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# Local Administration of ReveromycinA Ointment Suppressed Alveolar Bone Loss in Mice

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Presented by: Samiya Majid
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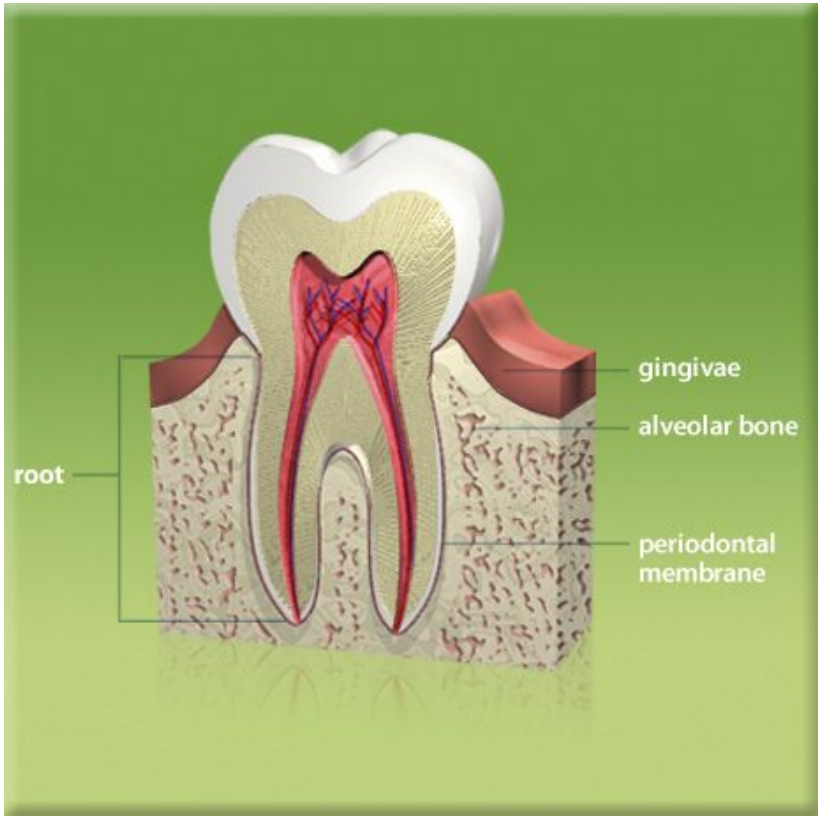
# Introduction

