Apatone Treatment Enhances Cell Proliferation and Reduces Inflammation Following Cobalt-Chromium Exposure

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Statement of Purpose: There is evidence that supports the contention that prosthetic metal wear debris can instigate cytotoxic effects and periprosthetic inflammation from a host of cells (macrophage, fibroblast, osteoblast, and osteoclast).[1] The release of the down-stream proteins and enzymes (ie cytokines, chemokines, Cox 1 & 2, and prostoglandins) that are associated with the aseptic osteolytic process are all ultimately attributed to the cellular activation of nuclear-factor-kappa-B (NFκB). The amelioration of this cellular cascade of events following metal debris exposure could lead to greater joint tissue interface stability, and the extended functional life-span of the prosthetic implant.

Apatone®, an amalgam of vitamins C and K_3 (IC-Medtech, El Cajon, CA), a drug currently under FDA phase-II clinical studies as a chemotherapeutic adjuvant, has been shown to be an effective anti-inflammatory agent.[2] This is accomplished through the accumulation of vitamin C (AA) into the cell via the glucose transporters. Once the oxidized form of AA (DHA) enters the cell, it is reduced and retained as AA, thus increasing the intracellular vitamin C concentrations. This redox cycling allows the presence of AA to reduce reactive oxygen species (ROS) and the DHA to inhibit IKKβ by binding in or near the active site, thus preventing the activation of NFκB (Fig 1).

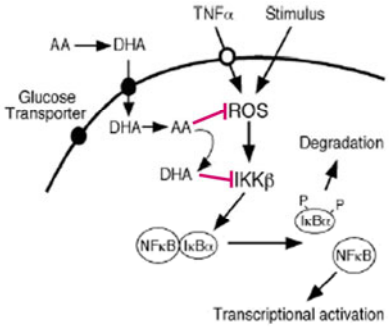

Figure 1

By reducing the oxidative cellular stress and diminishing the release of NFκB, we hypothesize that Apatone treatment of synovial fibroblasts will reduce, or retard, the cellular cytotoxicity and bio-reactivity customarily seen following cobalt-chromium-molybdenum (CoCrMo) particulate debris exposure.

Methods: Two ASTM F75 CoCrMo grade particulate powders (<10μ) were obtained through generous in-kind donations (Zimmer Inc, Warsaw, IN & Astro Met Inc, Cincinnati, OH). A metal mass dosage of 0.01g was used for all cell exposures.

Apatone was prepared in a 100:1 ratio (75.0μm of vitamin C and 0.75μm of vitamin K_3, respectively) for all experimental treatments.

Using an experimental protocol approved by the Institutional Review Board Committee on Human Research, synovial fibroblasts were harvested from the knee joint of a consented volunteer donor. The harvested tissue was processed in a customary manner, passaging the donor cell line once prior to the seeding of ≈1x10^6 cells into each of eight 75 cm^2 culture flasks. The flasks were then incubated over a 5-day period to render ≈5x10^6 cells. For each metal, four flasks were incubated for 24hrs and consisted of: a) control (cells only), b) cells exposed to metal only, c) cells treated with Apatone for 24hrs prior to metal exposure, and d) cells exposed to metal 24hrs prior to Apatone treatment.

Following the 24hr exposure, each flask was assessed for cell viability (hemocytometer with trypan blue exclusion) and NFκB levels (EZ-Detect NFκB p65 Transcription Assay, Thermo Fisher Scientific, Rockford, IL).

Results: Cellular proliferation and NFκB levels are summarized in Fig 2. When compared to the metal only exposures, the Apatone pre-treatment group appeared to enhance cellular proliferation by as much as 64% while also dramatically reducing the NFκB levels. When compared to the metal only exposures, the Apatone post-treatment group, a condition equivalent to symptomatic total joint recipients, also exhibited enhanced cellular proliferation by as much as 15%, while again showing a dramatic reduction of NFκB levels.

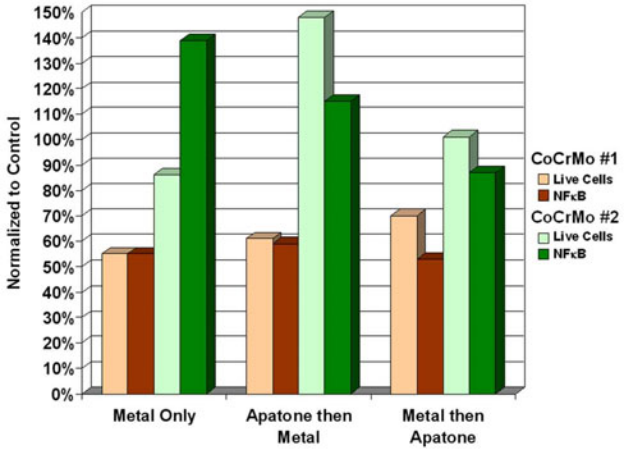

Figure 2

Discussion: While it has been shown that antioxidant enzymes and vitamins can return tumor cells states to that of normal cells[3], at present, this same approach has not yet been introduced to orthopaedics to lessen the commonly seen wear-debris-induced osteolytic effects.

Results based on this study indicate that Apatone treatment, following metal debris exposure, may prove to be an effective adjuvant in reducing the inflammatory induced reaction commonly seen following total joint replacement, and necessitates extensive evaluations.

References: 1) Tuan RS. J Am Acad Ortho Surg. 2008;16(1):S42. **2)** Jamison JM. In Lucas JM (eds). Trends in Prostate Cancer Research. Chapter VII. Nova Science, Inc 2005;189. **3)** Tareen RS. Int J Med Sci. 2008;5(2):62.