MK-7 enhances expression of genes related to bone, enamel and dentin, and reduces the expression of genes related to apoptosis in developing murine molars.

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Background

Menaquinone-7 (MK-7) it's a fat-soluble vitamin and a vitamin K2 homologe needed for post-translational modification of certain proteins required for blood coagulation, and in metabolic pathways in bone and other tissues.

Recent studies found an association between long-term anticoagulant treatment (OAC) and reduced bone quality due to reduction of active osteocalcin. OAC is often linked to an undesired soft-tissue calcification in both children and adults. OAC often leads to increased incidence of fractures, reduced bone mineral density/bone mineral content, osteopenia and increased serum levels of undercarboxylated vitamin K-dependent proteins, known as Gla-proteins. Litle is known about the effects of vitamin K2 during tooth development.

Materials and methods

Female Balb C mice, CD-1 strain were fed on a vitamin K deficient fodder (D10051601) for 2 weeks prior to pups being born. Control mice were similarly fed on the corresponding control fodder (D10012M).

New-born pups were given MK-7 (0.2, 2 or 10 mg/kg body-wt., Kappa Bioscience, Oslo, Norway) by subcutaneous injections in the right hand side mandible. Control groups were injected with vehicle (corn oil). At 24 h post-injection the pups were sacrificed and first right-hand side molar tooth germs extracted and transferred into 300mlRNA-Later solution. The dissected tooth germs were homogenized. Total RNA was extracted using the RNeasy mini kit (Sigma).

About 1 µg of total RNA was used to synthesize cDNA and labelled with Cy3 or Cy5 using the Genisphere Array 900™ Kit. cDNA was hybridized to Mouse OneArray® v2 (Phalanx Biotech Group).

Statistical analysis of microarray data was carried out for each dose using Spotfire v. 9 Microarray Analysis Software (TIBCO Software).

Genes found to be differentially expressed using the various dosages of MK-7 were subjected to bioinformatics core analysis using Ingenuity Pathway Analysis (IPA) (Ingenuity Systems Inc., USA)

Results

Treatment with 10 mg/kg body-wt. (Bw) MK-7, showed significantly altered expression ($p<0.05$) for 629 genes compared to the control group (Fig.1).

Treatment with 2mg/kg body weight MK-7 influenced the expression of 281 genes compared to the control group. Some of these 281 differentially expressed genes ($p < 0.05$) , were up-regulated more than 5 -fold (Figs.2 and 3).

227 genes were found differentially expressed in pups given 0.2 mg/kg Bw MK-7. These genes exhibited highly significant associations to apoptosis, polyamine metabolism (3 genes), retinoic acid-dependent regulation of apoptosis (3 genes) and to altered osteoclast and osteoblast signalling (6genes).

Treatment Comparison

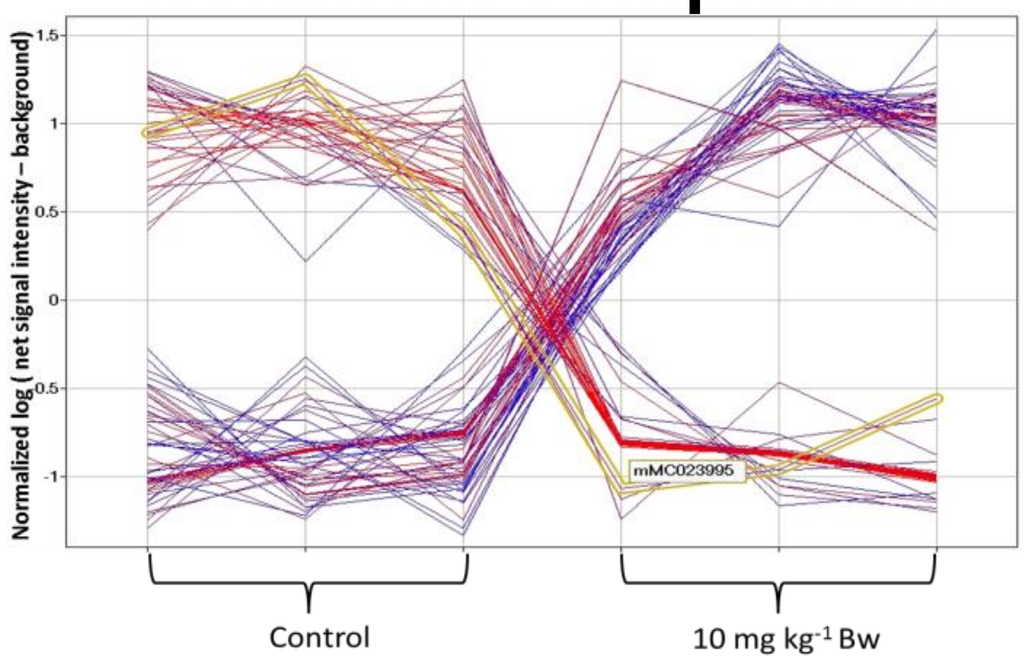

Fig.1. Differences in gene-expression in tooth germs from newborn mice treated with 10 mgkg-1-BW MK-1 compared to control.