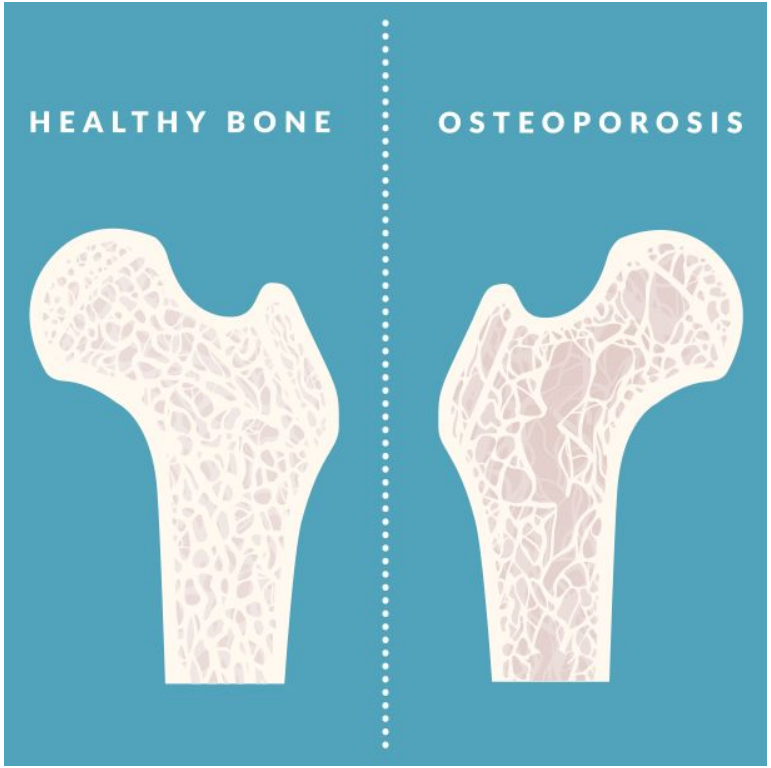



https://www.toothclub.gov.hk/en/pnc/en_pnc_2_2_1_6.html

# The Problem

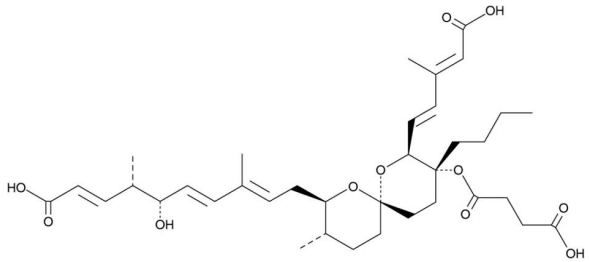
- **Normal periodontal tissue** - homeostasis is maintained by bone resorbing osteoclasts
- **Patients with osteoporosis** - impaired homeostasis

- NF-KB Ligand (RANKL)
- Osteoblasts produce osteoprotegerin (OPG)
- **OPG is protective against bone loss**




https://www.medicinenet.com/osteoporosis/article.htm

# Reveromycin A -
## osteoclast activity suppressor




https://www.caymanchem.com/product/17458/reveromycin-a

- 3 carboxylic acids

Taken up by acid-secreting osteoclasts

Induces apoptosis in these cells

Inhibits bone resorption

# Previous Study

Periodontal disease was induced in OPG KO mouse model and found intraperitoneally administered RMA inhibited alveolar bone resorption

Researchers wanted to see if RMA could be administered non-invasively through the mouth

**OPG KO -** osteoprotegerin knockout (osteoprotegerin has been removed from mice)

**RMA -** reveromycin A

**Intraperitoneally -** through the stomach

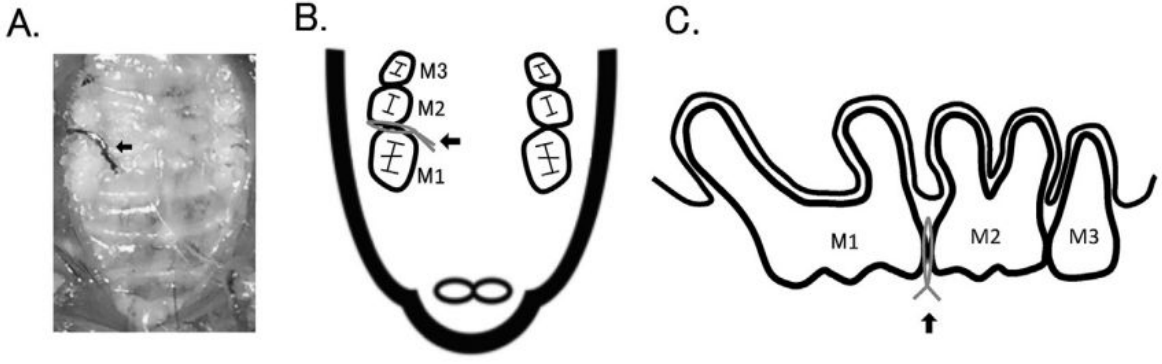
# Question to be answered:

Does RMA ointment reduce the number of osteoclasts and inhibit bone resorption in mice with periodontal disease?

**RMA -** reveromycin A

# Materials and Methods

- 64, 8-week old male wild type and OPG KO experimented on in temperature maintained conditions with constant light cycles. They had free access to food and water
- Periodontal diseased mice were prepared through ligation by placing wire as shown:



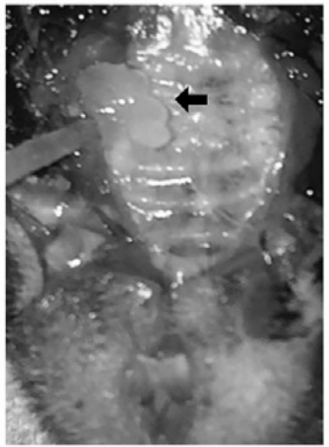

**OPG KO -** osteoprotegerin knockout (osteoprotegerin has been removed from mice)

**RMA -** reveromycin A

**Ligation -** surgical closing off of blood vessel

- Silicone -based ointment with 1% RMA was used
- Same ointment without RMA was used for control group
- Ointment was applied three times daily at:

D.



