In Vitro Stimulation of Oxygen Consumption by Lead May Elucidate the Role of Reactive Oxygen Species in Osteoarthritis

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ABSTRACT INTRODUCTION:
There is a disparity between our current knowledge of the etiology of osteoarthritis and its prevalence in the population. The non-inflammatory degeneration of joints characterized as osteoarthritis affects 80% of people over 55 to varying degrees; however, the putative causes for the disease have encompassed anatomical incongruities, excesses in mechanical loading, injury, infection and chemical/toxicant induction influences. It is this last cause that we have investigated at a cellular and molecular level in this study.

Clinical case studies and epidemiological studies have linked lead exposure to OA-like symptoms. Furthermore, several other reports have found increased levels of reactive oxygen species and their associated scavenging enzymes in the joint fluid of patients with osteoarthritis. These findings have led to speculation that reactive oxygen species may be responsible, in part, for joint degeneration.

These literature findings and our current results led us to formulate the hypothesis that the stimulatory effect of lead on the metabolism of articular chondrocytes disrupts their maintenance of the joint surface and overwhelms their ability to cope with oxidative stress. We believe that by investigating and delineating a specific molecular mechanism behind a chemical/toxicant induced-OA that novel treatments and preventions may be arrived at.

MATERIALS AND METHODS:
O2 consumption was measured in an airtight chamber thermostated at 37° C using a Clark type micro-electrode. Equal numbers of cells were used for control and treatment groups.

ATP levels were measured by Promega Kinase Glo. Chick ACs were isolated from the femora and tibiae of 4-6 week old Gallus domesticus chickens.

Isolated and purified protein extracts of known concentrations were run on a polyacrylamide gel and then transferred to a polynvinylidene fluoride membrane. The membranes were probed overnight at 4°C with the antibody of interest. The following day the corresponding HRP-conjugated secondary antibody was applied and the blots were visualized using ECL Plus.

Real Time PCR was used to quantify the mRNA expression of genes after 24 hours of lead or H2O2 treatment. The Sybergreen reagent was used according to suggested protocol. Gene expression was normalized to GAPDH.

DCFDA was used to determine general levels of reactive oxygen species. Mitosox was used to determine levels of superoxide. Amplex red was used to determine levels of peroxides. Each reagent is converted to a highly fluorescent product in the presence of the reactive oxygen species it detects.

RESULTS:
Lead rapidly stimulates oxygen consumption (Figure 1A) and ATP production (Figure 1B) resulting in a dose dependent generation of reactive oxygen species (ROS) as metabolic byproducts (Figure 2).

The TGF-β signaling pathway which maintains articular chondrocytes has been found to be highly sensitive to changes in intracellular redox state. In our experiments we have found that hydrogen peroxide can strongly inhibit TGF-β levels (Figure 3) as well as downstream signaling targets. The effect is partially rescued in the presence of the anti-oxidant, N-acetyl cysteine (NAC).

Given these findings, we hypothesize that lead initiates a cascade of reactions whereby elevated ROS depress TGF-β signaling, which in turn leads to an alteration in articular chondrocyte phenotype. The new phenotype (i.e. elevated MMP13, MMP9, colX and depressed col II) (Figure 4) resembles that which occurs in osteoarthritis. In data not shown we have also demonstrated that hydrogen peroxide mimics these effects.

DISCUSSION:
Our in vitro findings provide one molecular mechanism by which an environmental toxicant can induce or propagate the formation of an osteoarthritic phenotype in articular chondrocytes. By altering the underlying chondrocyte metabolism and changing the redox state of the cartilagenous matrix, we have found that lead depresses the TGF-β signaling pathways. This in turn leads to an up regulation of matrix degrading enzymes and type X collagen and a decrease in the matrix stabilizing collagen, type II.

While chronic lead exposure is by no means a factor in all cases of osteoarthritis, our data implicate ROS as a common causial intermediate in the initiation and progression of degenerative joint diseases.