Examining The Impact of Minocycline on Facial Nerve Regeneration In Rats Using LC-MS/MS Metabolomic Analysis

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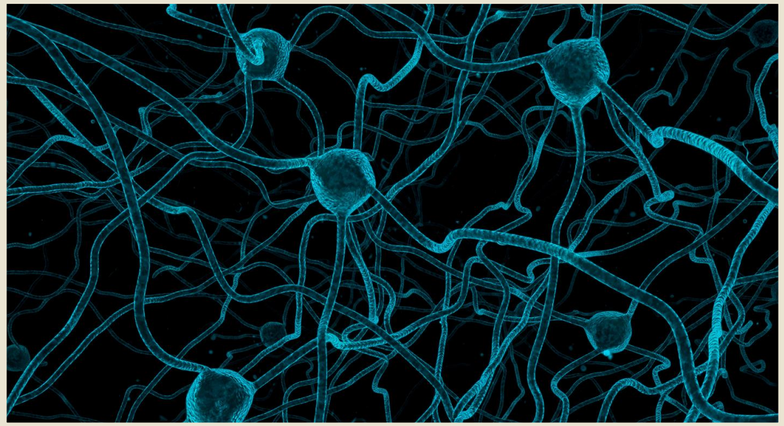


1.1 What Is Nerve Regeneration ?

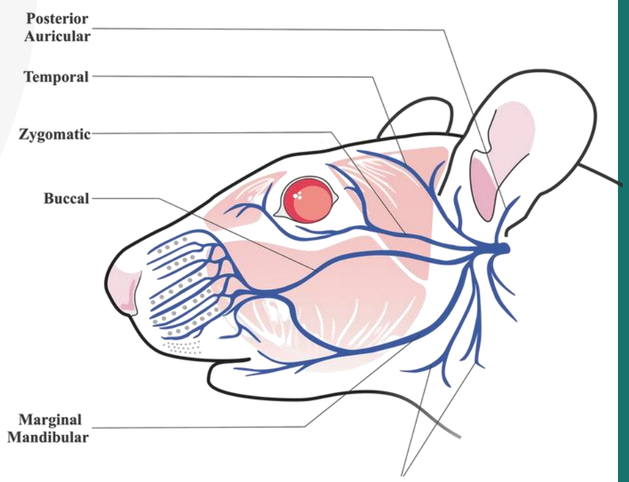
- It is a critical process that allows the intrinsic repair of injured nerve fibers and their functional restoration following trauma or chronic medical conditions.

- Neurons facilitate communication between the brain and body, regulating vital functions. Damage to these cells can disrupt these networks, potentially leading to severe illness.

- It involves intercellular interactions and regenerative protein production to restore damaged nerve connections. However, recovery is often slow, and severe damage can significantly impair nerve fiber reconnection.

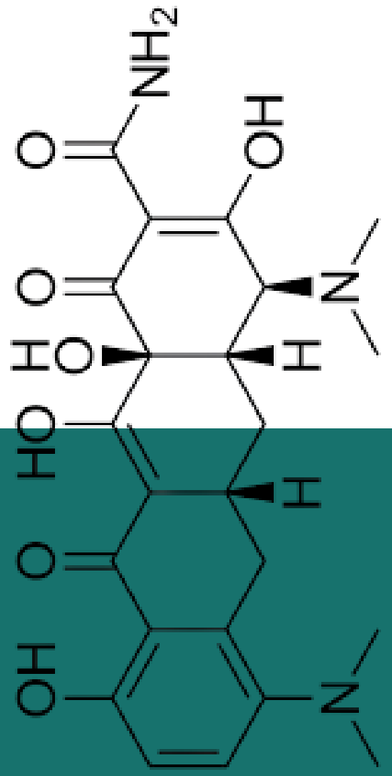
- Therefore, research on new treatment methods that can accelerate nerve regeneration and enhance its effectiveness holds great importance.

