ASSUMED GENOTOXIC EFFECTS OF VINCRISTINE-INDUCED TOXICITY IN MICE

Mohammad Chand Jamali [1], Hayate Javed [2], Shashi Kumar [3] & Shabnam Naaz [4]

1. Department of Health & Medical Sciences, Khwarizmi International College, 25669, Abu Dhabi, United Arab Emirates
2. College of Health & Medical Sc, UAE University, Al Ain, United Arab Emirates
3. Consultant Nephrologist, Nephrology Department, Paras HMRI Hospital, Patna
4. Resident Medical Officer, Nephrology Department, Paras HMRI Hospital, Patna

(Received on Date: 20th July 2017 Date of Acceptance: 24th Oct 2017)

ABSTRACT

Cancer may affect people at all ages, even fetuses, but the risk for most varieties increases with age. Cancer kills more children aged three to 14 than any other disease. Cancer is the second leading cause of death behind heart diseases. Cancer causes about 13% of all deaths. Nearly all cancers are caused by abnormalities in the genetic material of the transformed. The most commonly occurring cancer in men is prostate cancer (about 25% of new cases) and in women is breast cancer (also about 25%). The common treatment plan against cancer is chemotherapy. Doxorubicin is one of the mostly used drugs against cancer. This drug is using against different cancer types including the prostate and breast cancer. But it has severe side effects including cardiac problems. So in this situation the study on the genotoxicity of Vincristine and the possible recovery by the use of any other drug has great importance. The main objectives of this study are as follows: To find out the genotoxicity of Vincristine in mice Bone marrow cells of in which drug was administered in different dosages.

Keywords: Vincristine, Cancer, Chemotherapy.

Number of Tables: 1 **Number of References**: 34

INTRODUCTION

Synthetic pyrethroids have emerged as a new class of agricultural pesticides and have found wide use over organochlorine, organophosphate, and carbamate pesticides. Currently, they have broad-spectrum use in agriculture, domestic, and veterinary applications due to their high bioefficacy, enhanced stability, and comparatively low-mammalian toxicity. There are serious concerns on the potential risks of exposure to pyrethroid insecticides with increasing production and application (Adelsbach and Tjeerdema 2003). Vincristine, a member of the family of synthetic pyrethroids, belongs to type II class pyrethroids and is widely used in agricultural and other domestic applications. The absorption of vincristine from the digestive tract and its excretion takes a quick course. It is well established that vincristine, both cis- and trans-isomers are metabolized to phenoxybenzoic acid and cyclopropane carboxylic acid (Lukowicz - Ratajczak and Krechniak 1991). Populations at the highest risk of high-dose exposure are producers, hygienic, and pesticide workers, and small farm owners applying vincristine for plant protection. Low-dose exposure originates mainly from the household application of insecticides, contaminated food, and water (Gorell et al. 1998).

It has been well-documented that a galaxy of xenobiotics to which humans and animals are acutely or chronically exposed may potentially damage the immune system through a variety of distinct mechanisms (Dean and Vos 1986). The immune toxicological effects of vincristine have been investigated in different animal species. When male Wistar rats were treated with 6.25, 12.5, and 25 mg kgÀ1 of vincristine for 6 or 12 weeks, reduction in anti-sheep red blood cells (SRBCs) and anti-ovalbumin titer as well as the autologous rosette formation of splenic lymphocytes were observed (Desi, Dobronyi, and Varga 1986; IPCS 1989). Varshneya et al. (1992) demonstrated a dose-dependent decrease of delayed type hypersensitivity (DTH) in rats following a 2-month oral treatment with 20 and 40 mg kgÀ1 body wt perday of vincristine. Khurana, Chauhan, and Mahipal (1999) have reported that vincristine given in feed at a concentration of 100 ppm over a period of 8 weeks caused significant depression in DTH reaction in broiler chickens.

Institoris et al. (1999) reported that oral treatment with 55.4 and 22.2 mg kgÀ1 body wt dayÀ1 of vincristine for 28 days decreased DTH reaction in rats. These doses of the insecticide also decreased the mean cell volume of erythrocytes and white blood cell count in the peripheral blood.

The class of chemotherapy drugs which has been most closely linked to the development of second cancers is the alkylating agents, which work by inserting foreign molecules into the genetic material of dividing cancer cells. These foreign

molecules kill cells by disrupting their normal function and by preventing their further growth and multiplication. However, these chemotherapy drugs not only affect the cancer cells but also disrupt normal cell growth in progress in the lining of the gastrointestinal tract, blood cells, hair, nails, and any other part of the body where cells happen to be growing when the drugs are given. In addition to killing cells, the alkylating agents can produce mutations not unlike those produced by radiation. These mutations occasionally lead to cancer.

