Primate Femur Histomorphometry and Gene Expression: Effects of Chronic Alcohol Abuse on Bone

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Introduction

- Problem: Alcohol abuse is a widely recognized health concern that negatively impacts many organ systems.
- Chronic alcohol consumption leads to secondary osteoporosis with decreases in bone formation, bone mass, and bone mineral density.
- Specific mechanisms must be better understood to optimize therapeutic intervention.

Objective: This study was designed to relate the specific histomorphometric changes with osteoclastic and osteoblastic gene expression alterations in a primate model of alcohol abuse.

Materials

- Seven 3-4 year old male rhesus macaques: 19 months of treatment via indwelling intra-gastric tube
- 3 primates received isocaloric sucrose diet
- 3-14 kg/week (30% W/V in water)
- 4 primates received isocaloric sucrose diet
- Proximal femora were harvested for analysis
- Left femur: qRT-PCR mRNA analysis
- Isolated greater trochanter
- Stored in RNA Later (Qiagen)

Histomorphometry: Methods

- Two methods used for analysis:
  - Manual: Merz Grid
  - Semi-automated: ImagePro v.5.01
- Five Outcome Measures
  - Bone volume/tissue volume (BV/TV)
  - Mineralized portion of total tissue volume
  - Resorptive surface length/bone area
  - Cortical activity
  - Non-resorptive surface length/bone area
- Lack of osteoclastic activity
- Total surface length/bone area
- Mineralized bone surface area
- Cement line interface length/bone area
- Remodeling activity
- Two-way RM-ANOVA to assess region and diet effects on each parameter (p<0.05).

Bone Regions

- 16 bone regions examined for each parameter: 945 individual measurements

Histomorphometry: Results

- Bone Volume/Tissue Volume
  - Little change
  - Possible ↑ in mineralized portion of total tissue volume

- Resorptive Surface Length/Bone Area
  - Mixed results for osteoclastic activity

- Non-resorptive Surface Length/Bone Area
  - ↑ areas without osteoclastic activity

- Total Surface Length/Bone Area
  - Significant ↑ in cancellous bone of the alcohol group using Merz grid data

- Cement Line Interface Length/Bone Area
  - ↑ remodeling activity

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Conclusions

- This study was limited by sample size, but the preliminary results suggest that disruption of bone homeostasis at the mRNA level by chronic alcohol exposure contributes to the specific histomorphometric alterations of secondary osteoporosis.
- While larger studies are warranted to further examine the effects of alcohol ingestion on bone remodeling, potential gene targets for treatment of patients suffering from the homeostatic bone alterations due to alcoholism are suggested by these study results.

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RT-qPCR: Methods and Results

- Total RNA isolated from the left greater trochanter
- Messenger RNA levels of osteoblastic and osteoclastic target genes quantified with RT-qPCR using SYBRGreen technology
- Trends associated with alcohol consumption:
  - ↑ expression of osteoblastic genes
  - Altered expression of osteoclastic genes
  - Activation: overall ↑
  - Migration: ↓
  - Resorption: possible net ↑

Osteoblastic Gene Expression

- Osteocalcin (OCAL) ↓
- Collagen 1 alpha 1 (COL1A1) ↓
- Alkaline phosphatase (ALP) ↓

Osteoclastic Gene Expression

- Colchicine K (CTS) ↓
- Matrix metalloproteinase 9 (MMP9) ↓
- Tartrate resistant acid phosphatase (TRAP) ↓
- Receptor activator of nuclear factor kappa beta (RANK) ↓
- RANK ligand (RANKL) no change

References

- Bisphosphonates may also be effective in reducing osteoclastic activity.
- Monoclonal antibody blocking RANKL (Denosumab) may reverse the effects of ↑ OPG and recombinant parathyroid hormone (teriparatide) may help to ↓ lost osteoblastic function (for high risk cases).
- Bisphosphonates may also be effective in reducing osteoclastic activity.

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