INTERNALIZATION OF CHROMIUM IONS BY MACROPHAGES IN VITRO

*Catelas, I; **Vali, H; *Petit, A; *Zukor, DJ; *Antoniou, J; +*Huk, OL
+*Division of Orthopaedic Surgery, McGill University, Lady Davis Institute for Medical Research, Montreal, Quebec, Canada

Introduction

In order to minimize periprosthetic osteolysis initiated by polyethylene wear particles, metal-metal (MM) hip implants, made of CoCr alloys, have been considered as an alternative to conventional metal-polyethylene bearings. However, metal ion toxicity remains a major cause for concern.

Metal ions can bind to proteins or enzymes and circulate locally within adjacent cells and extracellular matrix media and systematically. Indeed, high levels of both cobalt and chromium ions have been found in red blood cells and urine of patients with a MM bearing compared to those with a metal-polyethylene bearing (1). These ions can therefore be mediators of both local and remote tissue responses.

However, the exact mechanism by which these ions exert their cellular and tissue responses are unknown. Recent studies conducted in our laboratory have demonstrated that Cr³⁺ induces an early inflammatory response in macrophages, including TNF-α secretion as well as macrophage mortality in a dose- and time-dependent manner (2). In addition, the valence state of the ions may influence cellular and protein binding. Indeed, Cr⁶⁺ ions have been reported to bind poorly to cells and to remain on the cell membrane, whereas Cr⁴⁺ would be more permeable to the cell membrane (3).

To better understand the cellular effects of Cr³⁺, the aim of the present study was to analyze the internalization of Cr³⁺ by macrophages in vitro.

Materials and Methods

J774 mouse macrophages (ATCC, Rockville, MD) were cultured in RPMI 1640 medium supplemented with 5% fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin. Monolayers of confluent cells were scraped, resuspended in fresh culture medium, and incubated overnight at 37°C in 5% CO₂ to 35 mm Petri dishes at a concentration of 5 x 10⁵ cells/ml. Cell monolayers were then washed once and exposed to 10-250 ppm Cr³⁺ (CrCl₃, Sigma Chemicals, Oakville, Ontario, Canada) for 1 to 24h in order to analyze the effects of ion concentration and incubation time. Macrophages without ions served as negative control.

The supernatants were discarded, and the cells were washed once with PBS (pH=7.4), fixed overnight at 4°C in 2.5% glutaraldehyde, treated with 1% tetroxyde osmium for 30 min, dehydrated in a series of ethanol solutions (25% to 100%), and embedded in epoxy resin. Ultrathin sections (70 – 90 nm thick) were cut using a diamond knife. The microstructure of the precipitates containing Cr³⁺ and their distribution within the cells were studied in TEM. The elemental composition of the precipitates was determined using energy dispersive X-ray analysis (EDS).

Results

Figure 1 shows a typical TEM micrograph of macrophages exposed to 50 ppm of Cr³⁺ and incubated for 1h. It is evident that spherical nanoforms were internalized as early as 1h incubation. The size and concentration of these nanoforms increased with increasing ion concentration. Some appeared to be organized and grouped into a membrane-bound structure (Figure 1B). After 24h, a degradation of some nanoforms was apparent, suggesting a partial dissolution of these nanoforms by the cells. EDS of the nanoforms revealed a chromium phosphate composition. As there is no evidence for a crystalline or an amorphous exclusively inorganic phase, it is most likely that Cr³⁺ are bound to organic molecules (proteins?). Moreover, their appearance also suggests the presence of an organic phase, most likely proteins. With increasing ion concentration, the composition of the nanoforms remained the same but their increasing size suggests increasing ion-protein interactions.

An ancillary experiment was conducted to determine whether the nanoforms consisting of Cr³⁺, phosphate, and organic components (proteins?) formed outside the cells and subsequently internalized, or rather, formed within the cells after internalization of Cr³⁺. Fifty (50) ppm of Cr³⁺ were incubated in the culture medium containing 5% serum without cells for 1h and 24h, respectively. The samples were processed as described above. TEM analysis (Figure 2) revealed that nanoforms were formed in the growth medium without cells and had identical structure and composition to those observed inside the cells, as documented in Figure 1. It was concluded that these structures were formed outside the cells and phagocytozed by the macrophages.

The presence of such nanoforms attached to the membrane of macrophages incubated with ions (Figure 3) confirms this result.

Discussion and Conclusion

This study is the first attempt to demonstrate that Cr³⁺ can be internalized by macrophages, after possibly binding to phosphorus or phosphoproteins, to create chromium phosphate nanoforms containing an organic phase. These nanoforms are formed outside the cells, are rapidly phagocytozed by macrophages, and may subsequently group in a membrane-bound structure. Identification of the proteins involved in this process, and more importantly, the fate of these membrane-bound structures laden with ions, remain to be investigated.

Incubation with up to 10 ppm Co³⁺ reveals a completely different mechanism of internalization without the formation of visible aggregates as observed with Cr³⁺. A recent study in our laboratory demonstrated that 50 ppm of Cr³⁺ induced a very low macrophage mortality compared to that observed with 10 ppm Co³⁺ (5% vs. 25% at 24h) (2). This suggests that the formation of nanoforms made of chromium phosphate and proteins may have a protective effect on the cells, at least up to a certain concentration. Further experimental data are required to better understand the ultimate fate of these nanoforms.

References


Figure 1: TEM micrograph of a macrophage after 1h incubation with 50 ppm Cr³⁺, x8500 (A) and X85k (B)

Figure 2: Nanoforms formed in the growth medium without cells.

Figure 3: TEM micrograph (x85k) of the nanoforms attached to the membrane of a macrophage incubated with 50 ppm Cr³⁺ for 1h.

**Department of Anatomy & Cell Biology, McGill University, Cell Biology, McGill University.

49th Annual Meeting of the Orthopaedic Research Society
Poster #1458