There are now reliable data showing that the risk of bladder cancer is elevated after treatment with cyclophosphamide (Pedersen *et al.,* 1988), and some preliminary evidence that the risk of bone sarcomas may be elevated after treatment of childhood cancers with alkylating agents (Tucker *et al.,* 1987).

Chemotherapy, in its most general sense, refers to treatment of disease by chemicals that kill cells, both good and bad, but specifically those of micro-organisms or cancer. In popular usage, it will usually refer to antineoplastic drugs used to treat cancer or the combination of these drugs into a cytotoxic standardized treatment regimen. Chemotherapy acts by killing cells that divide rapidly, one of the main properties of cancer cells. This means that it also harms cells that divide rapidly under normal circumstances: cells in the bone marrow, digestive tract and hair follicles; this result in the most common side-effects of chemotherapy–myelosuppression (decreased production of blood cells), mucositis (inflammation of the lining of the digestive tract) and alopecia (hair loss). Newer anticancer drugs act directly against abnormal proteins in cancer cells; this is termed targeted therapy (Hirsch, 2006).

Most chemotherapeutic drugs work by impairing mitosis (cell division), effectively targeting fast-dividing cells. As these drugs cause damage to cells they are termed cytotoxic. Some drugs cause cells to undergo apoptosis (so-called "programmed cell death"). This means that other fast-dividing cells, such as those responsible for hair growth and for replacement of the intestinal epithelium (lining), are also often affected. However, some drugs have a better side-effect profile than others (Goodman *et al*, 1946).

Accordingly, the aim of this study was to examine the vincristine-induced alterations in hematological, biochemical, and immunological parameters of mice.

MATERILAS & METHODS

Selection of Animals:

The Animals (Mice) considered in the present study of genetic analysis were 8-10 weeks old random bred Swiss albino male mice with an average body weight of 22-24g, maintained under standard laboratory conditions at temp 220c ± 20c, relative humidity 50 ± 10% and 12h photoperiod. Commercial pellet diet and deionized water were fed. Around 12 mice

of approximately equal weight were taken for the experiment.

Selection of test compound:

The test compound vincristine were purchased from local pharmacy shops.

Toxicity evaluation:

Naturally living organism in general protect themselves from the potentially harmful chemicals, it is their inherent tendency. A few animals may respond to low doses of toxic chemicals and others at high doses. Toxicity of the drug depends upon the concentration of the compound. The toxicity of the drug is usually expressed in terms of LD50/LC50 (for lethal dose). LD 50 (lethal dose 50) represents amount of poison/ unit weight, which killed 50% of population of animal species employed for the test. Several reports are available on the toxicity and LD50 values of drugs.

Doses selected:

Vincristine was administered orally for 28 days through food of dosage 5mg/kg ,7.5mg and 10mg/kg) body weight

All other chemicals used in the present experiment were of analytical grade. This includes, KCl from S.D fine chemie, Giemsa, Colchicines from Himedia, Fixative (Acetic Acid and Methanol).

Glass Ware:

Petri dishes, Microscopic Slides, Conical Flasks, Standard Flasks, Pasture Pipette, Syringe, 26g needles, Droppers.

Experimental Design:

Analysis of Chromosomal Aberrations in Bone Marrow of Mice

In the present study on dose effect relationship the animals were supplied with vincristine (5mg/Kg 7.5mg /kg and 10 mg/kg), or both For Control group, 3 Mice were also maintained which received equal volume of distilled water. All the control and treated animals were sacrificed by cervical dislocation after the administration of the test compound. 0.05% of colchicine was added to all the incubated mice 2hrs before scarifying to inhibit the spindle formation. Animals were dissected out for femur bones and flushed out bone marrow into a Petridish containing 0.75m KCl (hypotonic) solution to get a homogenous suspension. The cell suspension was collected in clean centrifuge tubes and incubated at 370c for 45minutes. After incubation the tubes were centrifuged for 10minutes at 1000rpm. The supernatant was discarded and to the pellet 5ml of freshly prepared pre-chilled fixative (3:1 methanol and acetic acid) was added and allowed to stay at room temperature for 10minutes. This step was repeated 4 to 5 times. Finally, the cells were fixed in fresh fixative.