**RMA** - reveromycin A

# Experimental Groups

**WC OC**

WT and OPG KO mice without ligature or RMA

**WR- OR-**

WT and OPG KO mice with ligature and without RMA

**WR+ OR+**

WT and OPG KO mice with ligature and RMA

**WT** - wild type

**OPG KO** - osteoprotegerin knockout (osteoprotegerin has been removed from mice)

**RMA** - reveromycin A

**Ligation** - surgical closing off of blood vessel

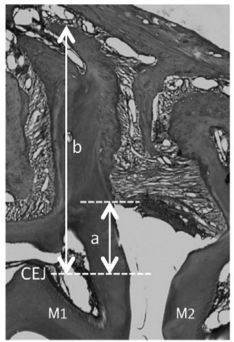
- Mice were sacrificed 8 weeks after ligature
- Samples were examined using micro-computed tomography (u-CT)
- Alveolar bone volume was analyzed using TRI/3D BON software
- % of remaining alveolar bone was calculated as shown:

E.


$$\text{The ratio of remaining alveolar bone} = \frac{\text{Remaining alveolar bone(a)}}{\text{Total alveolar bone space(b)}} \times 100(\%)$$

**Ligation -** surgical closing off of blood vessel

F.




- Maxillae were collected and fixed in 10% neutral buffered formalin, then decalcified in 10% EDTA for 4 weeks
- Paraffin blocks were prepared and tissue observation sites were selected based on where molar roots were found
- Hematoxylin-eosin (H&E) staining was performed
- % attachment was determined using:

$$\text{The percentage of attachment level (\%)} = \frac{\text{A length of CEJ the bottom of the gingival sulcus (a) (mm)}}{\text{A length of CEJ to the root apex (b) (mm)}} \times 100(\%)$$

- TRAP stain was also performed

**Maxilla -** upper fixed bone of jaw

**H&E -** provides detailed view of the tissue

**TRAP -** checks for reduced resorptive activity

- Tnf-a and IL-1B immunostaining was performed
  - Stained sections were scored as follows:
    - 1 (0-20% positive staining)
    - 2 (21-40% positive staining)
    - 3 (41-60% positive staining)
    - 4 (>60% positive staining)
- Slides were evaluated by 2 different examiners

**Tnf-a and IL-1B -** used for checking cell responses (like apoptosis)

# Side Question: Does swallowing excess RMA ointment have an effect?

- 4 groups - ointment free, peroral administration, local administration with non-RMA ointment, local administration with RMA ointment
- WT and OPG KO mice used
- Blood samples collected and TRAP concentrations were measured using ELISA kit

**WT -** wild type

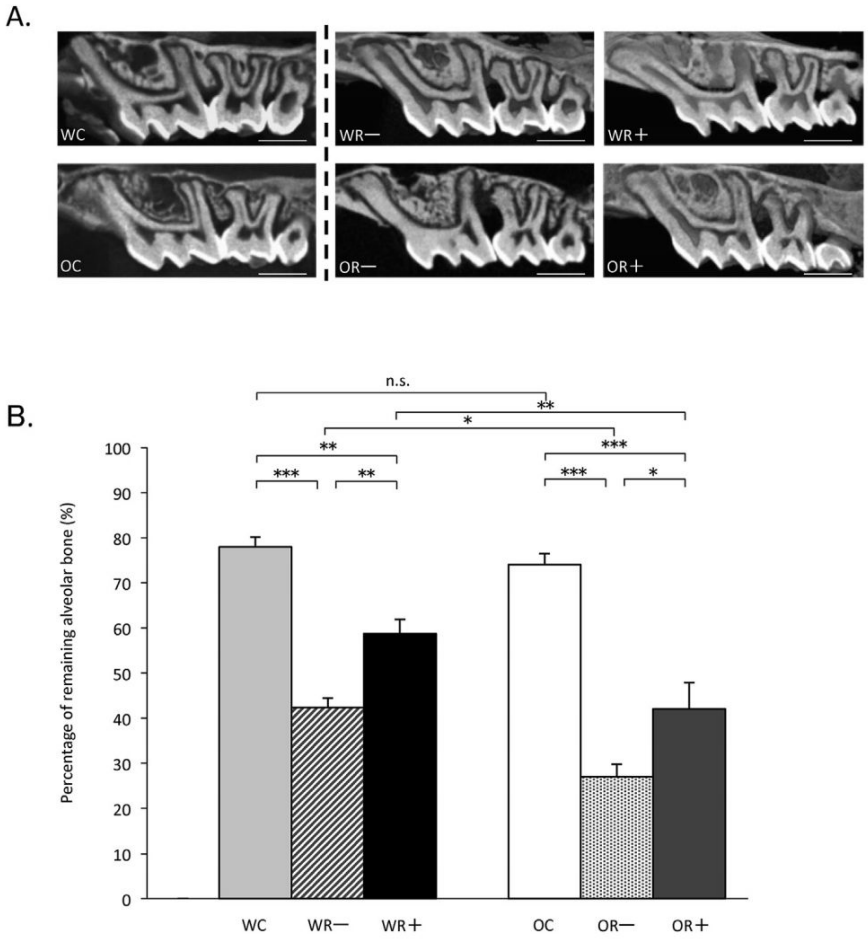
**OPG KO -** osteoprotegerin knockout (osteoprotegerin has been removed from mice)