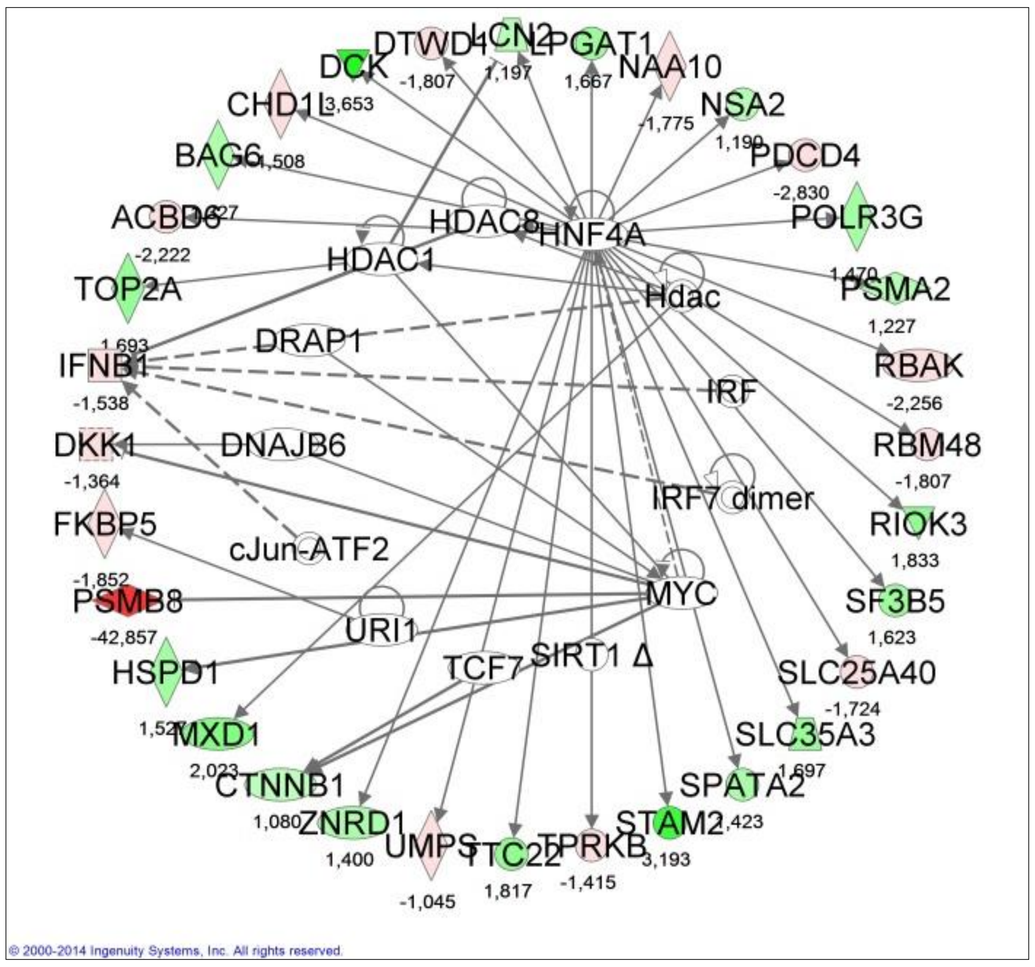

Fig.2 Bioinformatics core and transcriptionfactor analysis performed using Ingenuity Pathway Analysis (IPA) for the 281 genes exhibited a p-value of overlap <0.01 associating significantly 33 genes with 13 transcription factors.

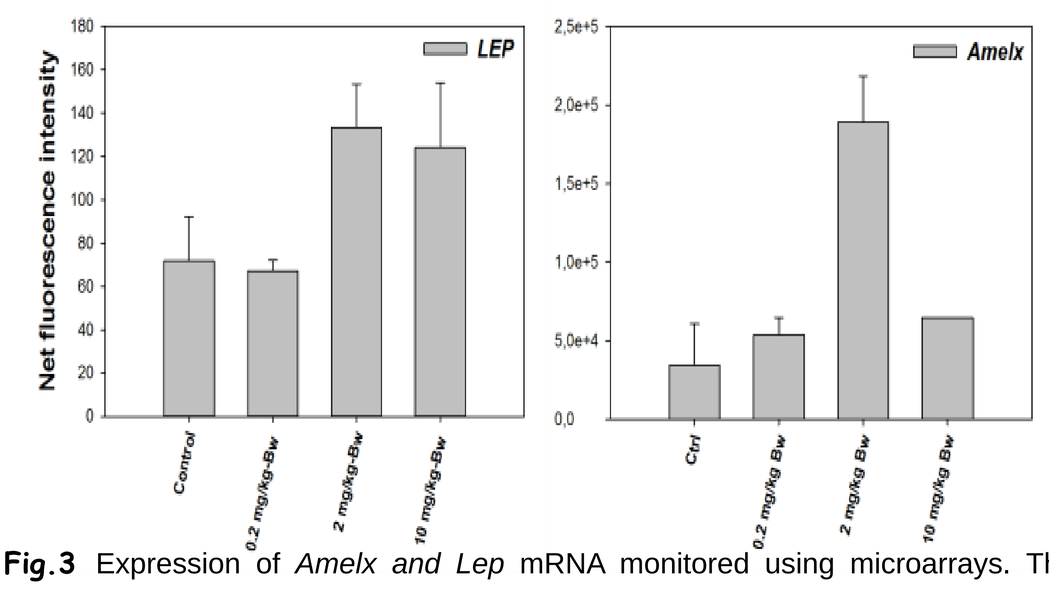

Fig.3 Expression of *Amelx and Lep* mRNA monitored using microarrays. The plotted normalized net fluorescence intensities (raw fluorescence intensity minus background) are means derived from biological triplicates.

Conclusions

A clear effect on gene expression in the developing tooth germ was apparent after 24 h at all dosages. The results indicate increased transcription of genes involved in development of bone, increased biosynthesis of important carbohydrates and of enamel/dentin, and reduced expression of apoptosis related proteins. Further investigations are, however, required to elucidate these findings. Such experiments will likely entail the establishment of a clear dose-response relationship and long term use of MK-7. Also, effects of oral administration should be studied.

References: Barnes C, Newall F, Ignjatovic V, Wong P, Cameron F, Jones G, Monagle P. Reduced bone density in children on long-term warfarin. Pediatr Res. 2005;57(4):578-81.
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