Preparation of slides:

Air-dried slides were prepared by dropping one or two drops of the final suspension on the grease-free, pre-chilled slides with the Pasteur pipette. The slides were dried immediately by air-drying method, coded and stained in 2% Giemsa (2mL of Giemsa

+ 2mL of Sorenson's buffer + 46 mL of distilled water) for 10 minutes.

Scoring of Chromosomal Aberrations:

For each mouse 100 well spread metaphase were screened and scored structural aberrations (gaps, breaks, fragments, exchanges and dicentrics) and numerical aberrations. However, well spread metaphases were micro photographed with Leica CW 4000 image analyzer.

The significance of differences for the number of chromosomal aberrations between the control and treated groups was tested using 2×2 contingency chi-square ($\chi 2$) test.

RESULTS & DISCUSSION

Clinical signs of toxicity such as slight nervousness, mild depression, reduced feed intake, rough hair coat, and abnormal gait were observed in vincristine-exposed mice. The animals of other groups including those treated with vincristine plus. There was no mortality in mice with any of the treatments. At term, there was a significant decrease in body weight of animals treated with vincristine alone as compared to untreated and vehicle control groups (both the control groups hereafter referred as control). Mice treated with vincristine alone treated group

Table 1: Frequency of chromosomal aberrations recorded in somatic cells of mice after treatment with various doses of Vincristine for 24, 48, 72hrs interval

Dose(mg/kg) and duration of treatment (hr)	24h		48h		72h	
	Normal metaphases scored (%)	Abnormal metaphases scored (%)	Normal metaphases scored (%)	Abnormal metaphases scored (%)	Normal metaphases scored (%)	Abnormal metaphases scored (%)
Control	489	11	489	11	488	12
	(97.80)	(2.20)	(97.80)	(2.20)	(97.60)	(2.40)
5 mg/kg	470	23	468	32	461	39
	(95.40)	(4.60)*	(93.60)	(6.40)*	(92.20)	(7.80)*
7.5 mg/kg	464	30	459	41	447	53
	(94.00)	(6.00)*	(91.80)	(8.20)*	(89.40)	(10.60)*
10 mg/kg	454	46	443	57	429	71
	(90.80)	(9.20)*	(88.60)	(11.40)*	(85.80)	(14.20)*

The values are in parenthesis are in percentages *P<0.01

Note: *Values are expressed as mean Æ SEM of six mice.*

In this study we analyzed vincristine induced genotoxicity in bone marrow cells of mice using CA's as end point. The actively proliferating cells from bone marrow provide maximum information on the effect of any test compound (Preston *et al* 1987). Chromosome aberrations observed in the present analysis were classified into structural, numerical and other abnormalities. These end points serve as indicators for evaluating the mutagenic potentials of test substances. Since they are considered as stable anomalies which continue to next generation.

The data on the genotoxic effects of vincristine evaluated from bone marrow cells of mice after 24, 48, 72hrs of administration of the drug was furnished in tables 1 and 2.These include changes in different types of chromosomal aberrations.

At 24hrs administration of vincristine , the total frequencies of chromosomal aberrations in the treated mice were increased to 4.60%, 6.00% and 9.20% when compared to controls were 2.20% respectively after the administration of 5, 7.5, 10 mg/kg body weight of vincristine.

At 48 hrs of administration the frequencies of chromosomal aberrations in the treated mice were 6.40%, 8.20% and

11.40% respectively when compared to controls were 2.20% for the various doses of vincristine with 5, 7.5, 10 mg /kg body weight of vincristine.

At 72 hrs administration of Vincristine the total percentage of chromosomal aberrations in the treated mice increased to 7.80%, 10.60%, 14.20% respectively when compared to controls were 2.40% after the administration of 5, 7.5, 10 mg /kg body weight of Vincristine.

The positive results with methotrexate in the bone marrow chromosomal aberrations test indicated that the chemical is capable of inducing cytogenetic effect. Similar results were recorded by Choudhury *et al* (2000) Who studied the cytogenetic effects of vincristine sulphate (VCR) in the bone marrow cells of male and female mice using three end points, were chromosomal aberrations and mitotic index at 24 hours post treatment and micronuclei (MN) at 30 hours post treatment in bone marrow cells of male and female mice after a single intraperitoneal exposure. In another study chromosome mutational activity in bone marrow of Swiss albino mice (Subramanyam *et al*, 1984). Gudi *et al* (1990) observed the induction of aneuploidy and chromosome breaks in bone-marrow erythrocytes of mice with vincristine. It also induces Micronuclei in bone marrow of mice (Maier P and Schmid W,1976). Changes of the mitotic index, induction of chromatid contraction and spreading and decrease of anaphase frequencies (These three criteria chosen are considered as an indicative pre-screening test for the aneuploidy inducing potency of a chemical in mitotic, but not in meiotic cells) were found positive in vincristine treated mouse bone marrow cells (B. M. Miller and I.-D. Adler 1989). Vincristine gave the highest incidences of aberrant micronuclei in the mouse bone marrow (Tinwell and Ashby, 1991).Vinca alkaloids vincristine (VCR), vinblastine (VBL) and vinorelbine (VNR) were induced genetic toxicity in somatic cells of *Drosophila melanogaster* (Marcelo *et al*, 2002).

ACKNOWLEDGEMENTS

We are gratefully to acknowledge Ms. Swaminathan for her advice, supervision, and crucial contribution in doing this experimental work. We also thank our parents, friends and colleagues.

REFERENCES

Adelsbach, T.L., and **R.S. Tjeerdema**. Chemistry and fate of fenvalerate and esfenvalerate. *Review of Environmental Contamination* **176**: 136-54,2003

Baligar, P.N., and **B.B. Kaliwal:** Induction of gonadal toxicity to female rats after chronic exposure to mancozeb. *Industrial Health* **39**: 235-43,2001

Beard, C.W. Serological procedure. Isolation and identification of avian pathogens. 2nd ed. 129-35. New York: Creative Printing Company,1980

Cantalamessa, F. Acute toxicity of two pyrethriod; permethrin and VINCRISTINEE in

neonatal and adult rats. *Archives of Toxicology* **67**: 510-13,1993

Choudhury RC, Das B, Misra S, Jagdale MB (2000). Cytogenetic toxicity of vincristine. J Environ.Pathol. Toxicol. Oncol. 19(4):347-55.

Dean, J.H., and **J.G. Vos**. An introduction to immunotoxicity assessment. In Immunotoxicology of drugs and chemicals, ed. J. Descotes, 3-11. New York: Elsevier,1986

Desi, I., **I. Dobronyi**, and **L. Varga**. Immuno, neuro and general toxicological animal studies on a synthetic pyrethroid: VINCRISTINEE. *Ecotoxicology and Environmental Safety* **12**: 220-32,1986

Doumas, B.T. Standards for total serum protein assay a collaborative study. *Clinical Chemistry* **21**: 1159-66,1975

El-Demerdash, **F.M.**, **M.I. Yousef**, and **F.M. Radwan**.Ameliorating effect of curcumin on sodium arsenite-induced oxidative damage and lipid peroxidation in different rat organs. *Food and Chemical Toxicology* **47**: 249-54,2009

Goodman LS, Wintrobe MM, Dameshek W, Goodman MJ, Gilman A, McLennan MT, , (1946), "Nitrogen mustard therapy". *JAMA* 132: 26–32.

Gautam, **S.C.**, **X. Gao**, and **S. Dulchavsky**. Immunomodulation by curcumin. *Advance Experimental and Medical Biology* **595**: 321-41,2007

Gorell, **J.M.**, **C.C. Johnson**, **B.A. Rybicki**, **E.L. Peterson**, and **R.J. Richardson**. The risk of Parkinson's disease with exposure to pesticides, farming, well water and rural living. *Neurology* **58**: 1346-50,1998

Gudi R, Sandhu SS, Athwal RS, (1990). Kinetochore identification in micronuclei in mouse bone-marrow erythrocytes: an assay for the detection of aneuploidy-inducing agents. Mutat Res. 234(5):263-268.

Hirsch J, (2006), "An anniversary for cancer chemotherapy". *JAMA* 296 (12): 1518–20.

Hongwei Chen, Doppalapudi S. Rupa, Rajpal Tomar, and David A (1994). Eastmond .Chromosomal Loss and Breakage in Mouse Bone Marrow and Spleen Cells. *Cancer Research 54*; 3533-3539.

Institoris, L., **U. Undeger**, **O. Siroki**, **M. Nehez**, and **I. Desi**. Comparison of detection sensitivity of immuno and genotoxicological effects of subacute VINCRISTINEE and permethrin exposure in rats. *Toxicology* 137: 47-55. IPCS. 1989. VINCRISTINEE. Enviornmental health criteria **82**. 85-122. Geneva: WHO,1999

Jain, N.C. Schalm's veterinary hematology. 4th ed. 4106-98. Washington quare, Philadelphia, USA: Lea & Febiger,1986

Khurana, S.K., R.S. Chauhan, and **S.K. Mahipal.**Immunotoxic effects of VINCRISTINEE on delayed type

hypersensitivity reaction in chicken. *Indian Veterinary Journal* **76**: 1055-57,1999

Lukowicz-Ratajczak,J., and **J. Krechniak.** Effect of deltamethrin and VINCRISTINEE in kidney function and metabolism. *Bromatologia Chemia Toksykologiczna* **24**: 133-37,1991

Maheshwari,R.K.,K.A.Singh,J.addipati, and **C.R. Srimal**. Multiple biological activity of curcumin: A short review. *Life Science* **78**: 2081-87,2006

Marcelo Tiburi, Maria Luiza Reguly, Gilberto Schwartsmann, Kênya Silva Cunha, Maurício Lehmann and Heloísa Helena Rodrigues de Andrade(2002). Comparative genotoxic effect of vincristinee, vinblastine, and vinorelbine in somatic cells of *Drosophila melanogaster*. *Mutation Research* 519(1-2): 141-149. (2002).

Comparative genotoxic effect of vincristinee, vinblastine, and vinorelbine in somatic cells of *Drosophila melanogaster*. *Mutation Research* 519(1-2): 141-149.

Naik, R.S., A.M. Mujumdar, and **S. Ghaskadbi.**Protection of liver cells from ethanol cytotoxicity by curcumin in liver slice culture in vitro. *Journal of Ethnopharmacology* **95**: 31-7,2004

Pedersen & Bjegaard (1988) . Free radical formation by antitumor quinones. Free Radic. Biol. Med., 6, 63–101,

Rivarola, V.A., and **H.F. Balegno.** Effect of 2,4-dichloro phenxyacetic acid on polyamine synthesis in Chinese hamster ovary cells. *Toxicology* **56**: 151-57,2001

Shakoori, A.R., F. Aziz, J. Alam, and **S.S. Ali.**Toxic effect of talastar a new synthetic pyrethroid, on blood and liver of rabbit. *Pakistan Journal of Zoology* **23**: 289-93,1990

Stelzer, K.J., and **M.A. Gordon.**. Effects of pyrethroids on lymphocyte mitogenic responsiveness. *Research Communication and Chemical Pathology and Pharmacology* **46**: 137-50,1984

Subramanyam S, Laxminarayana D, Helen KD(1984). Evaluation of genotoxic potential of vincristinee from multiple parameters. *Mutat Res*. 138(1):55-62.

Tamang, R.K., G.J. Jha, M.K. Gupta, Tucker MA, Meadows AT, Boice JD(1987) Jr, : Leukemia after therapy with alkylating agents for childhood cancer. *J Natl Cancer Inst* 78:459-464

H.V.S. Chauhan, and **B.K. Tiwari.** In vivo immunosuppression by synthetic pyrethroid (VINCRISTINEE) pesticides in mice and goats. *Veterinary Immunology and Immunopathology* **19**: 299-305 ,1988

Varalakshmi,C., A.M.Ali, B.V.ardhasaradhi, R.M.Srivastava, S. Singh, and **A. Khar.** Immunomudulatory effect of curcumin: In vivo. *International Immunophramacology* **8**: 688-700,2008

Varshneya, C., T. Singh, L.D. Sharma, H.S. Bhaga, and **S.K. Garg.** Immunotoxic

response of VINCRISTINEE in rats. *Indian Journal of Pharmacology* **36**: 123-26,1992

Venkateshwarlu, **P.**, **B.J.R**. **Sharma**, **B**. **Kalakumar**,**K.S.Reddy**,and**P. Ravikumar**. Comparative evaluation of toxicity of carbaryl, VINCRISTINEE and malathion of testis in mice. *Indian Journal of Toxicology* **4**: 33-37,1997

Yousef, **M.I**., **H.Z**. **Ibrahim**, H.M. **Salem**, **G.A. Hassan**,**S. Helmi**, and**K. Bertheussen**. Heamatological and biochemical changes induced by carbofuran and glyphosate in rabbits. *Environmental and Nutritional Interaction* **3**: 179-94,1999.

Yousef, **M.I**., **H.Z**. **Ibrahim**, **H.M. Yacout**, and **A.A Hassan**. Effects of VINCRISTINEE and dimethoate on some physiological and biochemical parameters in Barki sheep. *Egyptian Journal of Nutritional Feeds* **1**: 41-52,1998