderson CD, Easley ME, Lam PW, Trnka HJ, Lumsden DB, et al. Surgical treatment of chronic lower extremity neuropathic pain. *Clin Orthop Relat Res.* 2001. 10.1097/00003086-200108000-00022.156-64.
7. Sotereanos DG, Giannakopoulos PN, Mitsionis GI, Xu J, Herndon JH. Vein-graft wrapping for the treatment of recurrent compression of the median nerve. *Microsurgery.* 1995;16:752–6.
8. Varitimidis SE, Riano F, Sotereanos DG. Recalcitrant post-surgical neuropathy of the ulnar nerve at the elbow: treatment with autogenous saphenous vein wrapping. *J Reconstr Microsurg.* 2000;16:273–7.
9. Hirosawa N, Uchida K, Kuniyoshi K, Murakami K, Inoue G, Miyagi M, et al. Vein wrapping facilitates basic fibroblast growth factor-induced heme oxygenase-1 expression following chronic nerve constriction injury. *J Orthop Res.* 2018;36:898–905.
10. Hirosawa N, Uchida K, Kuniyoshi K, Murakami K, Inoue G, Miyagi M, et al. Vein wrapping promotes M2 macrophage polarization in a rat chronic constriction injury model. *J Orthop Res.* 2018. 10.1002/jor.23875.
11. Murakami K, Kuniyoshi K, Iwakura N, Matsuura Y, Suzuki T, Takahashi K, et al. Vein wrapping for chronic nerve constriction injury in a rat model: study showing increases in VEGF and HGF production and prevention of pain-associated behaviors and nerve damage. *J Bone Joint Surg Am.* 2014;96:859–67.
12. Devesa I, Ferrándiz ML, Terencio MC, Joosten LA, van den Berg WB, Alcaraz MJ. Influence of heme oxygenase 1 modulation on the progression of murine collagen-induced arthritis. *Arthritis Rheum.* 2005;52:3230–8.
13. Hervera A, Leáñez S, Negrete R, Motterlini R, Pol O. Carbon monoxide reduces neuropathic pain and spinal microglial activation by inhibiting nitric oxide synthesis in mice. *PLoS One.* 2012;7:e43693.
14. Megías J, Guillén MI, Clérigues V, Rojo AI, Cuadrado A, Castejón MA, et al. Heme oxygenase-1 induction modulates microsomal prostaglandin E synthase-1 expression and prostaglandin E(2) production in osteoarthritic chondrocytes. *Biochem Pharmacol.* 2009;77:1806–13.
15. Motterlini R, Haas B, Foresti R. Emerging concepts on the anti-inflammatory actions of carbon monoxide-releasing molecules (CO-RMs). *Med Gas Res.* 2012;2:28.
16. Castany S, Codony X, Zamanillo D, Merlos M, Verdú E, Boadas-Vaello P. Repeated Sigma-1 Receptor Antagonist MR309 Administration Modulates Central Neuropathic Pain Development After Spinal Cord Injury in Mice. *Front Pharmacol.* 2019;10:222.
17. Chen Y, Chen H, Xie K, Liu L, Li Y, Yu Y, et al. H₂ Treatment Attenuated Pain Behavior and Cytokine Release Through the HO-1/CO Pathway in a Rat Model of Neuropathic Pain. *Inflammation.* 2015;38:1835–46.

