

# ALCOHOL-INDUCED ADIPOGENESIS IN BONE AND MARROW: A MECHANISM FOR OSTEONECROSIS

+\*Wang, Y (A-Zimmer); \*Cui, Q; \*\*Li, Y; \*\*Mao, K; \*Balian, G; \*Wang, G (A-Zemmer)

+\*Orthopaedic Research Laboratory, Department of Orthopaedic Surgery, University of Virginia, School of Medicine, Box 800374, Charlottesville, VA 22908, 804-924-1716, Fax: 804-924-1691, yw4n@virginia.edu

**Introduction:** The mechanism of action of alcohol on bone and marrow cells has not been defined. In this study, we investigated the effect of alcohol administration on rabbits and determined the effect of ethanol treatment on gene expression by bone marrow mesenchymal cells in vitro. Our experiments are directed towards understanding the role of alcohol on the development of a fatty marrow and the ensuing osteonecrosis and the changes in gene expression by bone marrow cells during adipogenesis and osteogenesis in vitro.

**Materials and Methods:** A multipotential D1 cell, which was cloned from bone marrow, was maintained in culture and treated with 0, 0.09, 0.15, or 0.21 M ethanol daily for 14 days. The effect of ethanol on adipogenesis was examined by determining the accumulation of triglyceride vesicles by phase contrast microscopy and by staining with Sudan IV. Osteogenesis and adipogenesis in vitro were measured using northern blot hybridization to detect 422(aP2) and osteocalcin mRNA's.

A rabbit model was established to study osteonecrosis in vivo (approved by institutional animal review board). New Zealand white rabbits were divided into two groups: Group A(n=40) received (10ml per Kg. body weight) 45% ethanol intragastrically daily. Group B(n=40) received 10ml/Kg glucose solution. In ten animals from each group, serum lipid peroxides (LPO), superoxide dismutase (SOD), triglyceride (TG) and cholesterol (CHO) were determined at 1, 2, 3 and 6 months after treatment. The femoral heads and livers were removed and examined histologically after staining with H & E and Sudan IV, and by electron microscopy.

**Results:** D1 cells were treated with increasing (0.09, 0.15, and 0.21M) concentrations of ethanol for 3 days. The cells accumulated triglyceride vesicles, which were small initially and increased in size with time. The number of adipocytes in culture increased when the cells were treated at the higher concentrations of ethanol. Adipogenic changes were not detected in cells that had not been treated with ethanol (Fig.1).

Northern blot hybridization with osteocalcin cDNA and 422(aP2) cDNA showed that treatment with 0.15M ethanol decreased expression of osteocalcin mRNA by 85% compared with control cells that were not treated with ethanol (Fig. 2).

Rabbits treated with ethanol showed significantly increased serum LPO, TG, CHO, and reduced SOD activity. At 6 months, LPO and SOD activity in Group A was 3.81  $\mu\text{mol/ml}$  and 28.84 Nu/ml respectively, in Group B they were 2.52  $\mu\text{mol/ml}$  and 31.68 Nu/ml. TG and CHO in Group A was 5.87mmol/L and 2.54mmol/L respectively, and that in Group B was 1.35mmol/L and 1.64mmol/L.

Fatty changes in the liver and bone marrow of Group A were found histologically after ethanol treatment (Fig.3). Fat cell hypertrophy and proliferation, and diminished hematopoiesis in the subchondral bone area of the femoral head was observed throughout the experiment. The percentage of empty osteocyte lacunae was increased and trabeculae became thinner and sparse at 2, 3 and 6 months. The femoral head became loose, and could be easily cut in Group A at 6 months (Fig. 3). By contrast, no abnormal changes were detected in the livers and femoral heads of animals in the control group.

**Discussion:** Results from our study show that alcohol can induce adipogenesis in bone marrow in vivo, and in cells in vitro. Analysis of gene expression demonstrated diminished expression of osteocalcin in cells treated with ethanol. Decreased osteogenic properties and increased adipogenesis were detected in cells treated with ethanol. Our study indicates that the rabbit is a suitable animal model in which to study osteonecrosis and alcohol-induced adipogenesis in bone marrow stroma. Systemic changes in lipid levels may be a direct result of treatment with ethanol and may contribute to alcohol-related osteonecrosis. Inhibition of osteocalcin gene expression without an increase in 422(aP2) mRNA suggests that adipogenesis i.e.

increased triglycerides and vesicles, is brought about by the action of ethanol downstream in the fatty acid metabolism pathway.

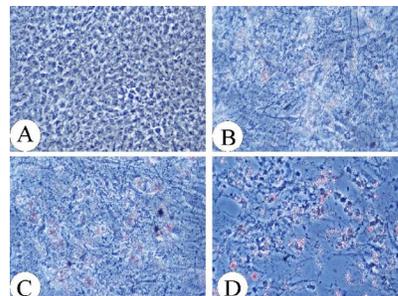


Fig. 1. Staining with Sudan IV showing no triglyceride vesicles in D1 cells in the control group (A) and accumulation of triglyceride vesicles in D1 cells that were treated with 0.09 (B), 0.15 (C) and 0.21 M ethanol (D) at 14 days.

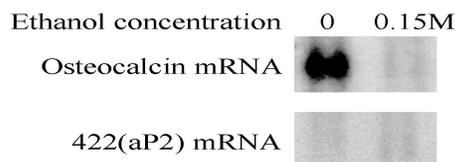


Fig. 2. Northern blot hybridization for osteocalcin and 422(aP2) mRNA. Treatment with 0.15M ethanol decreased osteocalcin mRNA to 15% compared with cells that were not treated with ethanol (0) at 14 days. 422(aP2) mRNA did not change in the cells treated with 0.15 M ethanol for 14 days.

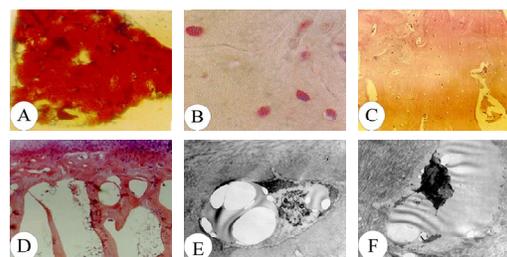


Fig. 3. Subchondral bone marrow of the femoral head. Group A: Staining with Sudan IV showed accumulation of fat cells (A) and triglyceride vesicles in osteocytes (B). At 6 months, histology showed increased empty osteocyte lacunae (C) and thinner, sparse trabeculae (D). Transmission electron microscopy showed large triglyceride vesicles in osteocytes (E) and pyknosis (F).

## References:

1. Cui, Q, et al.: Clin Orthop 344: 8-19, 1997
2. Wang, G.J., et al.: J Bone Joint Surg 59-A: 729-735, 1977
3. Cui, Q., et al.: J Bone Joint Surg 79-A: 1054-1063, 1997
4. Jones, J.P., Jr.: Clin Orthop 277: 41-53, 1992

\*\*Henan Medical University, Zhengzhou, Henan, P.R.China.