Original article

Genotoxic effect of an organophosphorus compound (Phosphamidon) on the Bone Marrow of Albino Rats

R. B. Kuwar, C. B. Jha, A. K. Saxena, S. Bhattacharya

Department of Anatomy , Kist Medical college, Imadol- 6 Lalitpur, Department of Anatomy B.P.Koirala Institute of Health Sciences Dharan, Department of Experimental Medicine and Surgery Institute of Medical Sciences, Banarus Hindu University, U. P, India, Department of Anatomy B. P. Koirala Institute of Health Sciences Dharan , Nepal

Correspondence to: Ram Bahadur Kuwar, Department of Anatomy, Kist Medical College, Imadol- 6 Lalitpur, Nepal

e-mail: rbkuwar@yahoo.com

Introduction: The present study shows the genotoxic effect of phosphamidon on bone marrow of albino rats and this study was conducted in the cytogenetic laboratory, at B.P. Koirala Institute of Health Sciences, Dharan, Nepal.

Materials and Methods: Rats were treated by drinking water containing 35 parts per million and 50 parts per million concentration of phosphamidon(an organophosphorus compound) for 30 days. After 30 days, they were sacrificed. The femur was dissected out and the bone marrow from femur was aspirated. The bone marrow was immediately processed and then slides were prepared and then observed in 100X oil immersion microscopy for chromosomal aberrations.

Results: Different type of Chromosomal aberrations were observed in both experimental groups like gaps, accentric fragments, containing single and double minutes, fragmentation, centromeric breakage and premature condensation of chromosomes.

Conclusion: Phosphamidon has profound genotoxic effect in experimental albino rats.

Key words: Phosphamidon, Bone marrow, chromosomal aberration, genotoxicity, chromosome

Introduction

Phosphamidon is widely used pesticide like other organophosphorus .It exerts its toxic effects to the pests by phosphorylation of serine residues, in the active centre of acetylcholinesterase which leads to accumulation of acetylcholine[1].Phosphamidon has been detected in the environment as a contaminants in the drinking water and pesticide residues in the food stuffs because of their extensive used. Thus human exposure to these compounds may occur through the occupation setting as well as via a variety of environmental sources[2].

In genotoxicity studies special attention is focused to cytogenetic assays. Chromosomal breakage and loss have been giving attention for long times and these are also associated to the disease sometimes the development of cancer.[3, 4]

Genetic material is also susceptible to most commonly used organophosphorus compounds and long term exposure may lead to either numerical (Aneuploidy or polyploidy) or structural aberration (Deletion, Duplication, Inversion and Translocation) phosphamidon is a potential toxic compound and is likely to be associated with chromosomal damage in bone marrow cells.

Material and Methods

Thirty healthy adult male weighing 160 -200 gms albino rats were randomly selected from the colony of the animal house of the Anatomy Department at B. P. Koirala Institute of

Health Sciences, Dharan, Nepal. They were given standard pellet diet and housed in well- ventilated room at control ambient temperature (25± 5°C) and natural day light cycle.

These rats were equally divided into 3 groups .Each group comprised 10 rats. The first group was provided the tap water which act as control while the second and third groups were provided the tap water containing 35 and 50 ppm doses of phosphamidon *ad libitum* for thirty days, and were designed as experimental groups. The phosphamidon used was commercially manufactured by Anu Product Ltd, India. The formulated chemical was diluted in drinking water to make 35 & 50 ppm solution of phosphamidon.

After thirty days, all the rats along with their control were sacrificed by cervical dislocation. Two hours prior to the sacrificed 2.5mg/kg, body weight of colchicine was injected intraperitonally to stop the mitosis in metaphase. As soon as the animals were sacrificed the thigh region of the animal was dissected . Two end of femur of each animal was cut and taken out. The bone marrow was aspirated using sterile syringe in the phosphate buffer.

Processing of bone marrow and slides preparation

1. The bone marrow was centrifuged at 1000 rpm for 10 minutes.
2. The pallet obtained after centrifugation was mixed in aqueous solution of KCl (0.56%w/v) and left for 10 minutes at 37°C(This step is called hypotonic treatment).
3. Cells were recentrifused , fixed in acetic acid and methanol (1:3).
4. After fixation, cells were dropped in the pre cleaned and chilled slides.
5. Slides were air dried and stained with 5% Geimsa (for 5 minutes) solution and chromosome analysis was carried out under 1000X magnification for each data point.

Statistical Analysis

x^2 tests were used for the statistical analysis.

Gaps, both chromosomes/ chromatid type ACF =acentric fragment , includes both single minutes and double minutes,Frag= fragmentation : PMC=premature condensation of chromosomes CENT BRK=centromeric breakage

Low Dosec^2=3.97 P=0.047; High dosec^2=6.34 P=0.012

Result

Chromosomal analysis was carried out by observing the metaphase after direct harvesting of bone marrow cells. Cells containing chromosomal aberrations were observed. 25 metaphases per rat were observed in both experimental and control groups. So in each group 250 metaphases are studied. In low dose group 24%chromosomal aberration were observed while in high dose 40% aberrations were present. Different types of chromosomal aberrations were observed in both experimental groups as well as the controls like gap accentric fragments containing single and double minutes, fragmentation ,centromeric breakage and premature condensation of chromosomes .All these aberration were statistically significant at low dose and high dose. (P<0.05)

Discussion and conclusion

Phosphamidon is found to be mutagenic in nature . It can produce number of chromosomal aberrations such as gap in chromosome and chromatid, acentric fragments containing double and single minutes, premature condensation of chromosomes. Previous study in fish showed increase in numbers of aberration dose dependently up to a certain limit, then found to be decreased, which was explained as selective killing[5] .Since in this study only two doses were tested, such result was not obtained. In the present study, most of the observed deletions were gaps, breaks and fragments and this may be due to close interchromatid bridge. Similar types of finding have been reported earlier in high dose treatment of other organophosphorus[6].

In previous study it was mentioned to be a weaker mutagen.[7] In our study we found that the percentage of chromosomal

Table: showing the chromosomal aberration at control, low and high dose of phosphamidon

Types	Total no. of Cells/ metaphase	Total no of abnormal Cells/metaphase	Types of abnormal features & percentage frequency					
			GAP%	BREAK%	ACF%	CENT BRE%	FRAG%	PMC%
control	250	0.16(0.065%)	0.01	0.003	0 .05	-	-	0.002
low dose	250	60*(24%)	4	4	8	4-	-	4
high dose	250	100* (40%)	12	4	16	4	4	-

* P < 0.05 low Dose -35 PPM High Dose-50PPM

aberrations are higher in high dose which is already evident by previous researcher. The genotoxicity of phosphamidon was found dose as well as time -responsive and in chronic exposure its effect was less .[8]However in present study we found the chromosomal aberration even in chronic oral treatment.

Phosphamidon is widely used in agriculture to control pests and easily available to farmers. Most of the people are not aware of the genotoxic effect of the compound. The awareness regarding the impact of pesticides should be given to farmer and they should be encouraged to practice biological means to control pests and herbals instead of these harmful compounds.

If the foods and, vegetables contaminated by phosphamidon consume continuously for long time, It might cause genotoxicity in human being. So, this can be issue of public health hazard and food safety. The authorities should be concerned regarding this.

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