18. Hervera A, Gou G, Leáñez S, Pol O. Effects of treatment with a carbon monoxide-releasing molecule and a heme oxygenase 1 inducer in the antinociceptive effects of morphine in different models of acute and chronic pain in mice. *Psychopharmacology*. 2013;228:463–77.
19. Hervera A, Leáñez S, Motterlini R, Pol O. Treatment with carbon monoxide-releasing molecules and an HO-1 inducer enhances the effects and expression of μ -opioid receptors during neuropathic pain. *Anesthesiology*. 2013;118:1180–97.
20. Jang TH, Park SC, Yang JH, Kim JY, Seok JH, Park US, et al. Cryopreservation and its clinical applications. *Integr Med Res*. 2017;6:12–8.
21. Suto K, Urabe K, Naruse K, Uchida K, Matsuura T, Mikuni-Takagaki Y, et al. Repeated freeze-thaw cycles reduce the survival rate of osteocytes in bone-tendon constructs without affecting the mechanical properties of tendons. *Cell Tissue Bank*. 2012;13:71–80.
22. Bennett GJ, Xie YK. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain*. 1988;33:87–107.
23. Mukai M, Uchida K, Hirokawa N, Murakami K, Kuniyoshi K, Inoue G, et al. Wrapping With Basic Fibroblast Growth Factor-Impregnated Collagen Sheet Reduces Rat Sciatic Nerve Allodynia. *J Orthop Res*. 2019;37:2258–63.
24. Dixon WJ. Efficient analysis of experimental observations. *Annu Rev Pharmacol Toxicol*. 1980;20:441–62.
25. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. *J Neurosci Methods*. 1994;53:55–63.
26. Masear VR, Colgin S. The treatment of epineural scarring with allograft vein wrapping. *Hand Clin*. 1996;12:773–9.
27. Ruch DS, Spinner RM, Koman LA, Challa VR, O'Farrell D, Levin LS. The histological effect of barrier vein wrapping of peripheral nerves. *J Reconstr Microsurg*. 1996;12:291–5.
28. Lemke A, Penzenstadler C, Ferguson J, Lidinsky D, Hopf R, Bradl M, et al. A novel experimental rat model of peripheral nerve scarring that reliably mimics post-surgical complications and recurring adhesions. *Dis Model Mech*. 2017;10:1015–25.
29. Vlodaysky I, Fuks Z, Ishai-Michaeli R, Bashkin P, Levi E, Korner G, et al. Extracellular matrix-resident basic fibroblast growth factor: implication for the control of angiogenesis. *J Cell Biochem*. 1991;45:167–76.
30. Vlodaysky I, Korner G, Ishai-Michaeli R, Bashkin P, Bar-Shavit R, Fuks Z. Extracellular matrix-resident growth factors and enzymes: possible involvement in tumor metastasis and angiogenesis. *Cancer Metastasis Rev*. 1990;9:203–26.

