Benfotiamine, a vitamin B1 derivative, inhibits VEGF-induced angiogenesis.

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Background

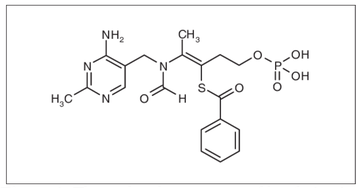

Figure 1. The molecular structure of benfotiamine (s-benzoylthiamine-o-monophosphate).

Benfotamine (S-benzoylthiamine-O-monophosphate) is a unique, lipid soluble derivative of thiamine which has been shown to have therapeutic effects on the body. From secondary diabetic complications to inflammatory diseases such as uveitis and endotoxemia, benfotiamine has demonstrated properties that make it a potent antioxidant and anti-inflammatory agent (1). However, its effects in the prevention of cell proliferation, migration, invasion, and metastasis have been minimally investigated. Thiamine deficiency and the use of thiamine supplementation to counteract deficiencies is noted sometimes in leukemias and gastrointestinal cancers . Other studies have discovered that some chemotherapies cause thiamine deficiency, however up to this point, benfotiamine has not be tested as an anti-carcinogenic drug (2,3). A recent study by Jonus et al, indicated the potential of benfotiamine in preventing cancer cell proliferation in vitro and in the tumor growth of nude xenograft mice (4). Angiogenesis plays a major role in the growth and spread of many cancers, and the role of vitamin B1, and more specifically benfotiamine, in neovascularization is not known.

Hypothesis

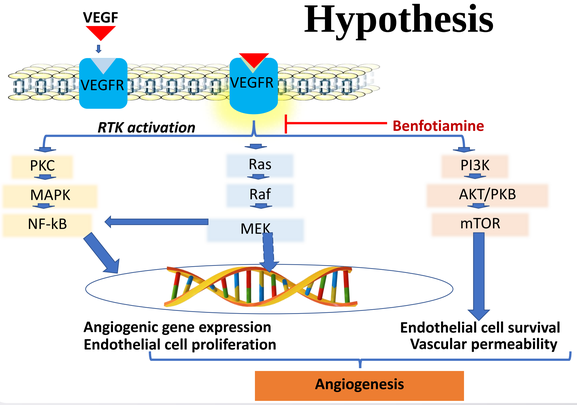

Figure 2. Possible anti-angiogenic mechanism of benfotiamine. Benfotiamine prevents VEGF-induced angiogenesis by blocking the RTK-mediated signaling via PKC/Ras/PI3K/NF-kB in endothelial cells.

Methods

- We treated human umbilical vein endothelial cells (HUVECs) with VEGF (Vascular Endothelial Growth Factor; 10 ng/ml) ± benfotiamine (0-100 uM) in a time and dose-dependent manner.
- We examined endothelial cell viability via MTT assay, apoptosis by Annexin-V staining and via in-vitro angiogenesis via tube formation assay.
- Activation of caspase-3, protein kinases, and transcription factors were examined by immunological methods as well as multiplex analysis.

Benfotiamine could be utilized as an anti-angiogenic and chemopreventive agent.

Results

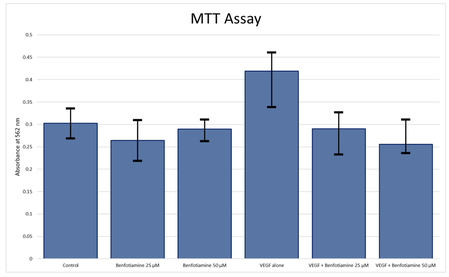

Figure 3. – Results of MTT Assay of HUVEC cells +/- treatment with benfotiamine. This indicates that benfotiamine does not affect cell viability.

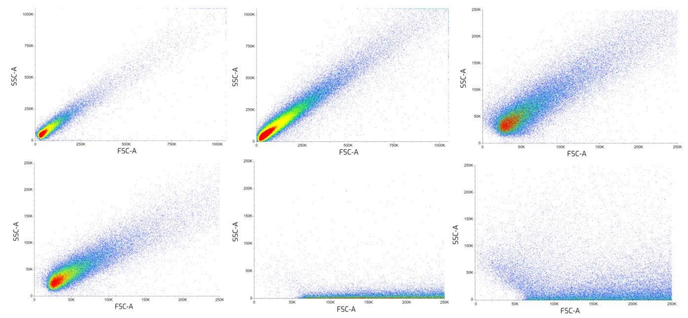

Figure 4. – Results of Flow cytometry HUVEC cells +/- treatment with benfotiamine with Annexin V/-ADD staining . This indicates that benfotiamine does not demonstrate apoptotic properties.

