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"A Mitochondrial Uncoupler, BAM15, Inhibits Liver Tumor Promotion in the Context of a High Fat Diet Enriched in Saturated Fat"

Liver cancer ranks as the third deadliest cancer globally and is on the rise due to the obesity epidemic. The incidence is higher in males than females. BAM15, a mitochondrial uncoupler, has demonstrated protective effects against weight gain in obesity models in mice. Our objective was to assess the effect of BAM15 on tumor promotion caused by a high saturated fat diet in mice. We also aimed to determine the role of Pparα, which is a transcription factor stimulating the expression of rate-limiting enzymes of fatty acid oxidation. Since bone marrow is enriched with adipocytes in adulthood, we finally assessed the role of BAM15 on bone turnover.

Experimental Design: Wild type (wt) C57Bl/6J and Pparα knockout (KO) mice were injected intraperitoneally with 20 mg/kg diethyl nitrosamine on postnatal day 13. From weeks 4-10, mice were fed a high saturated fat diet with cocoa butter as a saturated fat (CB diet). At 10 weeks of age, mice were either continued being fed the CB diet or were fed a cocoa butter diet supplemented with 0.1% (w/w) BAM15 (BAM diet). The mice were sacrificed at 30 weeks of age with recording of visible liver tumors and collection of serum and tissues. From the serum, severity of liver tumorigenesis was determined by ELISA of the tumor stem cell marker alpha-fetoprotein (AFP) and liver injury by a kinetic enzymatic assay of alanine transaminase (ALT). Serum markers for bone synthesis (Procollagen 1a1) and bone resorption (collagen crosslinks CTX-1) were assessed by ELISA. Gene expression was determined by RNA isolation and qRT-PCR assays.

Results: For both wt and Pparα KO mice, the BAM diet led to significantly ($P < 0.05$) lower body weight and weight of gonadal fat pads. In males, the BAM diet significantly decreased the liver weight and hepatic steatosis. The number of tumors per mouse was significantly higher in male than female mice fed the CB diet. In male mice, the BAM diet led to significantly lower numbers of tumors, and significant decreases in serum AFP content and serum ALT activity. Surprisingly, knockout of Pparα did not stimulate hepatic steatosis. Yet, Pparα KO male mice fed the CB diet had higher ALT levels, but significantly lower AFP levels than wt males. In wt mice, the BAM diet had no effect on procollagen 1a1 abundance but caused a significant decrease in serum CTX-1 content in both sexes. Gene expression in femoral bone marrow of two marker genes of adipocytes (*Fabp4* and *Pparg*) was unchanged by the BAM diet.

Conclusion and Discussion: In addition to protection from obesity, BAM15 inhibits liver tumor promotion caused by a high-saturated fat diet, particularly in males. Pparα has a dual effect, with knockout of the gene promoting liver injury, but reducing the tumor severity. Finally, BAM15 may inhibit bone resorption without a decrease of bone marrow adiposity.

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