Figures

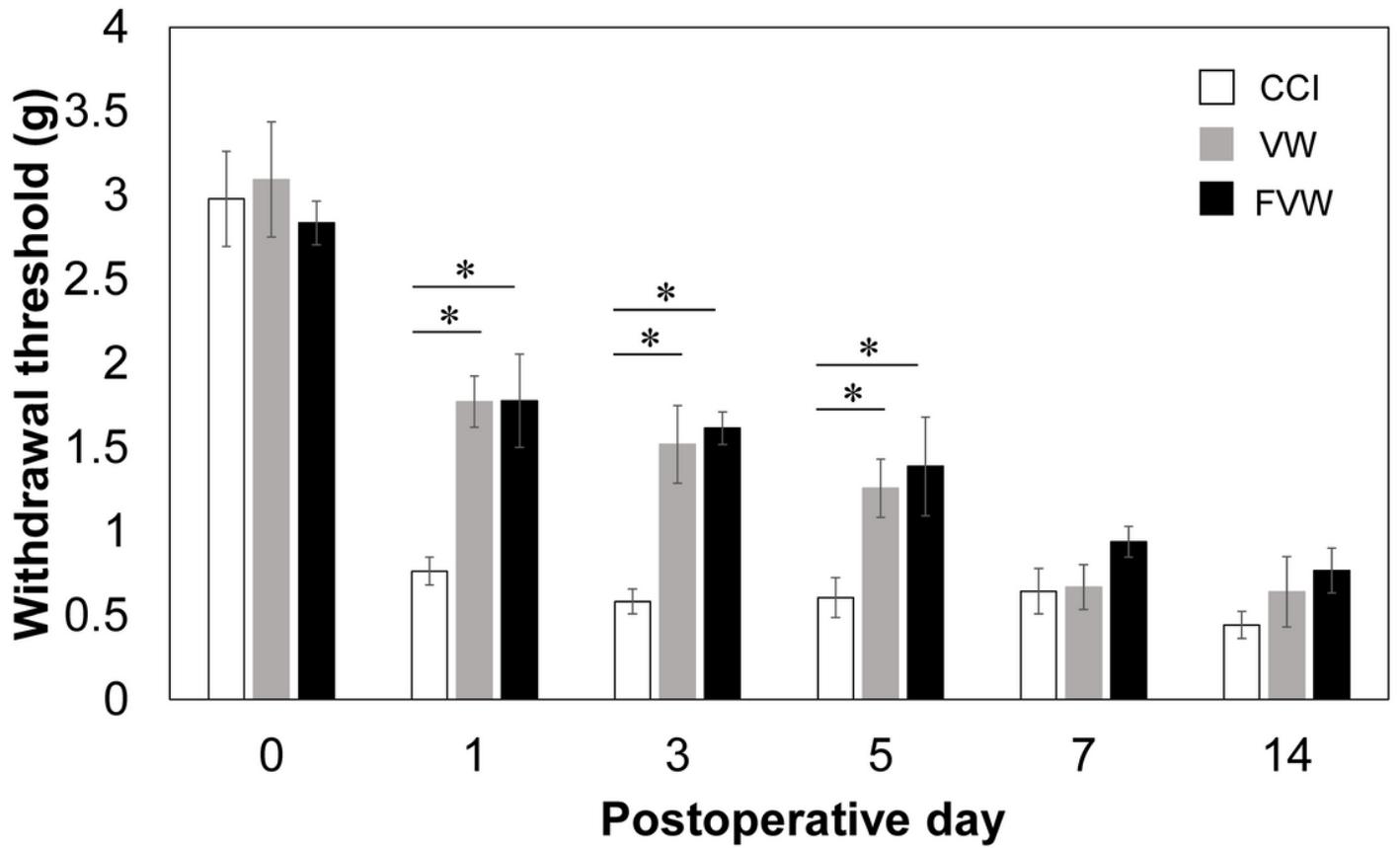


Figure 1

Withdrawal threshold in the CCI, vein wrapping (VW) and frozen vein wrapping (FVW) groups. Withdrawal threshold in the CCI, vein wrapping (VW) and frozen vein wrapping (FVW) groups. Data show mean \pm standard error (n = 5, each time point). *p < 0.05

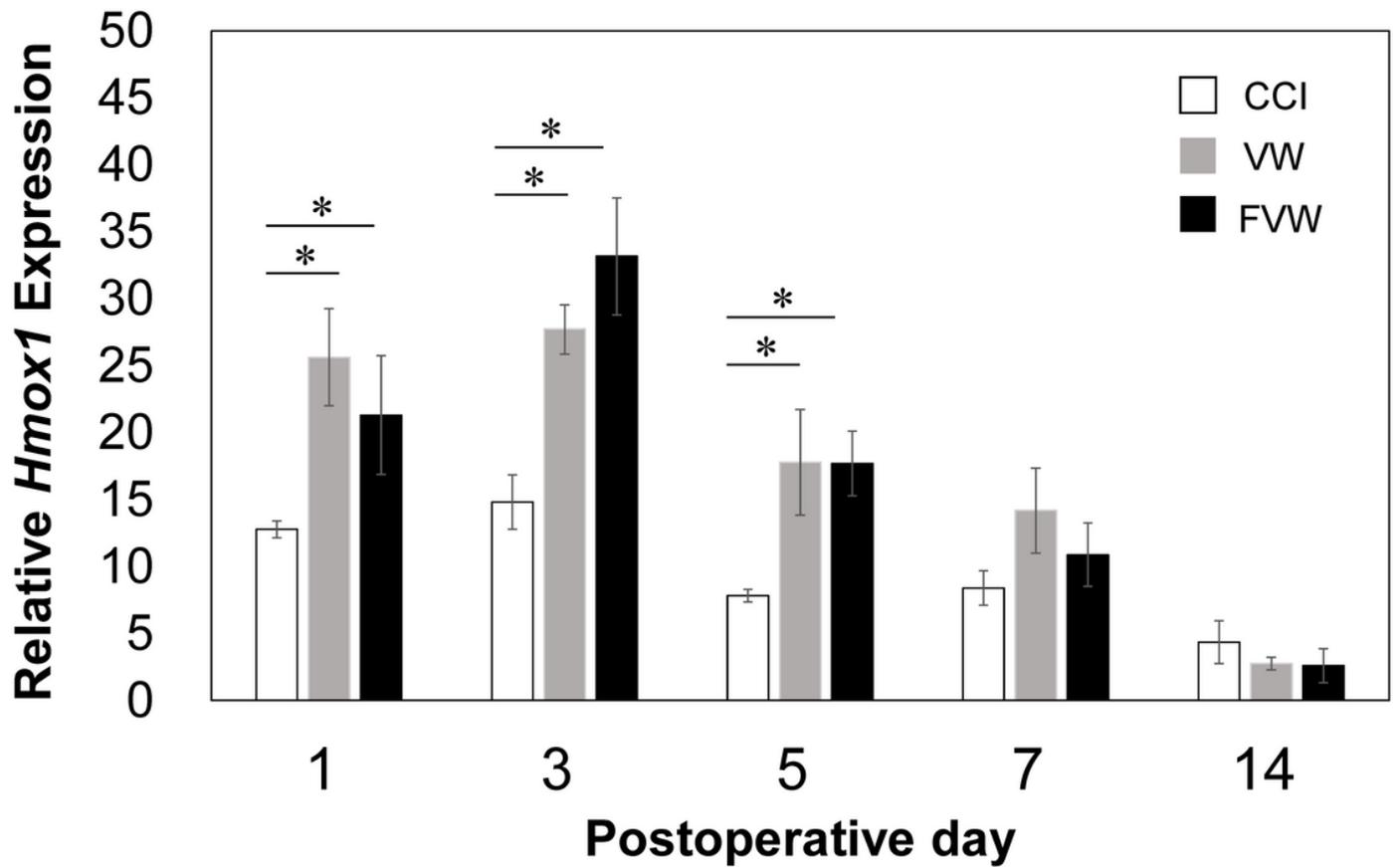


Figure 2

Effect of frozen vein wrapping on Hmox1 expression Effect of frozen vein wrapping on Hmox1 messenger RNA (mRNA) levels in sciatic nerve after chronic constriction injury (CCI). Data show mean \pm standard error (n=5, each time point). *p < 0.05