**RMA -** reveromycin A

**TRAP -** checks for reduced resorptive activity

# Results

# Comparison of u-CT



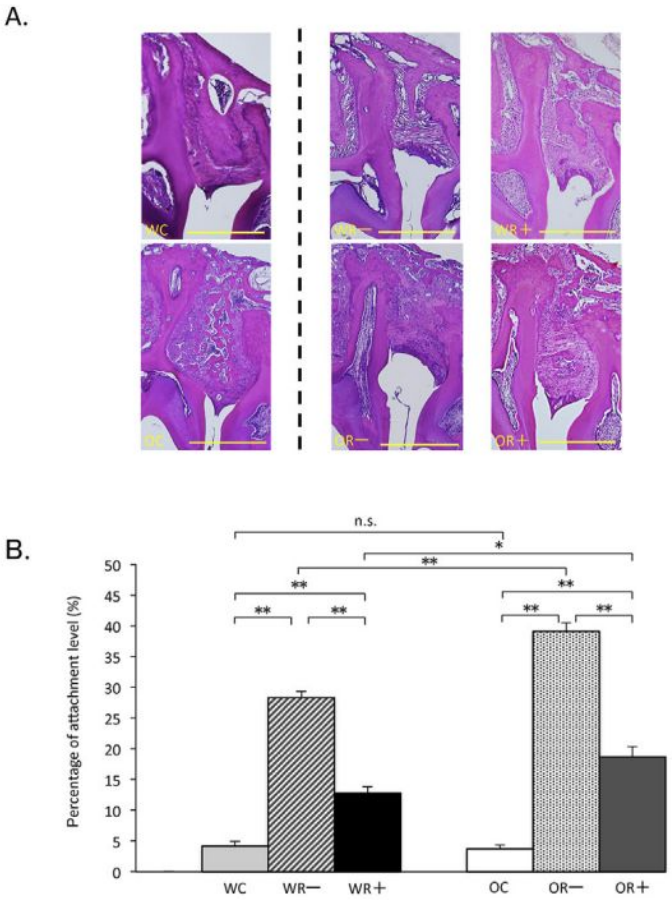

**u-CT-** micro-computed tomography

**WC and OC -** wild type and OPG KO mice w/o ligation or RMA

**WR- and OR- :** wild type and OPG KO mice w/ ligation and w/o RMA

**WR+ and OR+:** wild type and OPG KO mice w/ ligation and RMA

# H&E staining and