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Uptake and reversibility of uptake of nickel by human macrophages

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SUMMARY **The use of nickel-containing alloys in dentistry has been questioned because of the biological liabilities of nickel and the release of nickel ions from dental appliances into the oral cavity. The uptake of nickel by cells in the oral tissues is a critical factor in assessing the biological liabilities of nickel. Nickel uptake by macrophages may be particularly important because of the central role of macrophages in the inflammatory process and the known role of the macrophage in orchestrating the response to biomaterials. The aims of the current study were to assess the reversibility of the uptake of nickel from human macrophages and determine the portion of the nickel which reaches the nuclei as a function of time. Cellular nickel content was measured by means of atomic absorption spectrometry. Nuclear nickel content was assessed after fractionating cells. The results showed that nickel was rapidly taken up by macrophages and that the nickel accumulated in the nucleus in as little as 8 h. After 48 h, over 60% of the cellular nickel was in the nucleus. Once taken up, the nickel was lost at a lower rate. The rate of loss decreased as the initial exposure time to the nickel increased. Thus, the results indicated that macrophages may accumulate nickel if the time between exposures is insufficient to reverse the uptake. Further studies are necessary to correlate the retention of nickel with impaired function of macrophages and to further define the biological risks of using nickel in dental alloys.**

Introduction

Nickel-containing alloys have become common in dental applications. These alloys, which include the stainless steels, are used for crowns, partial denture frameworks, and orthodontic wires and appliances (Craig, 1993). The use of nickel-containing alloys has increased because of their low cost and excellent mechanical properties (Craig, 1993). The amount of nickel in these alloys ranges from 8% by weight in the stainless steels to 81% by weight in some partial denture and crown applications (Moffa, 1982).

The use of nickel-containing alloys in dentistry has been questioned because of the biological liabilities of nickel and the release of nickel ions from dental appliances into the oral cavity (Moffa, 1982; Mjör & Hensten-Pettersen, 1983; Black, 1984; Black, 1988). Nickel is a well-documented allergen (Moffa, 1982; Hildebrand, Veron & Martin, 1989; Goyer, 1986), carcinogen in the subsulphide form (Ni_3S_2) (Coogan *et al.*, 1989; Costa, 1989; Costa, 1991), and environmental toxin (Doll, 1984). The release of nickel ions from nickel-containing alloys in biomedical applications has been measured *in vitro* (Muller, Maessen & Davidson, 1990; Gerstorfer, Sauer & Pässler, 1991; Tai *et al.*, 1992) and *in vivo* (Black *et al.*, 1983; Woodman, Black & Nunamaker, 1983; Trainsnel *et al.*, 1990). The concentrations of nickel ions in tissues around implanted alloys has been measured as high as 123 μg/mL (normal ⩽ 2 μg/mL) (Bergman, Bergman & Söremark, 1980), but the release is dependent on pH and application (Muller *et al.*, 1990). *In vivo*, nickel

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concentrations have risen from 0·7–2·5 ng/mg of serum after implantation of a nickel-containing finger joint prosthesis for 12 months in cats (Woodman *et al.*, 1983). Since nickel is released into the body, the uptake and reversibility of uptake of nickel by cells is a central issue to the biological liability of such release. If nickel is taken up by cells, the risk of biological effects increases and if the uptake is irreversible, then the potential for accumulation to harmful levels in cells increases. Nickel uptake by macrophages may be particularly important because of the central role of macrophages in the inflammatory process (Auger & Ross, 1992) and the known role of the macrophage in orchestrating the response to biomaterials (Anderson & Miller, 1984). In dental applications, nickel-containing alloys are often in close proximity to the gingival tissues where macrophages are known to play an important role in the maintenance of the periodontal tissues (Page, 1991). The uptake of nickel by cells has been investigated for T cells, B cells, erythrocytes, macrophages and fibroblasts (Webb & Weinzierl, 1972; Nieboer *et al.*, 1984; Nordlind, 1985; Menon & Nieboer, 1986). Organic ligands limited or enhanced the uptake of Ni ions by macrophages or lymphocytes and accelerated or prevented the removal of Ni from these cells once taken up (Nieboer *et al.*, 1984; Menon & Nieboer, 1986). Compounds which form lipophilic complexes appeared to favour cell uptake and retention. However, T cells from individuals who were hypersensitive to nickel were no more prone to nickel uptake than those from unsensitized individuals (Nordlind, 1985; Menon & Nieboer, 1986). In mouse fibroblasts, the rate of nickel uptake was 0·043 femtomoles/cell per hour, which was low among eight metal ions which were tested (range was 0·012 for Pd^{2+} to $> 8{\cdot}0$ for In^{3+}) (Wataha, Hanks & Craig, 1993). Additionally, the nickel which was taken up by fibroblasts was not reversible at the same rate. Sixty per cent of the Ni ions taken up after 2 h remained in the cell 5 h after the nickel ions were removed from the medium. When the initial exposure time was increased to 6 h, 72% of the Ni remained after 5 h of reversal time (Wataha *et al.*, 1993). Thus, in fibroblasts the uptake rate of nickel was greater than the release rate, and the release rate slowed as the time of initial exposure increased.

