Incidence of Micronuclei and Abnormalities in the Head of Spermatozoa Caused by the Salts of a Heavy Metal, Nickel

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Micronuclei, the cytoplasmic chromatin masses that look like very small nuclei, arise from chromosomes lagging at anaphase or from acentric chromosomal fragments resulting from mitotic/meiotic disturbances. Although they were known for many years (Brenneke 1937, Mather 1937, Thoday 1951, Russel and Russel 1954, Evans *et al.* 1959), their real importance was recognized in the late 1960s and early 1970s when Heddle (1973) developed the bone-marrow micronucleus test. Today this is widely used as a screening system for the detection of compounds that cause chromosome mutations. Wyrobek and Bruce (1975) also indicated that the effect of environmental mutagens can also be assessed by determining the frequency of abnormalities in the head of spermatozoa. Although the exact genetic significance of this test is not known, it is being frequently used in the various screening programmes. During the present work we have used both of these parameters to determine the effect of the various salts of nickel on mice, because it is a suspect carcinogen (Doll *et al.* 1970, Sunderman 1978, 1984, Chovil *et al.* 1981) and the available data on its mutagenic potential are quite contradictory.

Material and methods

Mice of the lacca strain were purchased from the Central Research Institute, Kasauli (India) and were bred at $25\pm 2^{\circ}\text{C}$ in a specially equipped room. The stock solutions of the nickel compounds viz., NiCl_2 (CAS No. 7718–54–9), NiSO_4 (CAS No. 7786–81–4) and NiNO_3 (CAS No. 131–38–49) were made in distilled water and administered orally. A parallel control was kept in each case. For the detection of micronuclei, the animals were treated for 6 and 30 hours with the chemicals and smears of the bone-marrow were made in 1:1 phosphate buffer and fetal calf serum (Robert and Bernard 1982). The air dried slides were stained in May-Grunwald's Giemsa stain (Schmid 1973, 1975). The micronuclei were studied in young polychromatic erythrocytes (PCEs).

The smears of spermatozoa from the epididymis were made after five weeks of the last exposure (Wyrobek and Bruce 1975). These were stained in Giemsa. The abnormalities in the heads of spermatozoa were detected under the oil immersion lens of a Carl Zeiss photomicroscope.

Results

The data on the frequency of micronuclei and sperm head abnormalities are presented in Table 1. It is evident from this that all the three compounds induced a statistically significant number of micronuclei. In a few PCEs, two micronuclei were also seen (Figs. 1A–I). The effect in all the cases was dose related. Similarly the frequency of abnormalities induced in the head of the spermatozoa by these chemicals was also significant being 22.66 ± 2.179 , 25.66 ± 0.982 and 32.33 ± 1.870 with NiCl_2 , NiSO_4 and NiNO_3 respectively as against 8.66 ± 0.541

Table 1. Frequency of the micronuclei and abnormalities in the head of the spermatozoa induced by the various compounds of nickel

Assay	Chemical	Dose	Route	Time of sampling	Mean $\pm$ S.E.
Micronuclei	Control	H ₂ O	Oral	6 hours	1.33 $\pm$ 0.272
	NiNO ₃	72.2 mg/kg	"	"	2.66 $\pm$ 0.720*
	NiCl ₂	95 mg/kg	"	"	3.80 $\pm$ 0.815*
	NiSO ₄	73 mg/kg	"	"	2.33 $\pm$ 0.272*
	Control	H ₂ O	"	30 hours	1.66 $\pm$ 0.272
	NiNO ₃	72.2 mg/kg	"	"	4.66 $\pm$ 0.720*
	NiCl ₂	95 mg/kg	"	"	5.00 $\pm$ 0.948*
	NiSO ₄	73 mg/kg	"	"	4.33 $\pm$ 0.272*
Sperm head abnormalities	Control	H ₂ O	"	5 weeks	8.66 $\pm$ 0.547
	NiNO ₃	72.2 mg/kg	"	"	32.33 $\pm$ 1.870**
	NiCl ₂	95 mg/kg	"	"	22.66 $\pm$ 2.179**
	NiSO ₄	73 mg/kg	"	"	25.66 $\pm$ 0.982**

* p<0.05

** p<0.01

Fig. 1. A-I, polychromatic erythrocytes showing the presence of micronuclei.

in the controls. The different types of the abnormal spermatozoa were of Daphnia, polyp, amorphous, giant amorphous and anvil-shape (Figs. 2A-I).

