

PRUEBAS DE PLANAS

Cytotoxicity of copper and silver ions on specific osteoblastic properties

María Cecilia Cortizo,^{*,**} Mónica Fernández L. de Mele^{*} y Ana María Cortizo.^{**}^{*}Instituto de Investigaciones Físicoquímicas Teóricas y Aplicadas, Universidad Nacional de La Plata. ^{**}Cátedra de Bioquímica Patológica, Facultad de Ciencias Exactas, Universidad Nacional de La Plata.

Recibido: 14 de mayo de 2003. Aceptado: 26 de marzo de 2004.

Palabras clave: cobre, plata, citotoxicidad, liberación de iones metálicos.
Key words: cytotoxicity, silver, copper, metal ions release.

RESUMEN. La plata y el cobre son ampliamente utilizados como componentes de materiales dentales, aún cuando su resistencia a la corrosión es baja. Los iones liberados pueden producir varios efectos indeseables tales como reacciones tóxicas, de inflamación y mutagénicas. El objetivo del presente estudio fue evaluar la biocompatibilidad de los componentes de los materiales dentales con respecto al crecimiento y diferenciación de las células osteoblásticas UMR106, en respuesta a disoluciones salinas de los cationes metálicos y a la liberación de los iones (Cu^{2+} , Ag^+) a partir de los metales puros. Las células control UMR106 mostraron una morfología poligonal normal con procesos citoplasmáticos conectando las células vecinas. Después de 24 h en cultivo las células expuestas al cobre mostraron importantes cambios morfológicos. En períodos más prolongados (48 h) los iones cobre indujeron la casi completa muerte de las células. Las exposiciones a los iones plata por 48 h causaron menos reducción del número de células saludables. Los osteoblastos eran relativamente pequeños con cierta pérdida de procesos, observándose algunas figuras mitóticas y cuerpos apoptóticos. El efecto del cobre sobre la inhibición del crecimiento de las células era linealmente dependiente con el tiempo. Tanto las células sobrevivientes como el índice mitótico se correlacionaron significativamente con la cantidad de cobre en el medio, a pesar de que esta correlación era más fuerte cuando se analizaba el número de células sobrevivientes en comparación con el índice mitótico. Se pudo concluir que los iones cobre y plata son citotóxicos. En el caso de los iones cobre, se encontró una correlación de segundo orden entre la citotoxicidad y la concentración de iones liberados.

ABSTRACT. Copper and silver are widely used as components of dental materials even though their corrosion resistance is low. The released ions can produce several undesirable effects such as toxic, allergic, inflammatory and mutagenic reactions. The aim of the present study was to evaluate the biocompatibility of the components of dental metal materials on the growth and differentiation of UMR106 osteoblast cells in response to salt solutions of the metallic ions (Cu^{2+} and Ag^+) and to ions released from pure metals. The control UMR106 cells exhibited normal polygonal morphology with cytoplasmic processes connecting neighbouring cells. After 24 h in culture, cells exposed to Cu showed important morphological changes. After a longer incubation (48 h) the copper ions induced almost a complete cellular death of the culture. Exposition to silver for 48 h caused a lower reduction in the healthy cells in the culture. Osteoblasts were relatively small with lost of processes, although some mitotic figures and apoptotic bodies were still observed. The effect of Cu on the inhibition of cell growth was linearly time-dependent. Both cell surviving and the MI, significantly correlated with the Cu levels in the media, although this correlation was stronger when the number of surviving cells was analysed, in comparison with the mitotic index. It could be concluded that copper and silver ions are cytotoxic. In the case of copper ions a second order correlation between cytotoxicity and the concentration of the released metal ions was found.

INTRODUCTION

Copper and silver are widely used as components of dental alloys. Experiments made with noble dental alloys showed that gold, platinum, and palladium were not found in significant amounts within cellular cultures. However, among several alloys, those containing the highest amount of silver were defined as the least biocompatible considering both the cellular proliferation rates and the expression of an extracellular matrix protein.¹ Copper cations are also aggressive ions in the human body environment. Copper and zinc have been classified as the most cytotoxic elements in dental amalgams, followed by mercury and silver.²

Studies *in vitro* with osteoblast cell cultures can give very important and significant information to identify a metal as biocompatible, since they allow the evaluation of the behaviour of a homogeneous cell population.³

The aim of the present study was to study the biocompatibility of silver and copper on the growth and differentiation of UMR106 osteoblast cells in response to salt solutions and to ions released from pure metals (Cu^{2+} and Ag^+). The effect of copper and silver ions on specific osteoblastic properties was evaluated. The possibility of a correlation between the copper cation concentration and cytotoxicity was analysed.

MATERIALS AND METHODS

Experimental methods were described in detail previously.⁴ UMR106

Correspondence:

M. Fernández L. de Mele
Instituto de Investigaciones Físicoquímicas Teóricas y Aplicadas, Universidad Nacional de La Plata, C.C. 16, Suc. 4 (1900) La Plata, Argentina.
E mail: mmele@inifta.unlp.edu.ar

cells were cultured in Dulbecco's Modified Eagles Medium (DMEM) with 10 % fetal bovine serum (FBS). Cells were subcultured using trypsin-EDTA and plated into six well/plate or 10 mm dishes. When cells reached 70 % confluence, media was replaced by 0.5 % FBS-DMEM and incubated for 24 or 48 h in the absence or the presence of different dental metal materials.

0.942 cm² metal samples were immersed in sterile DMEM for different periods between 4 and 76 h to assay the metal ion release through atomic adsorption spectrophotometry.

The viability of osteoblast-like cells was determined by counting the number of cells pre-stained with Giemsa as it was previously described. A mitotic index (MI) was defined as the number of mitotic figures per field over the total number of cells per field.

Morphological changes induced by different metal alloys were observed by light microscopy after staining with Giemsa.

The activity of alkaline phosphatase (ALP) was evaluated as a specific marker of osteoblastic differentiation as we have previously described. The protein content was assayed by the method of Bradford.

Experiments were performed by triplicate for each experimental condition. Results were expressed as mean ± SEM. Statistical differences were analysed using Student's t test.

RESULTS AND DISCUSSION

Normal polygonal morphology with cytoplasmic processes connecting neighbouring cells were exhibited by the control UMR106 cells (Fig. 1a). Nuclei were well-stained, characteristically heterogeneous in size and showed several mitotic figures. After 24 h in culture, cells exposed to copper showed important morphological changes. Osteoblasts were absent in the vicinity of the wire. The number of cells increased progres-

sively at longer distance. Picnotic nuclei with distorted shape (Fig. 1b) and blebbing of the plasma membrane (Fig. 1c) were observed in the remaining osteoblasts. A longer incubation (48 h) copper ions induced almost a complete cellular death of the culture and signs of necrosis became more prevalent.

A slight reduction in the number of healthy cells in the culture was noticed after 48 h in silver containing culture media. The osteoblasts were relatively small with lost of processes.

In the presence of copper no mitotic figures were observed in the few cells that remain after 48 h. Cells exposed to Ag also show a small but not statistically significant inhibition of cell growth. However, the MI was significantly reduced ($p < 0.001$), suggesting an arrest of the proliferation induced by the silver on the osteoblastic culture.

The kinetic of ion-induced growth inhibition by culturing the osteoblasts with Cu- or Ag-wire for different incubation periods was also analysed. The release of metals was evaluated by atomic absorption spectroscopy of the culture media. The effect of Cu on the inhibition of cell growth was time-dependent (Fig. 2). This

effect was in parallel with the release of Cu from the dental material. Copper ions release increased linearly with time. Both, the number of dead cells and the MI, significantly correlated as a second order relationship with time (Fig. 2) and consequently, correlated with the copper ion concentration levels. However, this correlation was stronger when the number of surviving cells was analysed ($r = -0.936$, $p < 0.01$) in comparison with the MI ($r = -0.823$, $p < 0.05$).

