TOXIC EFFECT OF NICKEL IN AN *IN VITRO* MODEL OF HUMAN ORAL EPITHELIUM.

Cimino F., 2° anno del Dottorato di Ricerca in Tossicologia XVI ciclo. Durata del Dottorato in anni: 3. Sede di servizio: Dipartimento Farmaco-Biologico, Università di Messina

The physiological environment of the oral cavity stimulates the release of compounds from dental restorative materials which are placed into contact with oral tissue for many years. Thus systemic and local toxicity, allergy and carcinogenicity all might result from elements in the alloy being released into the mouth during corrosion. The occurrence of local toxic effects (adjacent to the alloy) is not well documented, but is a higher risk than systemic toxicity, because local tissues are exposed to much higher concentrations of released metal ions. Nickel represents a good example of a metal widely employed in dental alloys. However, nickel has a number of adverse biological effects that have made its use in biomedical implants controversial. In fact, among the known health related effects of nickel are skin allergies and stimulation of neoplastic transformation. Although not well understood, mechanisms involved in nickel toxicity include overproduction of reactive oxygen species and effects on cellular antioxidant systems (Stohs S.J. & Bagchi D., Free Radic. Biol. Med., 18, 321, 1995).

In the present study, by means of *in vitro* experiments, we have investigated the toxic effects induced by the exposure to low concentrations of nickel on human oral epithelium. At this aim a commercially available and well-characterized tissue culture model of human oral epithelium was employed (Schmalz G. et al., Eur. J. Oral Sci., 108, 442, 2000).

Samples of human reconstituted epithelium were purchased from SkinEthic Laboratories (Nice, France); this three-dimensional tissue culture model consists of TR146 cells grown on polycarbonate filters. Individual tissue cultures (0.50 cm^2) were placed into disks containing 1 ml of maintenance medium (MCDB 153 containing 5 µg/ml insulin, 1.5 mM calcium chloride and 25 µg/ml gentamycin). Serial dilutions of $NiCl_2$ (ranging from 7.6 to 0.05 mM) were prepared in the maintenance medium. At the beginning of the experiment, 100 µl of the solutions were applied topically onto the surface of the reconstituted epithelia. After incubation for 72 h in a humidified atmosphere at 37°C and 5% CO_2, the cultured tissues were rinsed with phosphate buffer saline. The cell survival in Ni-treated tissue cultures was measured by the quantification of mitochondrial dehydrogenase activities (MTT assay) and expressed as a percentage of the untreated control. The concentrations of PGE_2, IL-6, IL-8 and LDH in the incubation medium and the tissue amounts of GSH and GSSG were measured by commercially available kits. Finally, tissue

cultures were histologically examined by the hematoxylin/eosin staining and the TUNEL method .

Our findings have shown that exposure to 7.6-1.3 mM $NiCl_2$ significantly reduced cell viability in comparison with untreated cell cultures (tab. 1); at these concentrations large amounts of PGE_2 and IL-6 were released from tissue cultures in the incubation medium (tab. 1), although LDH and IL-8 concentrations appeared similar to those calculated in controls (data not shown). As shown in tab. 1, no statistically significant change in cell survival and in IL-6 and PGE_2 release was observed at $NiCl_2$ concentrations $\leq$ 1 mM .

As to tissue GSH/GSSG equilibrium, although, at all concentrations tested, $NiCl_2$ produced only a slight decrease in tissue GSH concentrations, a marked increase in tissue GSSG content was observed at metal concentrations low as 0.1 mM; in fact the GSH/GSSG ratio appeared similar to that calculated in controls only at 0.05 mM $NiCl_2$. This demonstrates that topically applied nickel can induce alterations in cellular redox state of oral epithelium also at concentrations which do not cause evident decrease in cell viability and in the release of proinflammatory mediators.

Finally, epithelium samples have been histologically examined. In comparison with control samples, we observed a higher rate of vacuolized cells (without evidence of cellular necrosis) and of apoptotic cells in $NiCl_2$ exposed tissue, at metal concentrations low as 0.7 and 0.3 mM (data not shown). Recent studies have implicated alterations in cellular redox state, production of reactive oxygen species and resulting oxidative modifications of proteins in normal physiological signaling; production and accumulation of apoptotic cells is one of characteristic features of tissue damage by oxidative stress that, in the absence of effective scavenging by macrophages, dramatically enhances oxidative damage and inflammatory response. While oxidative stress has been implicated as an initiator of apoptosis in at least some of these conditions, generation of reactive oxygen species and subsequent oxidative stress has also been demonstrated to be a component of a final common pathway of apoptotic execution via mitochondrial dysfunction (Kagan V.E. et al., Ann. NY Acad. Sci., 959, 188, 2002). Also if this hypothesis is merely speculative at the present time, one could suggest that, under non-toxic experimental conditions, nickel can affect cellular redox state and, thus, modify some cellular effectors involving in apoptosis pathway.

In conclusion, the present findings demonstrate that apparently non-toxic nickel concentrations can affect oral epithelium cells. Further studies are needed to investigate the biocompatibility of nickel-containing dental alloys.

Tab. 1 Results obtained in samples of reconstituted human oral epithelium treated with different concentrations of $NiCl_2$. Data are expressed as mean $\pm$ SD of six samples

Ni [mM]	**Cell survival** (%)	**PGE_2** (pg/ml)	**IL-6** (pg/ml)	**GSH** (nmol cm^{-2})	**GSSG** (nmol cm^{-2})	**GSH/GSSG**
Control	100.00	50.2 ± 5.6	4.24 ± 2.2	2.392 ± 0.25	0.874 ± 0.07	2.73 ± 0.21
7.6	33.21 ± 3.5	94.00± 4.3	31.00 ± 2.7	2.151 ± 0.18	1.430 ± 0.11	1.50 ± 0.17
3.3	76.60 ± 3.4	73.20 ± 8.7	14.07 ± 2.3	2.242 ± 0.23	1.364 ± 0.14	1.64 ± 0.15
1.3	90.2 ± 2.5	41.68 ± 10.4	4.08 ± 2.0	2.342 ± 0.26	1.230 ± 0.09	1.90 ± 0.22
1.0	94.21 ± 1.8	36.00 ± 12.7	4.998 ± 2.2	2.353 ± 0.15	1.151 ± 0.12	2.04 ± 0.18
0.7	97.80 ± 2.4	32.60 ± 16.65	5.75 ± 2.4	2.325 ± 0.12	1.078 ± 0.10	2.15 ± 0.23
0.3	98.50 ± 3.0	58.30 ± 8.5	9.27 ± 3.5	2.306 ± 0.21	1.052 ± 0.15	2.19 ± 0.19
0.1	99.00 ± 4.1	52.50 ± 5.7	12.61 ± 4.5	2.318 ± 0.23	1.002 ± 0.08	2.31 ± 0.22
0.05	100.00 ± 3.5	55.50 ± 6.5	5.32 ± 2.7	2.325 ± 0.24	0.896 ± 0.09	2.48 ± 0.25