The intracellular distribution of nickel into cells has also been investigated to some extent. In fibroblasts, $^{63}Ni^{2+}$ was distributed among all subcellular fractions, but primarily in the nuclei (Webb & Weinzierl, 1972). Nuclear accumulation of nickel has also been reported for human blood lymphocytes and rabbit alveolar macrophages (Nordlind, 1985; Nieboer *et al.*, 1984). The presence of nickel in the nuclei appears to have functional importance as well, as it has been shown to alter secretion of cytokines from macrophages (Wataha, Hanks & Sun, 1995; Wataha *et al.*, 1997) and DNA synthesis of lymphocytes (Nieboer *et al.*, 1984).

The hypothesis of the present study was that the time of exposure of nickel to macrophages is important in the reversibility of nickel uptake and the distribution into the nucleus. Since dental alloys may release nickel ions for short bursts following initial insertion or disruption of passivating surfaces, the factor of time is critical to the biological liability of these alloys. The specific aims of the study were to establish (i) the reversibility of nickel uptake by macrophages and (ii) distribution of nickel into nuclei, both as a function of time. This information will help define the risks of using nickel-containing dental alloys and other biomedical alloys containing nickel.

Materials and methods

The cells used were human THP-1 monocytes-macrophages*. They were grown in suspension in RPMI 1640† with 10% fetal bovine serum†, β-mercaptoethanol‡ (50 μmoL/l), penicillin† (100 units/mL), and streptomycin† (100 1 g/mL). Cells were plated at 45 000 cells/mL and allowed to grow for 7 days to approximately 700 000 cells/mL in 15 mL before exposure to nickel ions. Population doubling time of the cells was measured to be 43 h.

Nickel ions from $NiCl_2{\cdot}6H_20$§ were added to the cells from an aqueous solution. Fifty microlitres were added to each flask containing 15 mL of cell suspension to give a final nickel concentration of 10 μg/mL (170 μmol/L). This concentration was selected on the basis of pilot experiments which showed that the cells would not be lysed by this concentration after 48 h exposure. Water was added to the control cultures. In initial experiments, the exposure times ranged from 8 to 48 h, but exposures greater than 8 h made the cells too fragile to process for Ni content. Therefore, the

*ATCC #TIB 202; Rockville, MD, U.S.A.
†Gibco BRL, Gaithersburg, MD, U.S.A.
‡Sigma, St. Louis, MO, U.S.A.
§Aldrich, Milwaukee, WI, U.S.A

exposure times were shortened to 2–8 h. The cells were counted in a haemocytometer before and after addition of the nickel. After nickel exposure, the cells were washed five times with 5 mL of phosphate-buffered saline. Centrifugation at 600 × g for 10 min was used to isolate the cells between washes. The cells were counted again after the last wash to ensure that cells were not lost during the washing procedure. Water (1·5 mL) was added to the last cell pellet, and the resulting suspension was treated ultrasonically for 30 s, then assayed for nickel content by means of atomic absorption spectroscopy (Wataha, Craig & Hanks, 1991). To measure reversibility of the nickel uptake, nickel was removed from the cultures after the initial exposure and fresh medium without nickel was added for 4–48 h, after which the cells were processed for nickel content as previously described. Nickel content was calculated as pg/cell based on cell counts and the atomic absorption measurements. The detection limit for nickel was 0·0015 pg of nickel per cell. The solutions used to process the cells such as medium and saline had no detectable nickel (detection limit was 0·020 μg/mL).

To determine the distribution of nickel into the cell nuclei, the cells were centrifuged, the medium was removed, and 0·1% NP40 in phosphate-buffered saline‡ was added for 5 min to isolate the nuclei. The nuclei preparation was verified by microscopy, the ability of the preparation to take up propidium iodide (assessed by fluorescence microscopy), and the absence of mitochondria (lack of MTT staining). Nuclei were washed with phosphate-buffered saline and isolated by centrifugation at 600 × g for 10 min, after which 1·5 mL of water was added to the pellet and ultrasonic treatment was used to disperse the pellet. The nickel content was measured and the nickel content per nucleus was calculated as previously described. The nuclear nickel content was compared to parallel preparations of whole cells, and the percentage of nickel in the nuclei was calculated.

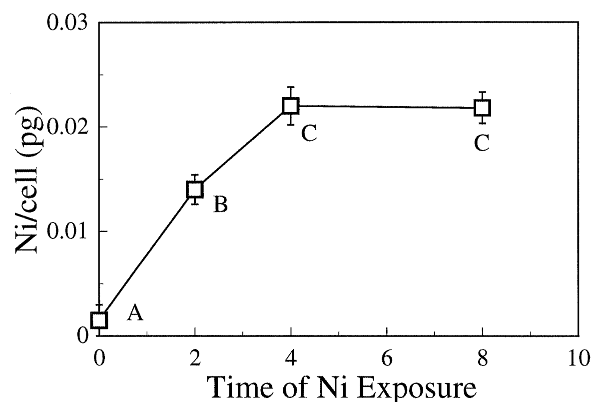

Fig. 1. Macrophages were exposed to nickel ions for 2, 4, or 8 h after which unbound nickel was washed away and retained nickel measured. Detection limit for the assay was 0·0015 pg/cell. Controls were 0 h readings without the addition of nickel to the cultures. Error bars indicate standard deviations of $n = 3$. Different capital letters indicate statistical differences (ANOVA, Scheffe comparison intervals, $\alpha = 0{\cdot}05$).

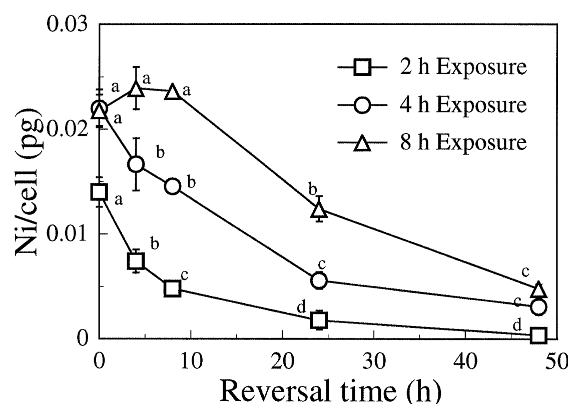

Fig. 2. Macrophages were exposed to nickel ions for 2, 4, or 8 h. After washing, the cells were incubated without nickel for 8–48 h, after which nickel which was retained in the cells was measured. Results are expressed in pg of nickel per cell. The detection limit for the assay was 0·0015 pg/cell; error bars indicate standard deviations for $n = 3$. Control cells did not contain detectable amounts of nickel. For each line, different letters indicate different statistical groups (ANOVA, Scheffe comparison intervals, $\alpha = 0{\cdot}05$).

Results

Nickel ions were rapidly taken up into the macrophages over the first 4 h of exposure, after which the uptake levelled off at about 0·023 pg/cell (Fig. 1). The rate of reversal of nickel uptake depended on the initial exposure time (Fig. 2). After 2 h initial exposure, the cellular nickel dropped significantly within 4 h of reversal time, and was essentially complete after 24 h. After 48 h of reversal time, approximately 3% of the original nickel was still present in the cells. When the initial exposure time was increased to 4 h, a similar pattern of reversal occurred, but at 48 h, 14% of the original nickel in the cells remained. When the initial exposure was 8 h, the pattern of loss changed significantly. No nickel was lost from the cells for at least 8 h, after which a gradual loss occurred. However, at 48 h, 22% of the original nickel remained per cell. The loss of nickel from the cells was complicated by the

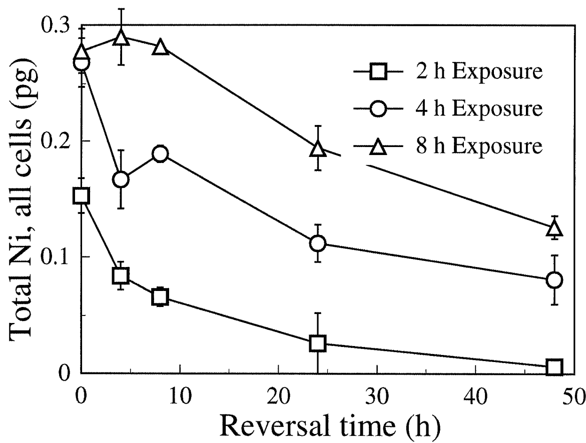

Fig. 3. Results are similar to those in Fig. 2, but are expressed as total nickel retained by the culture as a function of reversal time. This figure accounts for the increase in cell number during the reversal time. The detection limit for the assay was 0·0015 pg/cell; error bars indicate standard deviations for $n = 3$. Control cells did not contain detectable amounts of nickel. Statistical groups were identical to those in Fig. 2.

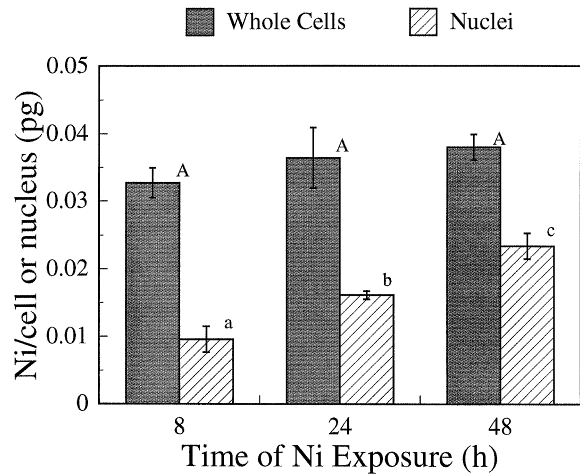

Fig. 4. Macrophages were exposed to nickel ions for 8, 24, and 48 h. Cells were then separated into two groups. One group was analysed for whole cell nickel content, and one for nickel content in the nuclei. The detection limit for the assay was 0·0015 pg/cell; error bars indicate standard deviations for $n = 3$. Control cells did not contain detectable amounts of nickel. For each set of columns, different letters indicate different statistical groups (ANOVA, Scheffe comparison intervals, $\alpha = 0{\cdot}05$).

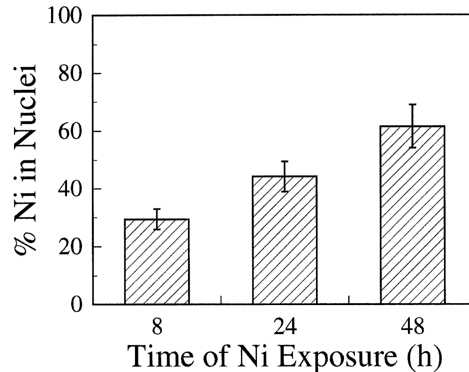

Fig. 5. The percentage of total cellular nickel retained by the nucleus as a function of exposure time. All groups were statistically different from one another (ANOVA, Scheffe comparison intervals, $\alpha = 0{\cdot}05$).

increased number of cells from growth during the period of reversal. Cell division during the reversal time tended to 'dilute' the amount of Ni per cell. If the increase in cells during the reversal time was accounted for, then the actual loss of nickel at 48 h was 96% after 2 h initial exposure, 70% after 4 h, and 55% after 8 h (Fig. 3). Thus, the actual reversibility of the nickel uptake was less than indicated in Fig. 2. The pattern of nickel loss was similar in Figs 2 and 3, but the nickel remaining in the cells after 48 h was different because of the change in total cell number during the period of reversal.

The amount of nickel which reached the nuclei of the cells increased as the time of exposure increased from 8 to 48 h (Fig. 4). After 48 h, 60% of the nickel in the whole cells were associated with the nuclei (Fig. 5). The increase in nuclear nickel content was significantly greater at each time of exposure (ANOVA).