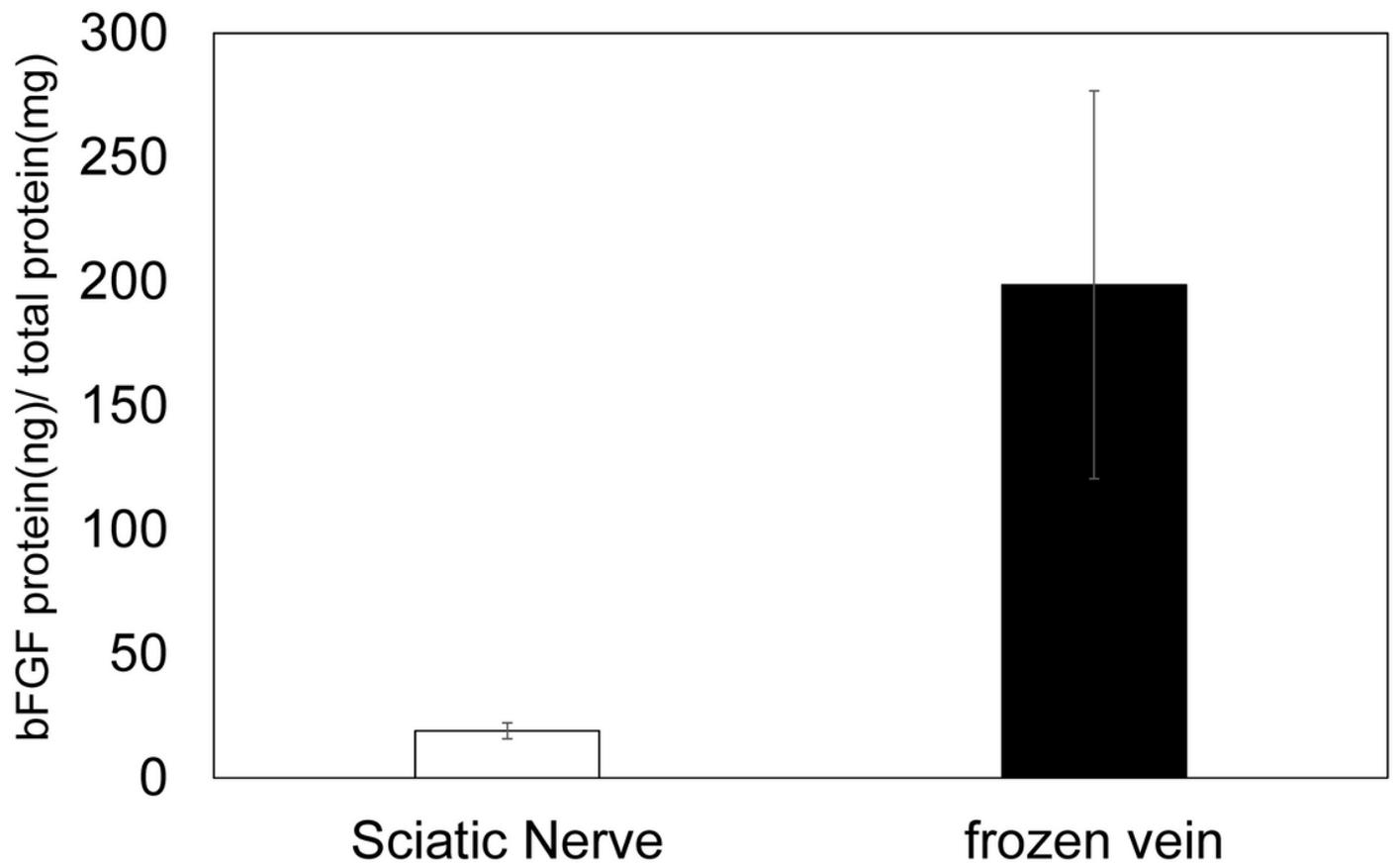


Figure 3

bFGF concentration in vein and sciatic nerve bFGF protein concentration (bFGF protein (ng)/total protein (mg)) in vein and sciatic nerve. Data show mean \pm standard error (n = 5). *p < 0.05