Discussion

The nickel compounds had failed to cause any mutations in *S. typhimurium* and *E. coli* (Sunderman 1981). However, in *Coryne bacterium*, Pikalec and Nacasek (1983) had observed

Fig. 2. A-I, various sperm head abnormalities caused by Nickel sulphate.

mutations. Nickel chloride caused mutations in cultured V-79 Chinese hamster cells resistant to 8-azoguanine (Miyaki *et al.* 1979). Treatment of nickel and other carcinogenic metal cations to the nucleotide bases, renders the DNA susceptible to strand scission or to elimination of bases by an unknown nuclease, resulting in mutations in the repair process. Hsie *et al.* (1979) used 6-thioguanine as a target and found nickel to be mutagenic although caution was indicated by them. In cells exposed to various concentrations of NiCl_2 for 3 hours, a dose dependent increase in the absolute number of mutants was found at the thymidine kinase

locus 51784 in mouse lymphoma cells. Nishimura and Umeda (1979) had reported that certain nickel salts were incorporated in the cells. *In vitro* exposure of the human lymphocytes to NiSO_4 , at a concentration as low as 2×10^{-6} M/litre, caused a significant increase in the SCEs (Wulf 1980). It caused chromosomal damage and increased SCE frequency in the Syrian hamster embryo cells and human lymphocytes in cultures (Larramendy *et al.* 1981). They had also noticed its inhibitory effect on the synthesis of proteins, DNA and RNA. Waksvik and Boysen (1982) and Waksvik *et al.* (1984) had observed an increase in the incidence of chromosomal aberrations in the peripheral lymphocytes of retired nickel workers. Similarly Saxholm *et al.* (1981) had recorded an elevated SCE frequency in the human lymphocytes exposed *in vitro* to crystalline Ni_3S_2 . Newman *et al.* (1982) have also reported an increased frequency of SCEs in the human lymphocytes, exposed to NiCl_2 in a range from 10^{-4} M to 10^{-6} M. Andersen (1983) accepted the nickel related SCE causation in P 388 chromosomes and human lymphocytes. Sen and Costa (1985) had studied the chromosomal damage in the Chinese hamster ovarian cells by soluble and particulate nickel compounds and noticed preferential fragmentation of the heterochromatic long arm of the X-chromosome by an increase in the aberration frequency due to nickel salts.

In the present studies, the clastogenic nature of the nickel compounds has been seen. The observations are in conformity with those of Sharma *et al.* (1987) in mice and mosquitoes. The high frequency of the abnormal spermatozoa observed also shows their effect on the gonial cells. As regards micronuclei, all the compounds showed dose related effect. The highest clastogenicity was seen with NiCl_2 followed by NiNO_3 . The values were 3.80 and 5.00 (after 6 and 30 hours respectively), whereas with these were 2.66 and 4.66. Statistically speaking, there appears to be not much difference in the value of real genomic loss caused by NiCl_2 and NiNO_3 . In any case, present observations and our earlier ones (Sharma *et al.* 1987) agree with most of the authors (Wulf 1980, Larramendy *et al.* 1981, Waksvik and Boysen 1982, Anderson 1983, Waksvik *et al.* 1984) that nickel salts do have mutagenic potential, though of variable degree.

Summary

All the three salts of nickel viz. nickel chloride, nickel nitrate and nickel sulfate induced micronuclei in the polychromatic erythrocytes of mice. These also caused various types of abnormalities in the head of spermatozoa. The frequency of spermatozoa with abnormal heads was maximum with nickel nitrate.

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