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Short Communication

Fatostatin delayed lip sensory recovery after inferior alveolar nerve transection by inhibiting sterol regulatory element-binding protein 1

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Peripheral nerve injury

Abstract Lipid metabolism is essential for nerve repair in damaged nerves. Fatostatin, a selective inhibitor of sterol regulatory element-binding protein 1 (SREBP1), could reduce cholesterol synthesis and disturb lipid homeostasis. However, whether fatostatin would delay lip sensory recovery after inferior alveolar nerve transection remains unclear. In this preliminary study, we investigated the effects of fatostatin on lip sensory recovery *in vivo* and axon growth *in vitro*. Fatostatin significantly delayed lip sensory recovery of mice after inferior alveolar nerve transection as evidenced by quantitative sensory testing. Fatostatin also reduced the average axon length of primary trigeminal neurons. Despite SREBP1, expressions of other lipid metabolism-related (including fatty acid synthase and ATP citrate lyase) and axon regeneration-related molecules (including activating transcription factor 3 and nerve growth factor) were also inhibited, as evidenced by the Western Blot and quantitative real-time PCR. Overall, fatostatin delayed lip sensory recovery after inferior alveolar nerve transection by inhibiting SREBP1.

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Introduction

The inferior alveolar nerve (IAN) is often injured during mandibular tumor ablation or removal of the third molar, leading to common lower lip numbness, which significantly affects patients' quality of life.[1] Our previous study found that many patients with IAN sacrifice exhibited varying degrees of sensory recovery spontaneously, and lipid metabolism plays an essential role in promoting lip sensory recovery.[2] Lipid metabolic processes, including lipid synthesis, breakdown, and peroxidation, play an essential regulatory role in nerve repair following injury.[3]

Sterol regulatory element-binding protein 1 (SREBP1) is a key regulator of lipid biosynthesis and is involved in several processes necessary for cellular function.[4] A previous study has suggested the potential connection between SREBP1, cholesterol synthesis, and axon growth.[5] Fatostatin, a selective inhibitor of SREBP1, could reduce its activity, thereby affecting cholesterol synthesis and lipid homeostasis.[6] Fatostatin had strong antitumor activity in cancer by inhibiting SREBP-regulated metabolic pathways.[6,7] However, no studies have explored its effects on lip sensory recovery. In this study, we explored the role of fatostatin in sensory recovery following inferior alveolar nerve transection.

Materials and methods

Animal models

This study was approved by the Institutional Review Board of Sichuan University West China Hospital of Stomatology (WCHSIRB-D-2024-706). Ten 8-week-old male C57BL/6J mice, weighing approximately 20 g, were purchased from Chengdu Dossy Experimental Animals Co., Ltd. (Chengdu, China). Mice were anesthetized with isoflurane. The inferior alveolar nerve transection model was established as we previously described.[2] 100 μM fatostatin (HY-12337, MedChemExpress, Monmouth Junction, NJ, USA) was administered to trigeminal ganglia through the infraorbital foramen with a 29 G syringe.

Cell culture and stating

The trigeminal ganglion tissues of postnatal mice were separated from the cranial base under a microscope. The tissues were placed in PBS containing 0.125 % trypsinase (Gibco, Waltham, MA, USA) for digestion (20 min, 37C). The cells were then mechanically dissociated with a Pasteur pipette in DMEM/F12 medium (Gibco) containing 10 % fetal bovine serum (Gibco) and 50 μg/ml ampicillin (Sigma—Aldrich, St. Louis, MO, USA). Once the trigeminal ganglion neurons adhered to the plastic or glass surfaces (about 3 h), the culture medium was changed to another one without fetal bovine serum. The TGNs were cultured in DMEM/F12 medium (Gibco) supplemented with N2 (1:100 dilution, Invitrogen, Carlsbad, CA, USA), B27 (1:50 dilution, Invitrogen), and 50 μg/ml ampicillin (Sigma—Aldrich) at 37 °C, 5 % CO2 for 2 days. For cells in the fatostatin group, 100 μM fatostatin was added into the cell culture system. NeuronJ was used to measure and record the longest axons in 30 cells in each image for analysis.

