INTERACTION OF CHROMIUM IONS WITH HUMAN SERUM PROTEINS

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INTRODUCTION

UHMWPE particles from metal-on-polyethylene prostheses are the main culprit in osteolysis. Due to their potential for improved wear performance, there has been a revived interest in metal-on-metal (MoM) prostheses. However, metallic wear particles are chemically active, leading to the release of cobalt and chromium ions that are found in high concentrations in the blood and urine of patients (1). Although chromium in its +3 oxidation state (Cr(III)) may act as an essential trace nutrient for glucose metabolism, some Cr complexes as chromate (CrO_4^{2-}) are known to cause allergic dermatitis, exert toxic and carcinogenic effects in animals and humans (2). Therefore, one of the major concerns regarding CoCrMo alloy prostheses is the biological and biochemical activities of Cr ions (3). Previous studies showed that Cr^{3+} ions form nanoclusters in cell culture media (4). To date, there has been little or no attempt to understand the nature of implant-derived metal ions in adjacent tissues or biofluid media.

The aim of this work was to examine the interaction of human proteins contained in serum with the nanoclusters generated by Cr^{3+} ions to better understand the role of proteins in the biological activity and toxicity of these Cr nanostructures.

MATERIALS AND METHODS

Fifty (50) ppm of Cr(III) (CrCl_3, Sigma-Aldrich, Oakville, ON) were incubated for 1h at 37°C in RPMI 1640 and DMEM supplemented with 5% human serum (HS). After incubation, structures were isolated by centrifugation and washed with water. Pellets were dried for 4h at room temperature, resuspended in ethanol (EtOH), and the solution was put on a copper grid to be analyzed by transmission electron microscopy (TEM). Structures obtained with human serum were also loaded on a 4-20% acrylamide gel and separated by SDS-PAGE. Proteins were stained by Coomassie blue. Gels were stained by Coomassie blue, cut in 2 mm pieces and incubated with 1% formic acid/2% acetonitrile for identification of proteins using liquid chromatography-quadrupole-time of flight-mass spectrometer (LC-Q-ToF-MS). Data was submitted to Mascot software (MatrixScience, London, UK) for a search against the NCBI nonredundant database.

RESULTS

Figure 1 shows that nanoclusters can interact with proteins from human serum. The molecular weights of the proteins were between 40 to 90 kDa on SDS-PAGE. The MS results suggest that Cr nanostructures are the result of the interaction with numerous proteins present in human serum. However, the complete analysis of results demonstrates that only two proteins (in both RPMI and DMEM) are implicated in these nanoclusters: albumin and transferrin. The MS results have shown important ion score for albumin for both RPMI and DMEM (105 for RPMI and 98 for DMEM) but the absence of transferrin.

DISCUSSION AND CONCLUSION

Results show that the composition of the cell culture media plays a key role on the complexation of Cr ions. These different complexes appear to interact differently with the cell membrane and appear to be internalized differently by human U937 macrophages. Macrophages are known to release different cytokines after engulfing a foreign body. The macrophage response to these different Cr nanostructures and their fate inside the cells remain to be investigated. In summary, these results suggest that caution must be exercised when interpreting results on the effect of Cr(III) ions in vitro.

Furthermore, we show that proteins from HS bind to these Cr structures. Although the nanostructures formed in the different media are different in shape and size, proteins implicated in their formation are the same. These results also show that human proteins bind in a specific way to nanoclusters generated by Cr(III) ions in cell culture media. It is known that HS contain more than 400 different proteins, albumin being the major one. It has been shown that albumin could play an immunological role by addressing signals to defense cells, such as macrophages. Transferrin may also specifically interact with the Cr-nanostructures as previously described with biological molecules such as Cr(III) transferrin.

Finally, albumin is the protein common to all chromium nanoclusters formed in both HS and FBS. This also highlights the limitation of the interpretation of results on the effect of Cr ions in vitro. In conclusion, the present study provides valuable information on the nature of human proteins involved in chromium nanostructures and the manner in which proteins interact with nanoclusters generated by Cr ions in cell culture media. The relation with Cr nanostructures in vivo remains to be determined.

REFERENCES


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