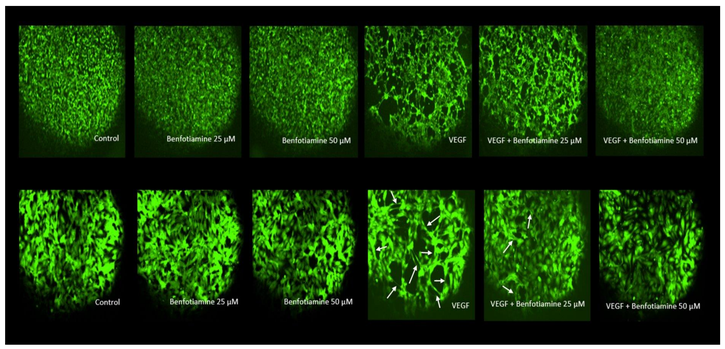

Figure 5 – Images from a tube formation Assay of HUVECs +/- treatment of benfotiamine with the top row of images under 10X magnification with the bottom row under 20X magnification. These images indicate that benfotiamine prevents VEGF- induced in-vitro tube formation.

Results (Continued)

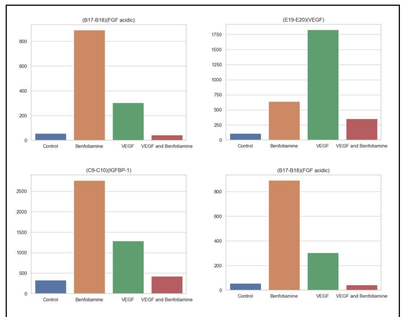

Figure 6. – Results of Proteasome Angiogenesis Assay of HUVEC cells +/- treatment with benfotiamine. These graphs indicate that benfotiamine regulates VEGF-induced growth factors.

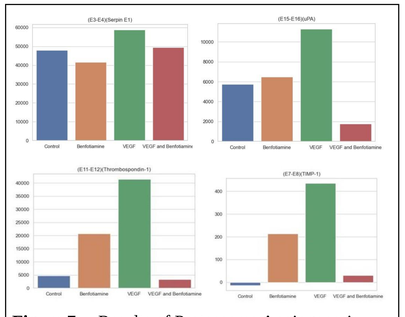

Figure 7. – Results of Proteasome Angiogenesis Assay of HUVEC cells +/- treatment with benfotiamine. These graphs indicate that benfotiamine regulates anti-angiogenic enzymes.

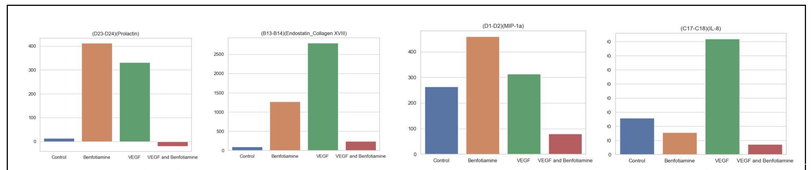

Figure 8. – Results of Proteasome Angiogenesis Assay of HUVEC cells +/- treatment with benfotiamine. These graphs indicate that benfotiamine regulates angiogenic cytokines.

Conclusion and Future Plans

Our current results indicate that benfotiamine has anti-angiogenic potential via inhibition of various pro-angiogenic growth factors and by promoting anti-angiogenic factors in vitro and in vivo. Thus, our studies demonstrate that benfotiamine could be further developed as a novel therapeutic agent to prevent cancer growth and metastasis. Next, we will examine how benfotiamine prevents in vivo angiogenesis by utilizing a rat Matrigel plug model of angiogenesis in rats. The Matrigel sections will be stained with CD36, vWF, and other angiogenic markers to visualize the effect of benfotiamine on VEGF-induced angiogenesis.