EFFECTS OF CERTAIN TRANQUILLIZERS ON SOMATIC CHROMOSOMES OF HIGHER LIVING ORGANISMS

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ABSTRACT

Four common tranquillizers containing diazepam or nitrazepam as active ingredients induced chromosomal abnormalities in dividing cells and alterations in mitotic index, when applied in high concentration, to plant and animal systems *in vivo*.

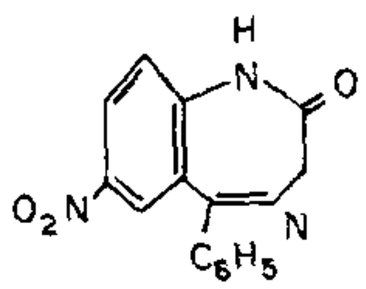
INTRODUCTION

TRANQUILLIZERS are used very commonly in combating human ailments, being available in a variety of commercial forms, either singly or in combination with other compounds. Lately, interest in the action of drugs on genetic systems has been increasing partly due to the fact that certain hallucinogenic compounds have been shown to exert harmful effects at the levels of cell and chromosome division *e.g.* mescaline and LSD in mammalian test systems leading to chromosomal alterations and changes in the mitotic frequency[1,2]. The effects of tranquillizers on metabolic pathways have been investigated by several workers, the test systems principally being mice and rats[3–5]. The *in vivo* bone marrow chromosomal study has been recommended as an ideal protocol among the cytogenetic assays to detect potential mutagenic effects of environmental agents[6,7].

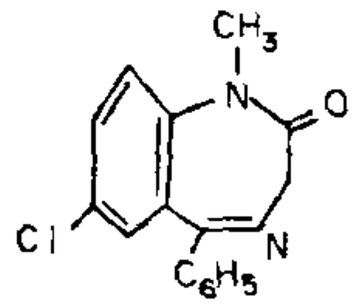
Benzodiazepines are among the most commonly prescribed drugs[8]. More than 8000 tons of benzodiazepines were prescribed in 1977 in the United States[9]. Reports have also indicated a significant association between maternal intake of benzodiazepines during the first trimester of pregnancy and oral clefts[10]. Chronic dietary administration of benzodiazepine tranquillizers to breeding pairs of Swiss Webster mice resulted in alterations in the normal patterns of reproductive behaviour and foetal growth, which significantly depressed body weights at birth[11]. The irritant effects of dilute solutions of benzodiazepines on the eye and tongue of rabbit, guinea pig and man has also been established[12]. Chlordiazepoxide was reported to induce cytogenetic effects and dominant lethality in mice[13,14] and gave a significantly high incidence of micronuclei in the bone marrow cells of mice[15]. The toxic effects of diazepam, chlordiazepoxide and nitrazepam on the spermatozoa of mice were studied with a daily oral dose of 0.5 mg[16].

The present investigation was undertaken to assess the effects of certain commercial tranquillizers on cell and chromosome division in animal and plant test systems.

The drugs used contained either of the two compounds diazepam and nitrazepam in combination with the usual pharmaceutical ingredients in different doses.



1,3-dihydro-7 nitro 5 phenyl 2 H-1,4 benzodiazepine-zone
$C_{15}H_{11}N_3O_3$
(Nitrazepam)



7 chloro-1,3 dihydro-1 methyl-5 phenyl-2 H-1,4 benzodiazepine-zone
$C_{16}H_{13}ClN_2O$
(Diazepam)[17]

MATERIALS AND METHOD

The actual commercial brands used were:—

(a) Nitravet (with 5 mg of nitrazepam/tablet) Mfg. by The Anglo-French Drug Co., Bangalore.

(b) Hypnotex-10 (with 10 mg of nitrazepam/capsule) Mfg. by The Pharmaceutical and Chemical Industries, Gujrat.

(c) Calmpose (with 5 mg of diazepam/tablet) Mfg. by Ranbaxy Laboratories Ltd., New Delhi and

(d) Valium-2 (with 2 mg of diazepam/tablet) Mfg. by Roche Products Ltd., Bombay.

All these chemicals were subjected to *Allium* test[18].

Dosages applied:

(1) Dosage applied to a human adult dissolved in 100 ml distilled water as stock solution.

(2) Half dilution of the stock solution.

For plant systems, *Allium cepa* bulbs were placed at the mouths of tubes containing these doses for 120 hr (figure 1). Root tips were removed after the complete treatment and squashed following acetic orcein schedule.

In the *animal experiments*, four different doses *i.e.* half, normal, double and fourtimes the normal human dose of calmpose and nitravet were force fed to four sets of mice (*Mus musculus*) daily for one month per kg. (figure 2) of body weight, taking 50 kg. as the standard human weight. A control set was fed distilled water in the same proportion. The animals were sacrificed after one month; bone marrow was aspirated and chromosomes were observed following colchicine hypotonic flame drying giemsa schedule[19].

Five thousand cells were scanned for each treatment for mitotic indices and 300 dividing plates were observed for percentages of abnormal cells. Statistical analysis was carried out to test for the significance of the data. Three replicates were made of each experiment and the consolidated data presented.

