Metal Ions Upregulate Vascular Activation, Lymphocyte Trafficking, and Angiogenesis: Potential Non-Allergic Mechanisms for the Tissue Responses to Metal on Metal Articulations in Total Joint Arthroplasty

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SIGNIFICANCE:
Increasing concerns have arisen regarding the use of metal on metal articulations in joint replacement surgery, leading to pain and early implant failures. This abstract describes mechanisms by which metal ions directly activate endothelial cells, resulting in vascular proliferation and the trafficking and accumulation of lymphocytes.

INTRODUCTION:
Metal-on-metal (MOM) articulations in total hip arthroplasty offer several theoretical advantages, including a slower wear rate as well as increased stability and resistance to dislocation. Despite these advantages, there is a growing body of literature suggesting that at least for some implant designs, the use of MOM may be associated with pain, instability, and early implant failure leading to revision surgery. Characteristically, histologic evaluation of the tissues surrounding these loose implants shows vascular proliferation and accumulation of lymphocytes, similar to that seen in a Type IV hypersensitivity reaction.

However, both clinical studies as well as in vitro evaluations have demonstrated that not all patients with failed implants have demonstrated hypersensitivity to the primary metal ions seen with MOM articulations, particularly cobalt and chromium.

Given that not all failed implants are associated with immune sensitization, we hypothesized an alternative mechanism where metal ions directly activate endothelium, resulting in lymphocyte migration via the upregulation of cell binding proteins, and subsequent transendothelial migration. We further hypothesized that metal ion induced activation of endothelium leads to upregulation of the transcription factor hypoxia inducible factor (HIF-1α), and subsequent increased activity of eNOS, producing an increase in nitric oxide induced signal transduction pathways.

METHODS:
Human vascular endothelial cells (HUVEC) were grown in tissue culture in EGM media, and lymphocytic Jurkat cells were grown in RPMI. Chloride salts of metal ions, cobalt and chromium, were added to the culture media, with NaCl as a control salt in equimolar concentrations. Alterations in the endothelial cell binding protein ICAM-1 were determined by Western blot. Upregulation of the the transcription factor HIF-1α was determined using Western blots with whole cell lysates from HUVEC.

Alterations in nitric oxide levels were assessed using immunohistochemistry and confocal microscopy with DAF, a nonfluorescent compound until it reacts with NO to form a fluorescent benzotriazole.

To assess the effects of metal ions on vascular permeability and transendothelial migration of lymphocytes, an ex vivo model of an intact vessel was created using a rabbit carotid artery. The arteries were harvested under sterile conditions and cannulated. All side branches were ligated, and the intact vessel was pressure tested. Culture media in the presence or absence of metal ions or lymphocytes were then perfused into the intact vessel via the cannula. Transendothelial migration of lymphocytes was assessed on the extraluminal surface of the vessels using lymphocyte-specific staining.

Effects of metal ions on angiogenesis were determined in tissue culture using a HUVEC based endothelial tube formation assay.

All experiments were performed with at least triplicate determinations, and statistical analysis was carried out using the analysis of variance with the Bonferroni-Dunn post hoc modification, with an alpha value of ≤0.05.

RESULTS:
Addition of both cobalt and chromium to endothelial cells upregulated the endothelial cell surface binding protein ICAM-1 in a dose dependent fashion. Western blot analysis of whole cell lysates from HUVEC demonstrated increased HIF-1α levels in response to cobalt, but not chromium ions. (Figure 1)

CONCLUSIONS:
These findings demonstrate that metal ions act directly on vascular endothelium, resulting in the upregulation of ICAM-1, upregulation of the transcription factor HIF-1α and nitric oxide, which leads to angiogenesis. This in vitro model effectively mimics ALVAL, and our data suggest that soluble metallic wear products from MOM articulations can result in implant loosening in the absence of an allergic response, and that this biologic response is largely elicited by cobalt ions over chromium. This implies that any large diameter metal on metal articulation, including resurfacing as well as total joint replacements, may be subject to similar biologic effects regardless of implant design.