1.2 The Facial Nerve



- Also known as the seventh cranial nerve, carries both motor and sensory fibers, enabling the control of facial expressions and reflex responses.
- Injury to the facial nerve can significantly affect both functional abilities and psychosocial health, often leading to long-term challenges for patients.
- This highlights the importance of **developing protective and rehabilitative strategies to preserve nerve function.**

1.3 Why Minocycline ?



Antibiotic Properties

Minocycline is a second-generation tetracycline with broad-spectrum antibiotic properties, used in treating acne vulgaris and various infections.
It also exhibits anti-inflammatory and immunomodulatory effects by inhibiting the increase in inflammatory parameters.

Neuroprotective Properties

Minocycline was reported to be protective in neurological diseases such as stroke, spinal cord injury and Parkinson’s disease.
These properties support the hypothesis that minocycline may have beneficial impact on nerve regeneration.

2 Aim of Our Study

- Our study aimed to reveal the potential of a new and effective therapeutic approach in the treatment of facial nerve damage by investigating the effects of locally applied minocycline in promoting nerve regeneration after facial nerve injury using LC-MS/MS metabolomic analysis, histopathological analysis and electroneurophysiological testing.

3 Materials & Methods

3.1 Groups & drug administration

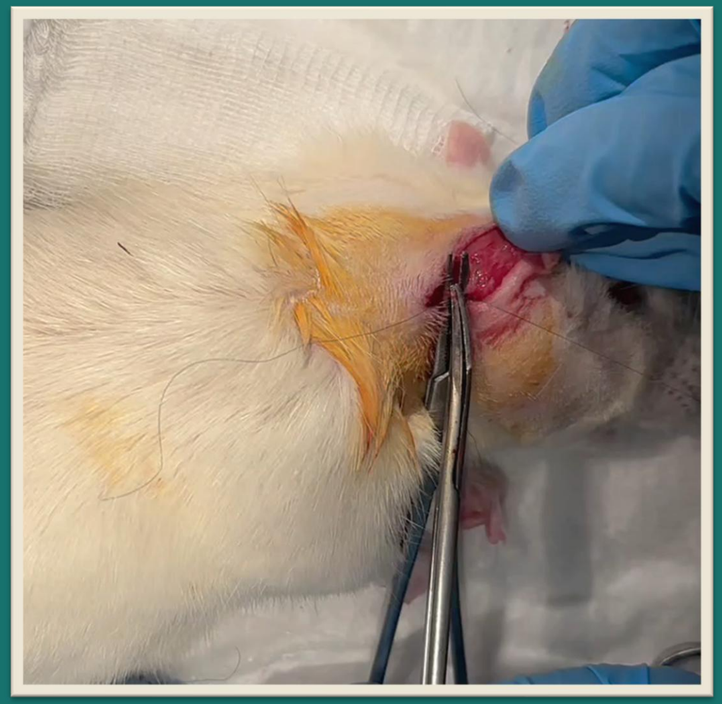
Groups	Doses and Agents	Administration Method
Group A (control)	Sterile water in 20 µL of fibrin gel	Delivered locally on the suture line of the reanastomosed facial nerve
Group B	40 µg of Minocycline in 20 µL of fibrin gel	Delivered locally on the suture line of the reanastomosed facial nerve
Group C	100 µg of Minocycline in 20 µL of fibrin gel	Delivered locally on the suture line of the reanastomosed facial nerve

3.2 Duration of the study

28 days

3.3 Surgical procedure

Facial nerve injury was induced through a full-thickness incision on the zygomatic and buccal branches of the facial nerve. Followed by suturing separated nerve ends to achieve reanastomosis.



4.1 LC-MS/MS Results

Compared groups; A and B

Compound.name	fc	p_value	Total.score
betaine	4,662555	0,079463	0,765685
Betaine	2,748739	0,068076	0,888173
N,N-Dimethyldodecylamine N-oxide	2,483427	0,367762	0,745644
Morantel	2,372279	0,076611	0,750811
ReSpect:PT110240 O-Phosphocholine|Phosphocholine chloride calcium salt tetrahydrate|Choline phosphate|Phosphorylcholine|N-Trimethyl-2-aminoethylphosphonate|trimethyl-(2-phosphonooxyethyl)azanium	1,913705	0,117158	0,791502
ReSpect:PT109980 O-Acety-L-carnitine hydrochloride|ALC|ALCAR|L-Carnitine acetyl ester|(R)-3-Acetoxy-4-(trimethylammonio)butyrate|Levacecarnine|[(2R)-2-acetyloxy-4-hydroxy-4-oxobutyl]-trimethylazanium	1,710933	0,116616	0,813206
PC(16:0/0:0) Phosphatidylcholine	1,683671	0,173378	0,832991
nicotinamide CollisionEnergy:102040	1,598168	0,192848	0,859502
FRUCTOSE	1,466286	0,210804	0,819333
Ketamine	1,45971	0,381347	0,828002
ReSpect:PT110160 Choline chloride|2-Hydroxyethyl trimethylammonium|Bilineurine|2-Hydroxy-N,N,N-trimethylethanaminium|Bilineurin|Hepacholine|Lipotril|2-hydroxyethyl-trimethylazanium	1,359901	0,184974	0,83194
DL-PHENYLALANINE CollisionEnergy:102040	1,236357	0,384734	0,874935
2-Amino-1-phenylethanol CollisionEnergy:205060	1,218093	0,422032	0,808354
Carnitine	1,165324	0,30121	0,862323
N,N-Dimethylarginine	1,144094	0,614179	0,859326
Arginine	1,071665	0,742884	0,864191
Erucamide	0,999361	0,99712	0,886858
Creatine	0,998383	0,995139	0,863082
Hexylamine	0,843734	0,288186	0,786833
.epsilon.-Caprolactam	0,815871	0,061826	0,848862
Creatinine	0,740337	0,412579	0,860085
Lauric acid diethanolamide	0,426223	0,12389	0,90838

4.1 LC-MS/MS Results

Compared groups; A and C

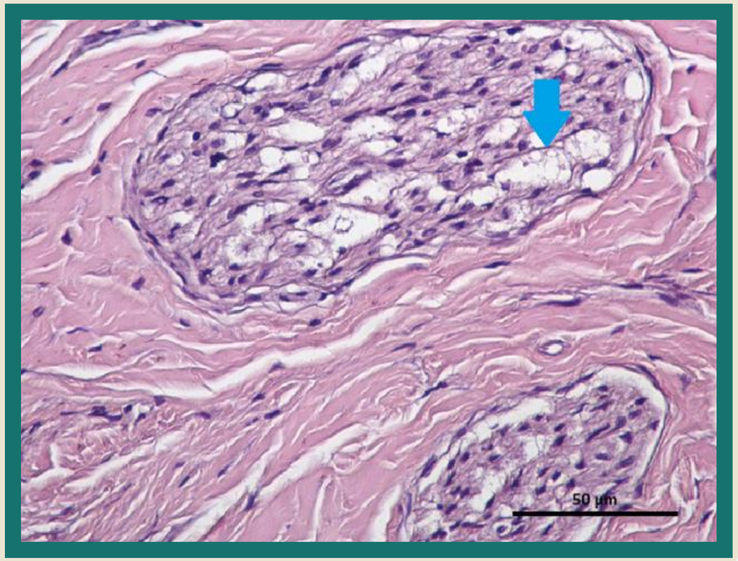
Compound.name	fc	p_value	Total.score
N,N-Dimethyldodecylamine N-oxide	3,338525	0,255172	0,753827
Thymine	2,607464	0,34974	0,86935
Lauric acid diethanolamide	2,290137	0,17786	0,884926
Melophlin M	1,910894	0,177084	0,726726
thymidine CollisionEnergy:102040	1,81522	0,446376	0,838752
PC(16:0/0:0)	1,413051	0,281073	0,844192
Proline	1,404925	**0,031766***	0,857096
Propionylcarnitine	1,367499	0,47104	0,866459
5-Methylcytosine CollisionEnergy:102040	1,269258	0,774368	0,888628
Carnitine; CE10; PHIQHXFUZVPYII-ZCFIWIBFSA-N	1,184962	0,564727	0,869365
Cytosine	1,156095	0,841546	0,861822
Arginine	1,082775	0,820663	0,892175
Hexylamine	1,066715	0,55235	0,786668
ReSpect:PT110160 Choline chloride|2-Hydroxyethyl trimethylammonium|Bilineurine|2-Hydroxy-N,N,N-trimethylethanaminium|Bilineurin|Hepacholine|Lipotril|2-hydroxyethyl-trimethylazanium	0,997946	0,992135	0,83194
ReSpect:PT109980 O-Acety-L-carnitine hydrochloride|ALC|ALCAR|L-Carnitine acetyl ester|(R)-3-Acetoxy-4-(trimethylammonio)butyrate|Levacecarnine|[(2R)-2-acetyloxy-4-hydroxy-4-oxobutyl]-trimethylazanium	0,951217	0,854372	0,812751
Erucamide	0,946482	0,779582	0,900876
Indoline	0,925232	0,701273	0,819134
Ketamine	0,905022	0,811913	0,828002
DL-PHENYLALANINE CollisionEnergy:102040	0,899478	0,596357	0,876755
Creatine	0,896317	0,732909	0,886642
FRUCTOSE	0,815152	0,399488	0,819333
nicotinamide CollisionEnergy:102040	0,796647	0,394976	0,859502
ReSpect:PT110240 O-Phosphocholine|Phosphocholine chloride calcium salt tetrahydrate|Choline phosphate|Phosphorylcholine|N-Trimethyl-2-aminoethylphosphonate|trimethyl-(2-phosphonooxyethyl)azanium	0,716518	0,328275	0,801732
Morantel	0,449435	0,057918	0,750811
Betaine	0,367427	**0,0242***	0,888173
betaine	0,182625	0,072821	0,765685