RESULTS

On an average both the animal and plant systems indicate that, when applied at sufficiently high concentrations these commercial products containing nitrazepam and diazepam are all capable of altering the mitotic index and increasing the number of chromosomal abnormalities in the dividing cells.

In animal experiments the mitotic indices for the four different doses of diazepam and nitrazepam were compared separately with a control. The treated series showed a suppression of mitotic index and a significant rise in total abnormalities in the chromosomes as compared to the control.

The results were slightly different with *Allium cepa*. The increase in abnormalities and decrease in mitotic index was dose dependent, as expected. However, nitrazepam gave an increase, at a moderate significant level in *A. cepa* when applied at the normal human doses.

The abnormalities observed were divided mainly into three different groups –

Group I – Abnormalities mainly due to spindle disturbances *e.g.* diplochromatin, C-mitosis, sticky bridge, lagging, polyploidy, multipolarity, etc.

Group II – Abnormalities due to direct action on chromosomes *e.g.* breaks, gaps, centric fusion, centric fission, telomeric fusion, lesions, etc.

Group III – Gross chromosomal abnormalities *e.g.* erosion, pulverisation, clumping, pycnosis, etc.

Abnormalities of all the three groups taken together gave the percentage of total chromosomal disorder. The histogram represents a comparison of abnormalities caused by each dose of diazepam and nitrazepam separately with the control.

It is suggested that when using tranquillizers care

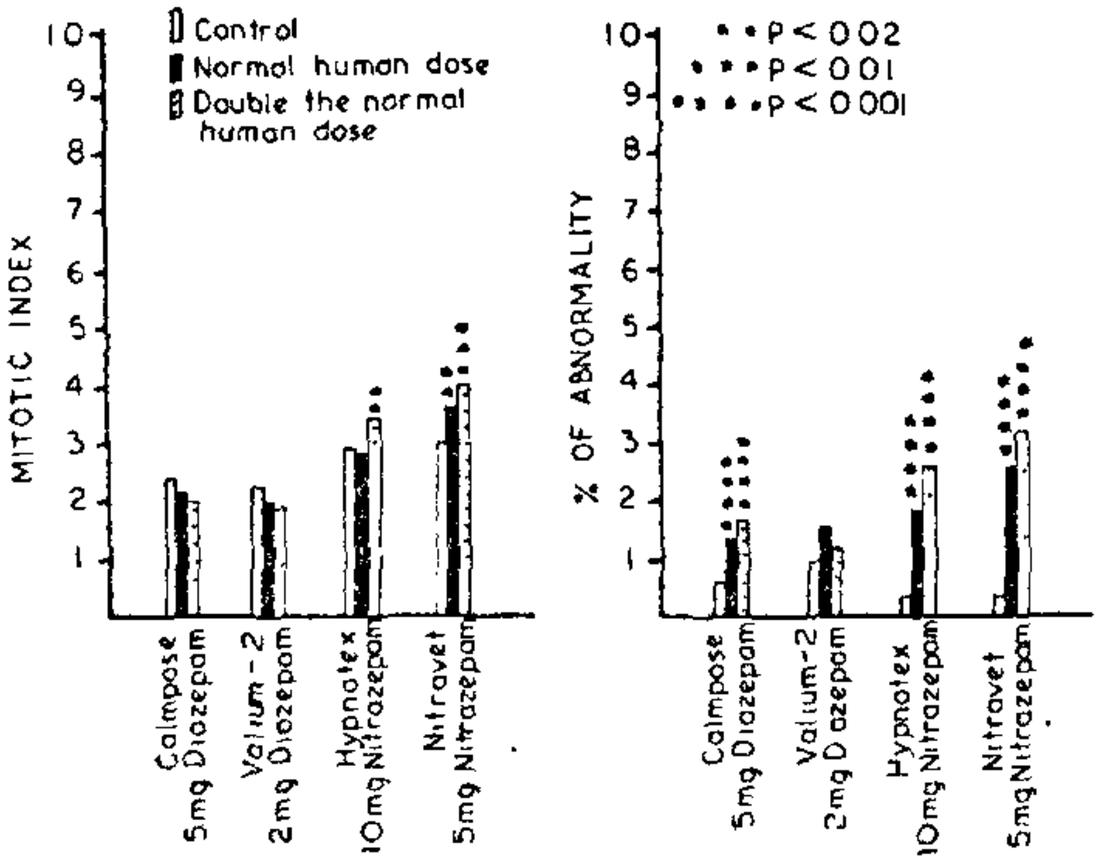


Figure 1. Treatment for 120 hr on somatic chromosomes of *Allium cepa*.

