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

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**Introduction**

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.

**Methods**

### 1.0 Knockout of PPARα

Gene expression was determined by PPARα KO. Weight loss of gonadal fat pads were not explained by changes in gene expression of BAM15.

**Examples of livers with tumors:**

- A large hepatocellular carcinoma
- Multiple small liver nodules

### Knockout of PPARα

- Wild type (wt) C57BL/6J and PPARα knockout (KO) mice were injected intraperitoneally with 20 mg/kg diethyl nitrosamine (DEN) 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 on the CB diet or were fed a CB diet supplemented with a 0.1% (w/w) BAM15.
- 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.
- RNA was isolated from randomly selected subsets of mice and RNA quality was validated by TapeStation analysis.
- Gene expression was determined by qRT-PCR assays.

**Results**

### Body weight

Body weight of mice fed a cocoa butter diet (CB), or a CB diet supplemented with BAM15 (BAM15) at sacrifice\(^1\).

### Liver weight

Liver weight of mice fed a cocoa butter (CB) diet, or a CB diet supplemented with BAM15 (BAM15) at sacrifice\(^1\).

### Gonadal fat pad weight

Gonadal fat pad weight of mice fed a cocoa butter (CB) diet, or a CB diet supplemented with BAM15 (BAM15) at sacrifice\(^1\).

### Gene expression in gonadal fat pad

mRNA expression in gonadal fat tissue from mice fed a cocoa butter (CB) diet, or a CB diet supplemented with BAM15 (BAM15) was determined by qRT-PCR relative to the expression of 18S rRNA. N = 6 per group. ANOVA of \(\Delta C_{\text{t}}\) values was performed. There were no significant effects of the BAM15 exposure\(^1\).

### Hepatic steatosis

Steatosis scores of H&E-stained liver sections from mice fed a cocoa butter (CB) diet, or a CB diet supplemented with BAM15 (BAM15) were recorded\(^1\).

### Visible tumors

Visible liver tumors and nodules were counted at sacrifice for mice fed CB diet, or a CB diet supplemented with BAM15 (BAM15)\(^2\).

**Summary**

- BAM15 led to significantly (\(P<0.05\)) lower body weight and weight of gonadal fat pads.
- Weight loss of gonadal fat pads were not explained by changes in gene expression of Fabp4, Pppla2, Lipe, Pparg, or Srebf1.
- In males, BAM15 significantly decreased liver weight and hepatic steatosis.
- The number of tumors per mouse was significantly higher in male compared to female mice fed the CB diet.
- In male mice, BAM15 led to significantly fewer tumors and significant decreases in serum AFP and serum ALT activity.
- Knockout of PPARα did not stimulate hepatic steatosis, but led to higher ALT levels and significantly lower AFP levels in males fed the CB diet.
- 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.
- Expression of two adipocyte marker genes (Fabp4 and Pparg) in femoral bone marrow was unaffected by BAM15.

**Conclusion**

- 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.
- BAM15 may inhibit bone resorption without a decrease in bone marrow adiposity.

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\(^1\) Data were analyzed by ANOVA followed by comparisons with Tukey’s adjustment. \(^2\) Data were analyzed by Kruskal-Wallis test and Dunn’s multiple comparisons test. \(\Delta C_{\text{t}}\), \(P<0.05\), 0.01, 0.001, 0.0001.