Sensory function assessment

The mice were placed in steel wire cages, with their head out of the cage. The measuring tip of the electronic Von Frey meter was used to vertically compress the same site of the mice' left and right lower lips, and the force was gradually applied. The behaviors of the mice, such as foot retraction, vocalization, head retraction, and avoidance, were regarded as signs, and the values of the five signs were recorded continuously for statistical analysis. Each mouse was measured several times before recording to make the mouse adapt to the contact of the measuring tip. We limited the maximum force to 100 gr to avoid the risk of puncturing the lower lip skin during the measurement (1 gr = 0.064 8 g). Five mice in each group were measured every four days for 40 days.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from mouse trigeminal ganglia samples or primary neurons using TRIzol reagent (15596026; Thermo Fisher Scientific, Waltham, MA, USA). Total RNA (1 μg) was used to synthesize the first strand of cDNA with the TaqMan MicroRNA Reverse Transcription Kit (RR047A, Takara, Shiga, Japan). Real-time PCR was conducted using SYBR Green (RR820A, Takara) on a 7900HT Fast Real-Time PCR system (4329001, Applied Biosystems, Waltham, MA, USA). The relative expression levels of target genes were normalized to GAPDH and calculated using the 2−ΔΔCt method. The sequence of primers are listed in Table 1.

Western Blot

Tissues and cells were lysed in cold RIPA buffer (BioSharp, Hefei, China), supplemented with 1 mM phenylmethylsulfonyl fluoride (Biosharp) and a protease inhibitor cocktail (MedChemExpress). Protein samples (30 μg per well) were loaded onto gels prepared using the Epizyme Gel Kit (Epizyme, Shanghai, China). Electrophoresis was performed at 80 V for 30 min, followed by 120 V for approximately 1.5 h. Proteins were transferred onto a 6 × 8 cm polyvinylidene fluoride membrane (Sigma—Aldrich) preactivated with methanol. The membranes were blocked in 5 % BSA in Tris-buffered saline with Tween 20 (TBST), and then incubated overnight with the primary antibody. The used primary antibodies included SREBP1 (sc-13551, Santa

Table 1 Sequences of primers used for qRT-PCR.

Gene	Primer sequences (5′-3′)
NGF	Forward: TGATCGGCGTACAGGCAGA
	Reverse: GCTGAAGTTTAGTCCAGTGGG
ATF3	Forward: TTTGCTAACCTGACACCCTTTG
	Reverse: AGAGGACATCCGATGGCAGA
FASN	Forward: GGAGGTGGTGATAGCCGGTAT
	Reverse: TGGGTAATCCATAGAGCCCAG
SREBF1	Forward: TGACCCGGCTATTCCGTGA
	Reverse: CTGGGCTGAGCAATACAGTTC
ACLY	Forward: ACCCTTTCACTGGGGATCACA
	Reverse: GACAGGGATCAGGATTTCCTTG

NGF, nerve growth factor; ATF3, activating transcription factor 3; FASN, fatty acid synthase; SREBF1, sterol regulatory element-binding transcription factor 1; ACLY, ATP citrate lyase.

Cruz, Dallas, TX, USA), ACLY (sc-517267, Santa Cruz), ATF3 (18665, Cell Signaling Technology, Boston, MA, USA), GAPDH (GB15002, Servicebio, Wuhan, China) After washing in TBST, membranes were incubated with the secondary antibody (ZSGB-BIO, Beijing, China), washed again in TBST, and visualized.

Immunofluorescence

Cells were fixed in 10 % formalin for 1 day, followed by gradient dehydration, paraffin embedding, sectioning, dewaxing, and rehydration. Antigen retrieval was performed using citric acid buffer (Beyotime, Shanghai, China) in an autoclave. Cells were treated with 3 % H_2O_2 to block endogenous peroxidase activity, washed with TBS 2–3 times, and blocked in 10 % goat serum (BioSharp). The primary antibody of NF200 (55453, Cell Signaling Technology) were diluted 1:100 and incubated overnight at approximately 50 μL per section. After washing with TBS, cells were incubated with secondary antibodies (1:200, ZSGB-BIO) and then washed in TBS. Slides were mounted with Antifade Mounting Medium for Fluorescence (with DAPI) (Biosharp).