attachment level




**H&E -** provides detailed view of the tissue

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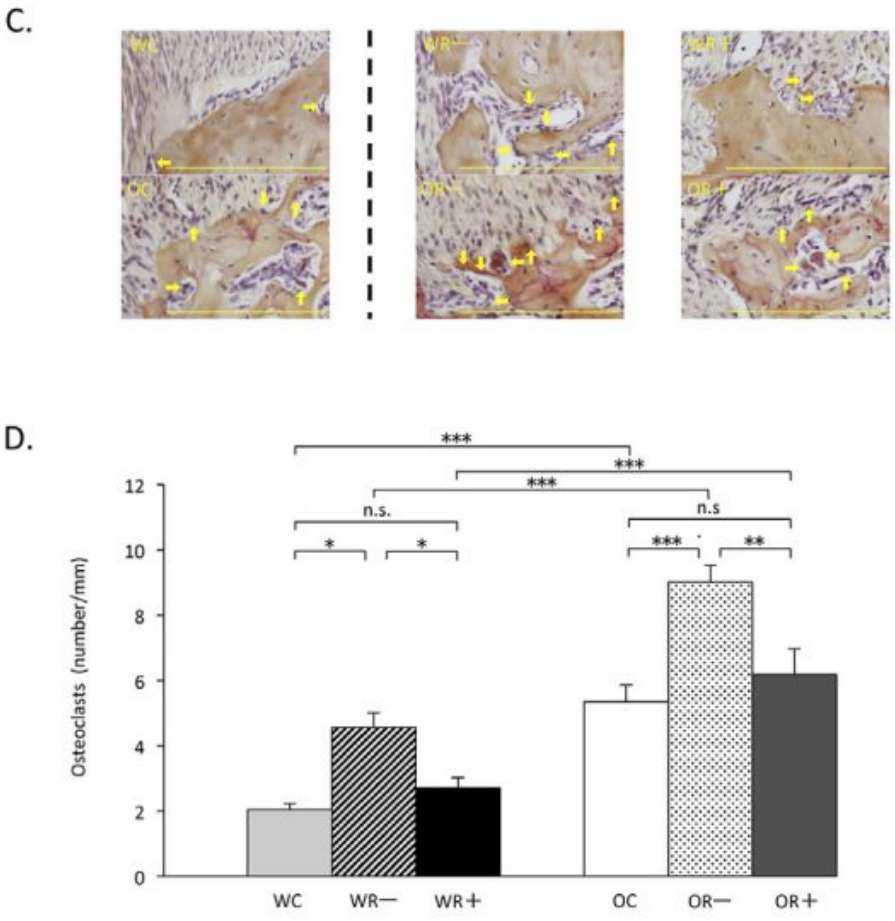
# Osteoclast Count




**WC and OC -** wild type and OPG KO mice w/o ligation or RMA

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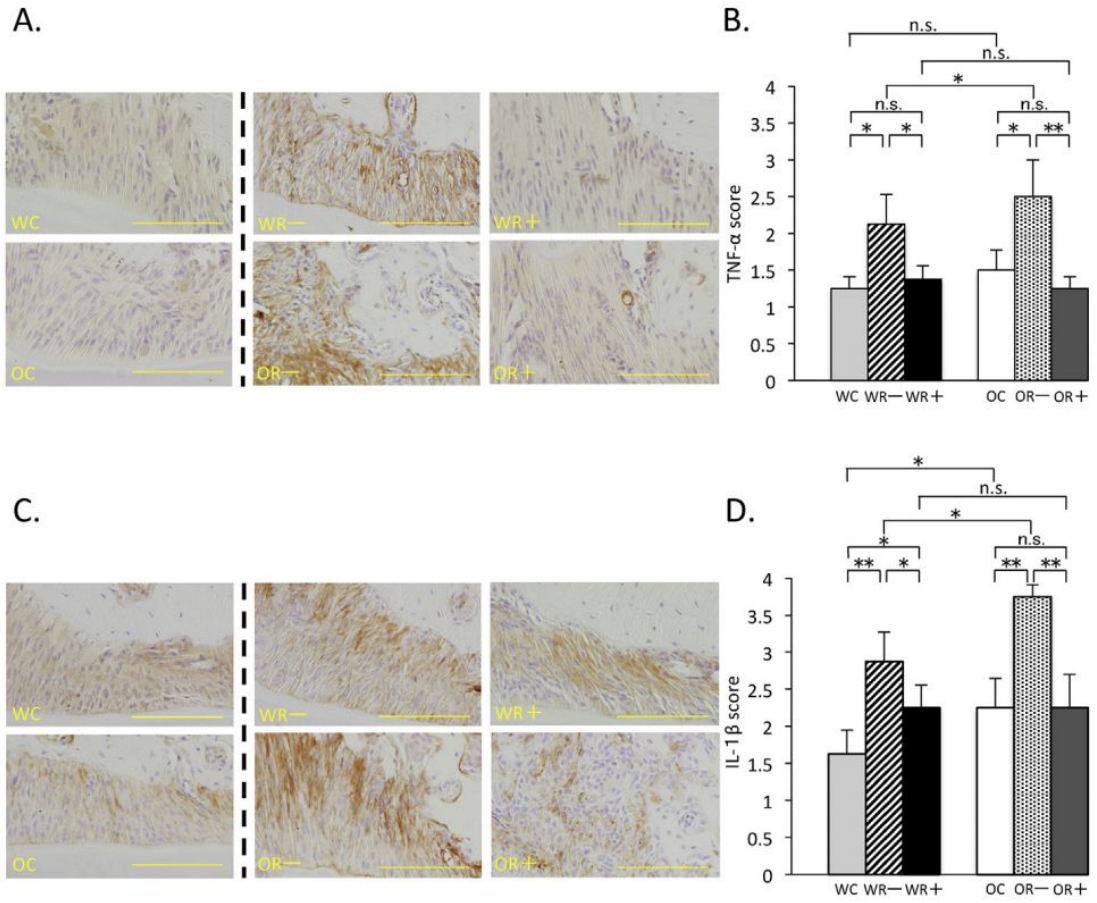
**WR+ and OR+:** wild type and OPG KO mice w/ ligation and RMA