The kinetic of Ag release into the culture media was also evaluated. Silver dissolution was lower (c.a. 0.001 mg/L) than that of copper. It was not significantly correlated with the number of dead cells.

CONCLUSIONS

Cu and Ag ions are cytotoxic. There is a second order correlation between cytotoxicity and copper ions release.

ACKNOWLEDGEMENTS

To CONICET, CIC, UNLP and ANPCyT for the financial support, and to R. Pérez for the spectrophotometric analysis.

BIBLIOGRAPHY

1. Grill V, Sandrucci M.A., Di Lenarda R., Cadenaro M., Narducci P, Bareggi R., Martelli A.M. Biocompatibility evaluation of dental metal alloys *in vitro*: Expression of extracellular matrix molecules and its relationship to cell proliferation rates. **J. Biomed. Mater. Res.**, 52, 479-87, 2000.
2. Cortizo M.C., Fernández Lorenzo de Mele M.A., Cortizo A.M. *In vitro* evaluation of biocompatibility of dental metal materials on osteoblast cells in culture. **Metal ions in Biology and Medicine**, 7, 149-153, 2002.
3. Kaga M., Seale N.S., Hanawa T, Ferracane J.L., Wite D.E., Okabe T. Cytotoxicity of amalgams alloys and their elements and phases. **Dental Materials**, 7, 68-72., 1991.
4. Cortizo A.M., Etcheverry S.B. Vanadium derivatives act as growth factor-mimetic compounds upon differentiation and proliferation of osteoblast-like UMR106 cells. **Mol. Cell Biochem.**, 145, 97-102, 1995.

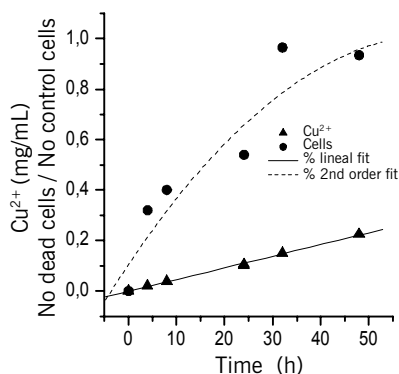


Fig. 2. Copper ions release (▲) and number of the dead cells/number of the control cells relationship as a function of time (●). Linear (—) and second order fit (---) of the data are also included respectively.

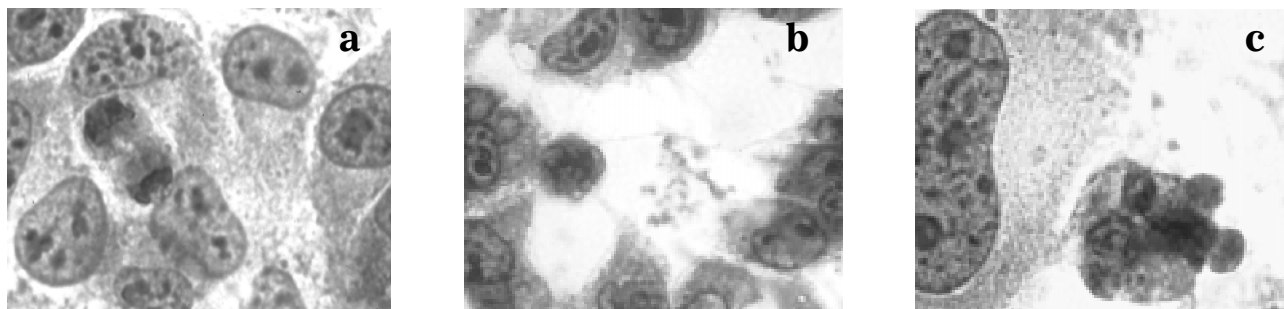


Fig. 1. UMR106 osteoblast-like cells incubated either with 0.5 % FBS-DMEM alone (control) (a), media plus the Cu-wire (b and c). Obj. x40 (a and b), x63 (c).