Statistical analysis

Statistical analyses were performed to evaluate the differences between experimental groups. ANOVA or t-tests were used to compare the outcomes of sensory recovery, gene expression, and molecular markers across groups. Data were reported as mean ± standard deviation (SD), and a *P*-value of less than 0.05 was considered statistically significant.

Results

Fatostatin impaired lip sensory recovery following inferior alveolar nerve transection *in vivo*

To evaluate the impact of fatostatin on sensory recovery after inferior alveolar nerve transection, we conducted quantitative sensory testing and molecular analysis. Lip sensory recovery of mice was significantly delayed by fatostatin compared to the control, with a higher force threshold observed in the fatostatin group over time ($P < 0.05$) (Fig. 1A). Additionally, fatostatin significantly reduced the mRNA levels of SREBP1, FASN, ACLY, ATF3, and NGF in the trigeminal ganglia ($P < 0.05$) (Fig. 1B). Western Blot analysis (Fig. 1C) further confirmed that the protein levels of SREBP1, ACLY, and ATF3 were significantly reduced by fatostatin, suggesting that fatostatin inhibits key molecules involved in lipid metabolism and neurotrophic signaling pathways. These findings indicate that fatostatin negatively impacts lip sensory recovery after nerve injury by disrupting lipid metabolism and neurotrophic factor production.

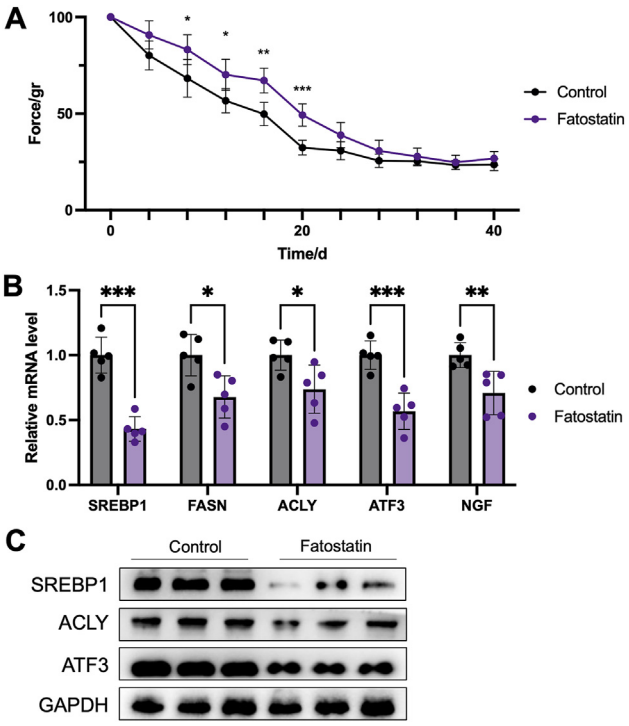

Figure 1 Fatostatin impaired lip sensory recovery following inferior alveolar nerve transection *in vivo*. (A) Quantitative sensory testing using Von Frey filaments showed the sensory recovery of the lower lip in the control and fatostatin-treated groups, measured every 4 days for 40 days. The results show that fatostatin significantly delayed lip sensory recovery compared to the control group. (B) Quantitative real-time PCR analysis of gene expression in the trigeminal ganglia demonstrated that fatostatin treatment significantly decreased the mRNA levels of SREBP1, FASN, ACLY, ATF3, and NGF. (C) Western blot analysis confirmed that fatostatin treatment reduced the expression levels of SREBP1, ACLY, and ATF3 in the trigeminal ganglia compared to the control group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. SREBP1, sterol regulatory element-binding protein 1; FASN, fatty acid synthase; ACLY, ATP citrate lyase; ATF3, activating transcription factor 3; NGF, nerve growth factor.