4.2 Histopathological Analysis

Performed using Hematoxylin-Eosin staining for microscopic evaluation.

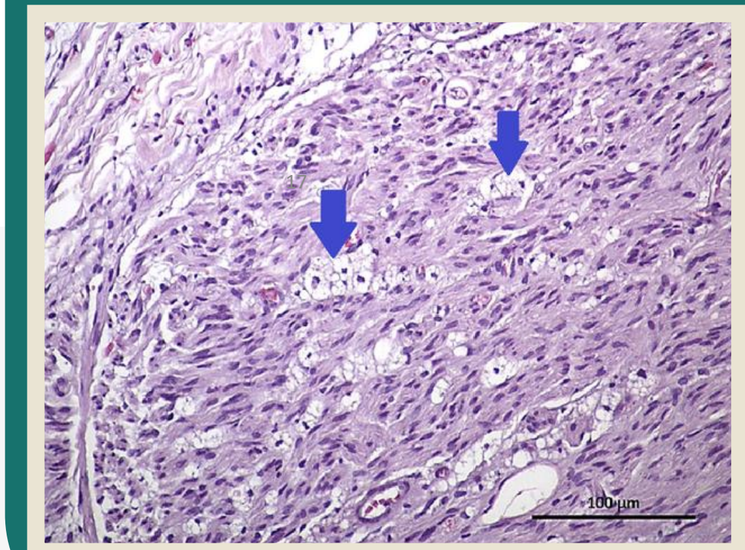
○ Group A:

Signs of edema and vacuoles in different sizes within the myelin sheath



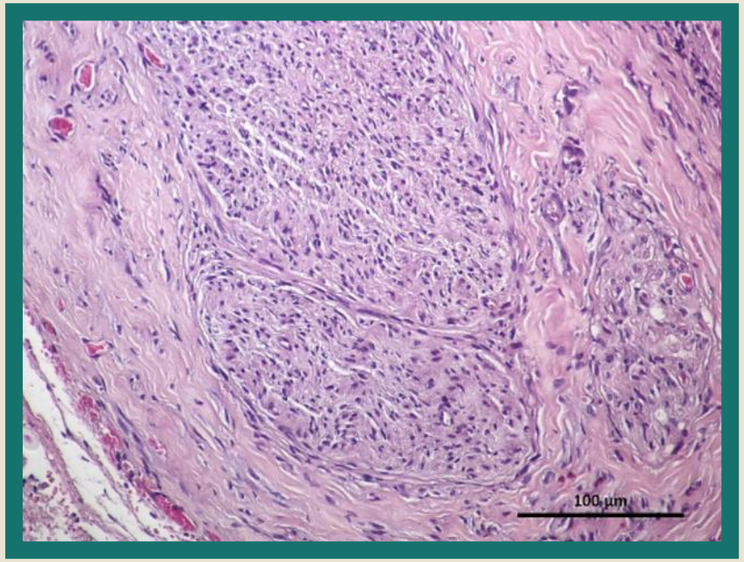
○ Group B:

Signs of minimal edema and rare vacuoles within the myelin sheath



○ Group C:

No signs of edema



4.3 EMG Analysis

DML: Distal Motor Latency
CMAP: Compound Muscle Action Potential

DML Pairwise Comparison Results

Comparison	Z	P
0W vs 1W	6	0.1763
0W vs 2W	3	**p<0.05**
0W vs 4W	10	0.1641
1W vs 2W	10	0.2614
1W vs 4W	13.5	0.5276
2W vs 4W	12.5	0.7995

***Wilcoxon test**

CMAP Pairwise Comparison Results

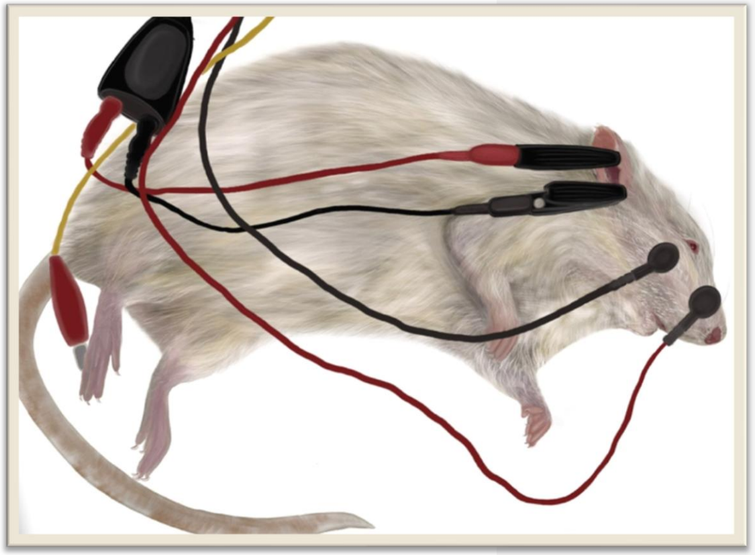
Comparison	Z	P
0W vs 1W	7	0.0977
0W vs 2W	1	**p<0.05**
0W vs 4W	6	0.0929
1W vs 2W	10	0.1641
1W vs 4W	18.5	0.6523
2W vs 4W	13.5	0.3008

***Wilcoxon test**

Pairwise Comparison of CMAP Values Across Different Time Points

Groups	χ^2	Kendall's W	p*
Group A (n=9)	5.9	0.17	0.165
Group B (n=9)	14.20	0.47	**0.013**
Group C (n=8)	8.47	0.29	**0.037**

***Friedman test**



05 Conclusions

In our study on nerve regeneration, we evaluated the cellular, metabolic, and electroneurophysiological effects of minocycline.

- Our histopathological analysis, metabolomics analysis, and EMG testing indicated that 40 μg and 100 μg of minocycline was effective in improving nerve function, and this effect was prominent by the end of week 2.
- Minocycline demonstrates promise in nerve regeneration, with two doses influencing functional recovery, metabolic processes, and structural repair.

06 Limitations & Strengths

Limitations

- For further histopathological analysis, immunohistochemistry and electron microscopy can be performed in future studies to identify regenerative proteins.
- Further studies are needed to optimize dosing strategies and better understand the underlying mechanisms.

Strengths

- First study in the literature to our review to investigate the effects of minocycline’s local application on facial nerve regeneration.
- Avoided the risk of systemic side effects by locally administrating minocycline in very small doses (40 µg and 100 µg).
- We evaluated the cellular, metabolic, and electroneurophysiological effects of minocycline on facial nerve regeneration.

Thank you

For your time and attention

For any questions, please feel free to contact me at:

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