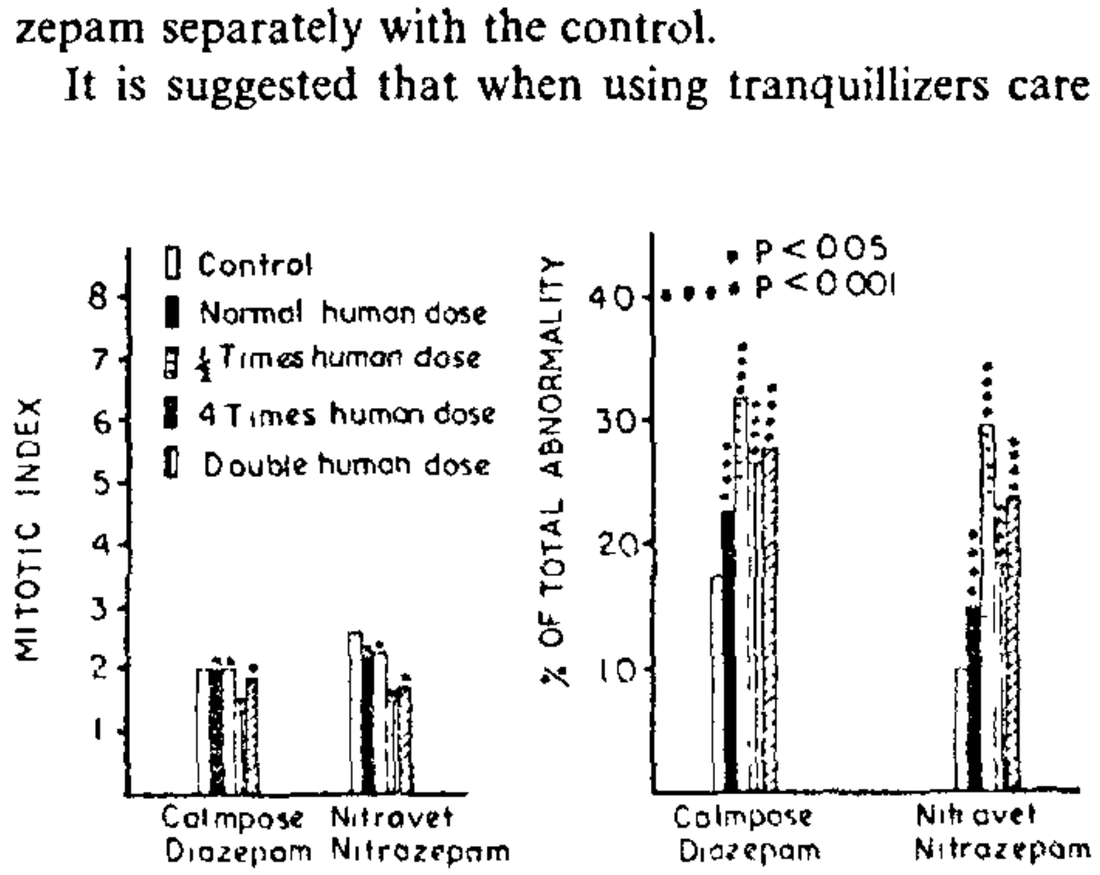


Figure 2. Treatment for one month on bone marrow chromosomes of *Mus musculus*.

should be taken not to exceed the prescribed dose and to avoid chronic treatments in order to prevent cumulative effects.

2 June 1983; Revised 26 March 1984

1. Long, S. Y., *Teratology*, 1972, **6**, 75.
2. Sharma, A., *Environmental chemical mutagenesis*. Perspect. Rep. Ser. **6**, 1984, Golden Jubilee Publ., Indian National Science Academy.
3. Bateman, A. J. and Epstein, S. S., In: *Chemical Mutagens – Principles & Methods for their detection*, 1971, **2**, 541.
4. Staiger, G. R., *Mutat. Res.*, 1970, **10**, 635.
5. Stenchever, M. A., Frakell, R. S., Jarris, J. A. and Veress, K., *Am. J. Obstet. Gynecol.*, 1969, **103**, 836.
6. Matter, B. E., In: *Progress in environmental mutagenesis*, (ed.) M. Alacevic, 1980, p. 191.
7. Matter, B. E. and Tsuchimoto, T., *Arch. Toxicol.*, 1980, **46**, 89.
8. Sellers, E. M., *Can. Med. Assoc. J.*, 1978, **118**, 1533.
9. Iverson, L. L., *Nature* (London), 1980, **285**, 285.
10. Saxen, I. and Saxen, L., *Lancet*, 1975, **2**, 498.
11. Guerriero, J. and Fox, K. A., *Res. Commun. Chem. Pathol. Pharmacol.*, 1976, **13**, 601.
12. Ballantyne, B. and Swanston, D. W., *Acta Pharmacol. Toxicol.*, 1974, **35**, 412.
13. Rao, P. K. and Rao, S. M., *Indian J. Med. Res.*, 1977, **66**, 847.
14. Kar, R. N. and Das, R. K., *Cytobios.*, 1983, **36**, 73.
15. Das, R. K. and Kar, R. N., *Cell Chrom. News Lett.*, 1979, **1**, 12.
16. Kar, R. N. and Das, R. K., *Cytobios.*, 1983, **36**, 45.
17. *The Merck Index*, (9th edn), Published by Merck & Co. Inc., N.J., U.S.A., 1976.
18. Levan, A., *Proc. Eighth Internat. Congr. Genet.*, Stockholm, 1949.
19. Yoshida, T. H. and Sagai, T., *Chromosoma*, 1973, **41**, 93.

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