Fatostatin inhibits axonal growth of primary trigeminal neurons *in vitro*

To examine the effects of fatostatin on axonal growth, we further performed the *in vitro* experiment. Fatostatin decreased axonal growth in trigeminal neurons compared

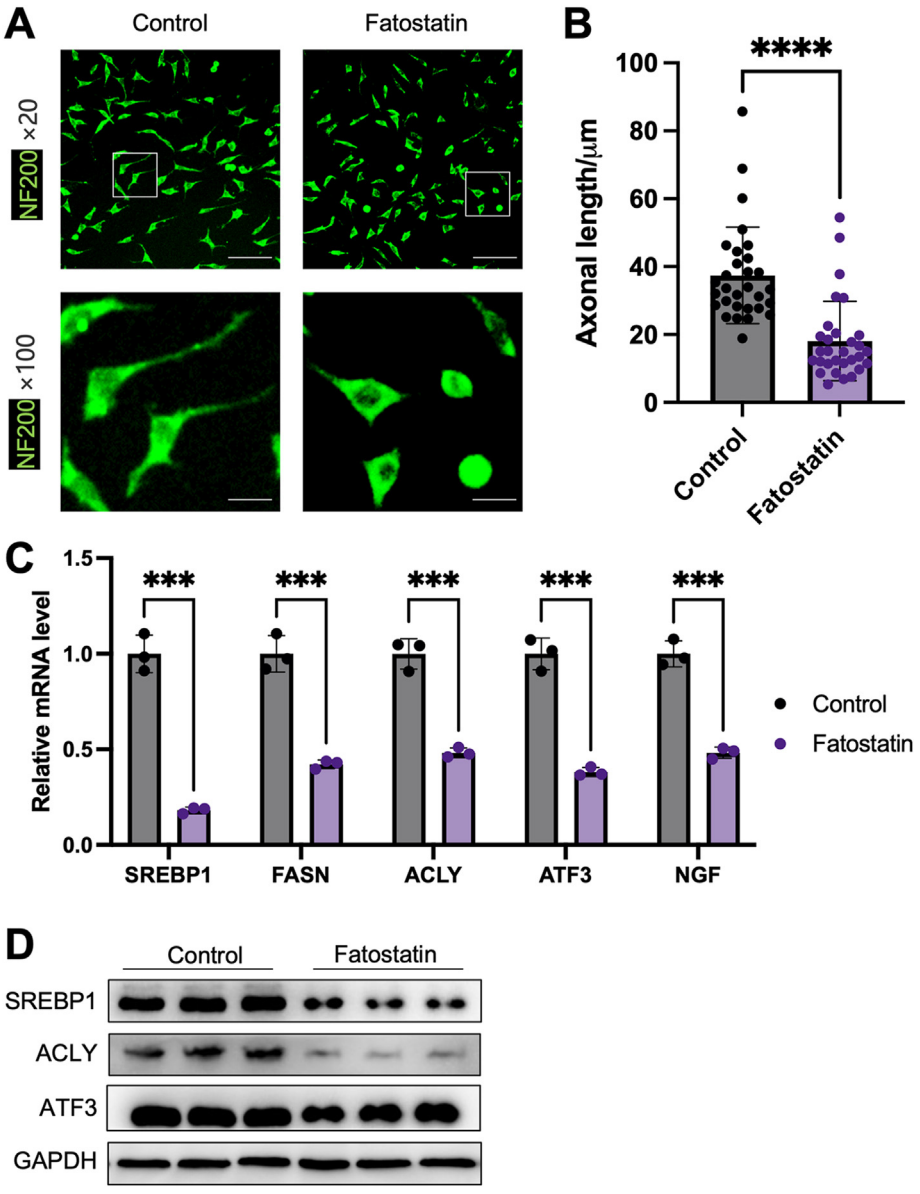

Figure 2 Fatostatin inhibits axonal growth of primary trigeminal neurons *in vitro*. (A) Images of cultured primary neurons stained with NF200. The upper panels show the control group (left) and the fatostatin-treated group (right) at 20 × magnification. The lower panels show higher magnification (100 ×) of neurons in both groups. Scale bars in the upper panels indicate 100 μm, and in the lower panels, 20 μm. (B) Quantitative analysis of axonal length in the top 50 neurons, showing that fatostatin significantly reduced axonal growth compared to the control group. (C) Quantitative PCR analysis of key genes related to lipid metabolism and axonal regeneration, including SREBP1, FASN, ACLY, ATF3, and NGF. The results show that fatostatin treatment significantly reduced the expression levels of these genes, with SREBP1 and FASN showing the most prominent changes. (D) Western blot analysis of SREBP1, ACLY, and ATF3 protein expression levels, demonstrating that fatostatin treatment decreased SREBP1, ACLY, and ATF3 protein levels compared to control. GAPDH was used as the loading control. ***, $P < 0.001$, ****, $P < 0.0001$. SREBP1, sterol regulatory element-binding protein 1; FASN, fatty acid synthase; ACLY, ATP citrate lyase; ATF3, activating transcription factor 3; NGF, nerve growth factor.

to the control group. Quantitative analysis (Fig. 2A and B) ($P < 0.05$). Similar to the *in vivo* results, qPCR and Western Blot analysis (Fig. 2C) demonstrated that fatostatin reduced the expression of key regulators of lipid metabolism including SREBP1, FASN, and ACLY, and also neurotrophic factors including ATF3 and NGF (Fig. 2C and D).

Discussion

In this study, we explored the role of fatostatin, a selective inhibitor of SREBP1, in modulating lip sensory recovery following inferior alveolar nerve transection. Fatostatin, a non-sterol diarylthiazole compound, was originally

identified as a molecule that blocks SREBP activation by targeting SCAP, thereby disrupting lipid metabolism and ER-to-Golgi trafficking.[6] It has shown therapeutic potential in cancer, obesity, metabolic syndrome, and inflammatory diseases.[7] However, its effect on the nervous system remains largely unexplored. Our findings suggest that inhibition of SREBP1 by fatostatin and the resulting alteration of lipid metabolism may impair sensory nerve regeneration after peripheral nerve injury.

