PROSTHETIC METALS HAVE A VARIABLE NECROTIC THRESHOLD IN HUMAN FIBROBLASTS: AN IN VITRO STUDY

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Introduction:
Aseptic loosening of prosthetic components is a problem of considerable concern in joint arthroplasty. It is generally agreed that this process begins with the generation and accumulation of fine wear particles from the metallic or polymer materials used in the arthroplasty. Beyond this, there may be a host of potential stimuli and causal factors including type and size of the particles, type(s) of synovial cell involved, and biological cascade factors stimulating the osteoclast for bone resorption leading to component loosening. We have sought to identify, for various metals, threshold wear debris levels that initiate progressive fibroblast necrosis. In an earlier publication, we exposed human fibroblasts in cell cultures to concentrations of commercially pure Titanium (cpTi) particles with total masses ranging from .0060g to .07g [1]. We reported that .009g was the beginning of a threshold, beyond which progressive fibroblast necrosis occurred. Another aspect of this earlier work was that there appeared to be a variation in the necrotic reaction to the cpTi particles among fibroblasts obtained from the tissue of different donors. This suggested that the necrosis response and aseptic loosening might vary not only among different metals but also among individual patients. A variation in the reactions to wear debris particles among individual patients might explain why some joint arthroplasty patients demonstrate extensive osteolysis while others do not. It may also provide a means of identifying pre- and post-operatively those patients who are at elevated risk of osteolysis.

Methods:
Cells - Human fibroblasts were obtained from grossly normal, non-inflamed tissue harvested from the superior medial plica of three volunteer donors undergoing diagnostic arthroscopic surgery of the knee. The Summa Health System Institutional Review Committee on Human Research approved the experimental protocol on 6/19/95 including an Informed Consent Form. The project has received annual approval for continuation. The harvested tissue samples were placed in ice-cold Delbeco’s modified Eagle media for transport. The cells were prepared for tissue culture in a standard manner [1]. CpTi and Tantalum (Ta) particles, with maximum diameters of 2.5m, were purchased from Johnson Matthey (Ward Hill, MA). CoCr and Ta particles, with a maximum diameter of 10m, were purchased from Astrolabs, Inc. (Cincinnati, Ohio). Based on the diameter and type of metal used were .007, .009, .02, and .04gms. Following one passage, the cells were placed in 25-cm2 culture flasks and were permitted to grow to confluency (normally 3-4 days) in a Forma Scientific water-jacketed incubator (Model 3103) at 37°C in a 5% CO2 atmosphere. The metal particle dosages were then added to flasks in duplicate. One set of duplicate flasks for each donor received no particles and served as the control. After a further 5 days in the incubator, one flask for each metal mass dosage for each donor and one control flask for each donor were removed from the incubator for cell counting. Interleukin 6 (IL-6) analysis was done on each tissue culture flask. Two ml of the media fluid were removed from the control and metal challenged flasks prior to cell counting, and stored in a 3ml centrifuge tube at -80°C. Quantimine assay kits were used which involved adding diluents to the media samples, followed by spectrophotometric readings at a wavelength of 450nm using a Sanofi Diagnostic Pasteur spectrophotometer Model LP400. The data were analyzed with general linear models and a 3-way Analysis of Variance (ANOVA), including interactions, using the statistical analysis software system, PC/SAS (SAS, Cary, NC). The Tukey Test was used, if needed, to show significant differences among means. Significance was accepted at p<0.05.

Observational results:
The ANOVA demonstrated that for both the dependent variables, the number of surviving cells and the IL-6 concentration, all the independent variables, with the exception of the time interval, had significant main effects (p<0.05). The only significant interaction between the independent variables was the tissue donor - time interval interaction (p<0.05). The cell counts in the control flasks for the three tissue donors differed significantly from each other. The percent of cells surviving varied significantly among the metal types and with the particle dosage for each of the metals. With blocking on the tissue donors and the particle dosages, the percentage of cells surviving in the CoCr flasks was significantly greater than that for the Ta flasks, which in turn was significantly greater than that for the cpTi flasks. With blocking on the tissue donor and the metal type, significant reductions in the percentage of cells surviving were seen for particle dosages of 0.009g or greater. Taking the metal types individually, the number of cells surviving trended downward as the particle dosage increased, but the decrease for the CoCr flasks did not become significant until the 0.02g and greater and 0.009g and greater, respectively. As stated above, the number of cells in the control flasks differed significantly among all three tissue donors. However, when the cell counts were normalized by those of the control flasks, there were no statistical differences between the tissue donors in terms of the percent of cells surviving at any of the particle dosages for any of the three metals. The IL-6 concentrations did not significantly vary with particle dosage for any of the three metals, but the IL-6 concentration was significantly lower following exposure to cpTi particles than following exposure to the latter two metals did not differ. In fact, the IL-6 concentrations after exposure to particles of these latter two metals did not differ from those of the corresponding controls.

Discussion:
With respect to the necrotic effects of the metals used in the experiment, cpTi and Ta particles both resulted in increasingly severe necrotic effects as the particle dosage increased. The threshold for the necrotic effect of Ta was a .02gm particle dosage. Ta has been thought to be a relatively inert metal from a biological view. These data clearly demonstrate that at the exposure levels used, Ta particles are deleterious to fibroblasts and produce statistically significant levels of necrosis. The threshold for the necrotic effect of cpTi was a .009g particle dosage. The threshold for the necrotic effect of CoCr was a .04gm particle dosage. We observed a significant change (a decrease) in IL-6 concentration from controls only following exposure to cpTi particles, but the concentration did not vary with increasing particle dosage. The tissue cultures for the three tissue donors demonstrated initial (control) values for cell counts and IL-6 concentration that differed significantly among the three tissue donors. The IL-6 concentrations did not significantly vary with particle dosage for any of the three metals. The IL-6 concentrations did not significantly vary with particle dosage for any of the three metals, but the IL-6 concentration was significantly lower following exposure to cpTi particles than following exposure to the latter two metals did not differ. In fact, the IL-6 concentrations after exposure to particles of these latter two metals did not differ from those of the corresponding controls.

In conclusion, fibroblast necrosis following metal particle exposure in cell culture was significantly affected by both the type and mass of the metal. Particles of cpTi produced fibroblast necrosis at lower mass exposures and resulted in a more severe necrotic reaction than did Ta or CoCr particles. The observed necrotic effect of Ta particles, commonly thought to be bio-inert, demonstrates that perhaps any metal or other material can be necrotic when presented to a cell membrane in particulate form. Normal cell vitality and IL-6 concentration vary considerably among individuals, but their response to exposure to metal particles in terms of cell necrosis and IL-6 concentration changes do not.

Acknowledgement:
This work was supported, in part, by The Summa Health System Foundation and The Robertson-Hoyt Fund.

References: