Comparative Effects of Chronic Treatment with Certain Metals on Cell Division

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Received March 4, 1983

Metals and their salts have been known to exert toxic effects at different levels on the tissue of higher organisms (Venugopal and Luckey 1978). Their chemical activity on biological macromolecules has been studied intensively (Siegel 1980), particularly with reference to carcinogenesis (Flessel et al. 1980, Leonard 1981). A number of variable parameters is known to influence the effects, including the test systems used (Sugimura et al. 1981, Hsu 1982). The present investigation was planned to compare the relative effects of certain representative inorganic metallic salts from different groups of the periodic table on cell and chromosome divisions in multiple test systems. The two parameters employed in measuring the relative clastogenic action of the chemicals were alterations in the mitotic index and changes in chromosome structure and behaviour during mitosis.

Materials and methods

The principal test systems used were: Allium cepa and Rattus norvegicus, in vivo. Standard Allium test was performed using different concentrations (0.00001 to 0.5%) of aqueous solutions of 18 metallic salts from groups I to VIII, namely:

Group I: Sodium selenite (a) molybdate (b) arsenate (c) rubidium chloride (d) copper sulphate (e)

Group II: Magnesium sulphate (f) zinc chloride (g) strontium nitrate (h) cadmium chloride (i) mercuric chloride (j)

Group III: Ceric sulphate (k) cerium nitrate (l)

Group IV: Lead acetate (m)

Group V: Vanadium pentoxide (n)

Group VI: Magnesium sulphate (o)

Group VIII: Cobalt chloride (p) cobalt sulphate (q) nickel sulphate (r)

Roots of Allium cepa bulbs were immersed in solutions for different periods ranging from 2 to 24 hours. After each treatment, the bulbs were allowed to recover in Knop's nutrient medium. At each interval for both direct and recovery experiments, the root tips were excised, fixed in acetic acid: ethyl alcohol (1:3) and squashed following the usual 2% acetic-orcein: N HCl (9:1) squash technique. The mitotic index and percentage of abnormalities induced were obtained from scanning 2000 cells and compared with controls maintained in distilled water.
In animal experiments, male albino rats were force fed with aqueous solutions of the different salts at the rate of 1 to 30 mg/kg of body weight for 7 to 21 days amounting to approximately one fourth of the LD$_{50}$ for a particular compound to the minimum concentrations tolerated without any change in mitotic index. Bone marrow chromosomes preparations were obtained by the usual hypotonic-flame drying-Giemsa technique at specified intervals and scanned for abnormalities. DNA was estimated by diphenylamine reagent (Burton 1956) and RNA by orcinol reagent (Fleck and Munro 1962) from different tissues like lung, kidney, brain and liver.

Fig. 1. Different chromosomal aberrations induced by metallic salts in root-tips of *Allium cepa*. 
In all experiments, the data were subjected to statistical analysis in order to determine the significance of the observations.

Results and discussion

A consolidated account of the data shows that differential activity of the different metallic salts in decreasing the mitotic index in plants within different groups is $b > a = c > d > e$ in group I and $i > j > f = g = h$ in group II. The mitotic index is significantly altered from the control by salts of groups IV and VII, while the difference is negligible with salts of groups II and VIII.

In animals the mitotic index was inhibited by the metals in the different groups as $b > a = c > d$ in group I; $k > j$ in group III and $p > q = r$ in group VIII. Salts of group V altered the index significantly from the control.

Chromosomal abnormalities, as induced by different salts showed the following results: In plants the relative effects were $c > a > b > d = e$ in group I; $j > i > f = g = h$ in group II and $p > q > r$ in group VIII. The number of abnormalities was significantly higher than the control with metals of groups IV and VII but not signi-
significant with group III.

In animals the action in increasing abnormalities was \( a > c > b > d \) in group I; \( l > k \) in group III and \( r > p = q \) in group VIII, while the overall increase was significant with group V.

The chromosomal abnormalities induced both in plants and animals involved (i) either alterations in the chromosomal structure such as fragmentation, inversion,
translocation etc., or (ii) spindle disturbances such as non-disjunction, diplochromatid formation, stickiness, polyploidy, C-mitosis etc. (Figs. 1, 2). Frequently the two types of abnormalities overlapped and it was difficult to associate a specific

Diagram 3, 4. 3, relative effects of the metals of group-V on DNA and RNA (µg/100 mg wet tissue) contents in different organs of rat. 4, relative effects of the metals of group-VIII on DNA and RNA (µg/100 mg wet tissue) contents in different organs of rat.

type with a particular chemical (Singh and Sharma 1980, 1981, 1982 and De and Sharma 1981). In general, at lower concentrations, the effects were directly proportional to the period of treatment. Higher concentrations, on the other hand, led to gradual toxicity and after a particular dose proved to be lethal (Giri et
al. 1978, 1979, 1980, 1981, Gajra et al. 1982). As expected, the plants showed a much greater resistance and could recover even after chronic treatments for relatively longer periods than the animals.

Estimation of the total DNA and RNA contents from different organs of treated animals did not show any significant difference within or between groups (Diagrams 1 to 4). In general, DNA and RNA contents decreased in most cases, often to a significant (at 5%) level (Sanyal et al. 1980). In a few cases, however, the results were variable. Such variability was relatively low as compared to the total amount of data accumulated and may be attributed to physiological conditions rather than to specific action of any particular metal. An overall assessment of the data collected shows that the effects of metals on the mitotic index and chromosome division are more general than specifically on any particular cell component. Chronic administration of any of the salts, even in very low dosages, leads to a gradual disruption of cellular activity, culminating in chromosomal aberrations and fall in mitotic index. If not continued beyond a particular threshold period the effects are reversible. But gradual accumulation of the metals ultimately leads to toxicity and lethality to the organisms. The inhibitory action on cell division usually increases with increasing molecular weight within the members of a particular group of the periodic table (Sharma 1983, 1984). A similar tendency has been exhibited in toxicology studies (see Siegel 1980).

Summary

A total of 18 metallic salts belonging to seven groups of the periodic table have been applied in sublethal chronic doses on plant and animal cells in vivo for their comparative action on cell division.

Usually within the members of a particular group inhibition of mitosis and induction of chromosomal abnormalities increased with increasing molecular weight. There was a general direct proportionality with dosage applied and the cytotoxic action, though occasionally there was an increase in very low doses. These effects were more general in nature and could not be specifically attributed to any particular cell component. The amounts of total DNA and RNA showed a general decrease with increased dosage and period of treatment.

Acknowledgements

The authors are grateful to Prof. A. K. Sharma, Programme Co-ordinator, Centre for Advanced Study in Cell and Chromosome Research, Department of Botany, University of Calcutta, for facilities provided. Part of this work was carried out in a project sanctioned by the Man and Biosphere Committee under the Department of Environment, Government of India.

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