The role of lipid metabolism in nerve regeneration is increasingly recognized, with lipids serving as crucial components in membrane repair and signal transduction during nerve recovery. SREBP1, as a master regulator of lipid biosynthesis, is involved in cholesterol synthesis, and its inhibition disrupts cellular lipid homeostasis. Moreover, a recent study demonstrated that SREBP-1 plays a crucial role in both neuronal and oligodendrocyte (OL) myelination.[10] Therefore, future studies may further expand the research about the impact of fatostatin other cell types in the peripheral and central nervous system.

Although the results are preliminary, the potential clinical implications of these findings are significant. While fatostatin is commonly used in experiments to influence lipid metabolism, other drugs that target lipid metabolism are widely prescribed for managing hyperlipidemia and cardiovascular diseases.[8] For example, statins, which inhibit the HMG-CoA reductase pathway, also impact cholesterol synthesis and lipid metabolism within neuronal cells.[9] Given the widespread use of statins in patients with high cholesterol, it is crucial to consider whether these drugs may impair nerve regeneration in patients who require peripheral nerve repair due to trauma or surgery. The potential impact of lipid-lowering drugs on neural regeneration requires further investigation in clinical and basic researches.

Our study does have several limitations. First, the sample size (5 mice per group) used for sensory testing was relatively small, and larger-scale animal studies are needed to verify our findings and improve statistical reliability. Second, our observation period was limited to 40 days, which may be insufficient to capture the complete trajectory of nerve regeneration. Future studies with longer follow-up durations are required to fully evaluate the recovery process. Lastly, the exact molecular mechanisms by which fatostatin impairs sensory recovery and axonal growth remain unclear. Further investigation into the downstream signaling pathways and interactions with other molecules would help elucidate these mechanisms.

In conclusion, our preliminary results suggested that fatostatin delayed lip sensory recovery after inferior alveolar nerve transection by inhibiting SREBP1. Further studies are needed to comprehensively assess the effects of lipid-lowering drugs on lip sensory recovery.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

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