# TnF-a and IL-1B




**Tnf-a and IL-1B** - used for checking cell responses (like apoptosis)

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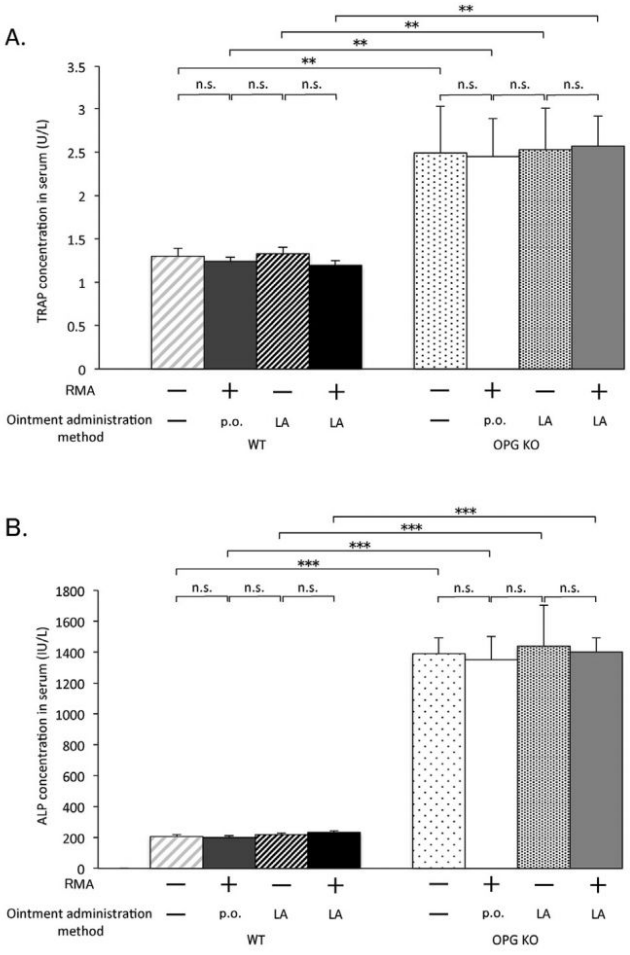
# Results of side question




**WT -** wild type

**OPG KO-** osteoprotegerin knockout

**p.o. -** peroral

**LA -** topical administration

# Discussion

WR+ and OR+ had smaller attachment loss than WR- and OR-

→ RMA-containing ointment inhibited alveolar bone resorption and alleviated periodontal disease progression

RMA ointment reduced immunostaining scores of TNF-a and IL-1B

→ RMA locally suppressed inflammation

- RMA ointment suppressed localized inflammation and alveolar bone resorption and decreased osteoclast count to a similar degree as it had when administered peritoneally
- These results suggest the effectiveness of RMA in treating patients with vulnerable periodontal tissue due to periodontitis and osteoporosis

# What's currently being used and why is RMA better?

# Final Conclusion

The study shows that RMA is an **effective non-invasive treatment** for:

- Preventing periodontitis
- Inhibiting alveolar bone resorption locally

Link:: https://www.sciencedirect.com/science/article/pii/S1347861321000530