Discussion

The current study confirmed the intercellular penetration of nickel ions. This result agrees with those of previous investigators who have studied the subcellular distribution of nickel (Webb & Weinzierl, 1972; Nieboer *et al.*, 1984; Menon & Nieboer, 1986). The current study also showed that the movement from the extracellular spaces to the nuclear compartment was rapid. After 8 h, 25% of the cellular nickel was in the nucleus (Figs 4, 5). Thus, although the mechanism of uptake of nickel was not determined, any uptake mechanisms must account for this rapid uptake. At 48 h, the nuclei contained more than 60% of the nickel. Therefore, it appeared from the current study that there was a tendency for nickel ions to accumulate in the nucleus of these macrophages with time. After 48 h, the concentration of nickel in the nuclei of macrophages was substantially higher than that seen by Webb & Weinzierl (1972) in fibroblasts (36%). This difference may reflect differences in passive diffusion of nickel

through the different cellular architectures, or may indicate differences in active transport of nickel.

Increasing the initial time of nickel exposure significantly slowed the loss of nickel from the cells (Figs 2, 3). Even when the initial uptake of nickel was equivalent at two exposure times, the longer exposure time resulted in a prolonged retention of nickel by the cells (Fig. 2). Thus, the longer retention was not simply a function of the increased nickel per cell with time, but must have been also a function of the distribution and binding of nickel. The observation that increased exposure times led to increased nuclear concentration (Fig. 4) supports this idea. It is likely that all binding sites for nickel are not of equal affinity, and it may be that over the time of exposure, the nickel tends to accumulate in the higher affinity binding sites. If this idea is true, then the nucleus would appear to be a higher affinity binding site since nickel accumulated there with time (Figs 4 and 5). Further studies should examine the reversibility of binding from subcellular fractions as well as whole cells.

The relationship of the external concentration of nickel to the rate of its uptake is critical to the *in vivo* risks that nickel poses. If high external concentrations lead to higher uptake rates, then cellular toxicity may develop more quickly because of the higher intracellular concentrations, and reversibility may be secondary because the cells die. The current study did not address the effect of the external concentration of nickel on its distribution and reversibility of uptake. However, previous studies with fibroblasts have shown that the rate of nickel uptake increases linearly with increases in external concentration (Wataha *et al.*, 1993). The fibroblasts are probably not representative of the macrophages used in the current study since the fibroblasts did not saturate with nickel over the 8 h uptake period (Wataha, Hanks & Craig, 1994). Macrophages used in the current study showed cellular saturation after only 4 h (Fig. 1).

The rate of nickel uptake by the macrophages was substantially lower than that seen with fibroblasts in other studies (Webb & Weinzierl, 1972; Wataha *et al.*, 1993). Previous work with Balb/c mouse fibroblasts (Wataha *et al.*, 1993) showed that Ni reached about 0·32 pg/cell after only 6 h whereas the macrophages in the current study reached just over 0·02 pg/cell after 8 h. Some of this difference may reflect the smaller size of the macrophages. The pattern of uptake in macrophages was also different from fibroblasts, which showed linear uptake with time for at least 8 h (Wataha *et al.*, 1993) and up to 24 h (Webb & Weinzierl, 1972) before saturation occurred. Differences in the uptake between cell types indicates that different mechanisms of uptake may occur for different types of cells. Some of these differences may be attributed to the anchorage dependence of the fibroblasts versus the anchorage independence of the macrophages, although no conclusions can be drawn from the current results. In both cell types, the rate of loss of nickel from cells decreased as the time of initial exposure increased.

To summarize, this study has shown that the time of initial exposure is a critical factor in the reversibility of the uptake of nickel into macrophages and the accumulation of nickel in the nucleus. As the time of nickel exposure increases, the loss of nickel from the cell slows and a greater percentage of nickel is present in the nucleus. The results imply that nickel which is released from dental alloys may accumulate in cells such as macrophages if the exposure of cells to Ni is of sufficient concentration and frequency. Based on previous reports (Bergman *et al.*, 1980; Covington *et al.*, 1985; Gerstorfer *et al.*, 1991) dental alloys are capable of releasing quantities of nickel similar to those used in the current study (10 μg/mL) especially under conditions such as low pH (4·0). Accumulation of nickel may lead to altered cell function in tissues in the oral cavity (Wataha *et al.*, 1994). Further study is necessary to define the risk of using nickel-containing alloys in biomedical applications.

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