PROTECTIVE EFFECT OF VITAMIN C ON VINCRISTINE-INDUCED TOXICITY IN MICE

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ABSTRACT

Effects of vincristine and their reversal by Vitamin C following oral administration were evaluated in mice. Mature male swiss mice were orally administered vincristine (15 mg/kg body wt), Vitamin C (50mg/kg body wt) or both daily for 4 weeks. At the end of fourth week, hematological & serum biochemical were studied. Sub chronic exposure to vincristine significantly reduced, when the co-contaminant Vitamin C administration restored the changes in the body weight, hematological parameters, and serum biochemical indices and significantly increased the antibody titer, and cell mediated immunity. These results suggest that concurrent Vitamin C treatment has a beneficial role in mitigating adverse effects of vincristine.

Keywords: VINCRISTINE, Vitamin C, oral administration

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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 per day 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 efficacy of Vitamin C in ameliorating 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 and Vitamin C 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 15mg/kg body weight and Vitamin C 50 mg /kg

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 (7.5mg /kg), Vitamin C (50 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% Geimsa (2mL of Geimsa

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

Scoring of Chromosomal Aberrations:

For each mice 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 (χ2) 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 Vitamin C did not exhibit any apparent signs of toxicity. 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 plus Vitamin C showed increased body weight as compared with vincristine alone treated group

Table 1. Effect of VINCRISTINE and VINCRISTINE plus vitamin C through oral route for 28 days on the TLC and DLC of male mice.

Group	TLC	Lymphocytes	Neutrophils	Monocytes	Eosinophipls
Contol	9.3 ± 0.1	78.1±0.6	17.9±1.1	4.6±0.8	0.5±0.3
VINCRISTINE	9±0.04	60±.05	15±.04	3±0.65	0.5±0.2
Vitamin C	9.5±.02	65±.45	18±.23	5±.70	0.5±.01
Vin+Vitamin C	9.2±.01	63±.04	17±.18	4±.68	0.5±.01

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

The value of TLC in vincristine plus Vitamin C-treated group was significantly higher as compared to animals exposed to vincristine alone. Lymphocytopenia was observed in group treated with vincristine as compared to control groups (Table 1). Vincristine plus Vitamin C-treated group showed no significant decrease in differential leukocyte count (DLC) as compared to control group. Significant increase in the neutrophil count was observed in rats treated with vincristine

alone. There was no significant change in monocyte and eosinophil counts in any of the treatment groups.

The skin thicknesses in various groups are presented in Table 2. Skin thickness at 0 h showed no significant difference among the groups. Skin thickness in vincristine-treated group after 24 and 48 h was found to be decreased as compared to control groups. In vincristine plus Vitamin C-treated group, there was a significant increase in the skin thickness as compared to vincristine alone treated group.

Table 2. Effect of vincristine and vincristine plus curcumin through oral route for 28 days on cell mediated immunity of male mice.

Treatment	Dose	0	24	48
Control	-	0.30±0.01	0.42±0.01	0.39±0.01
vincristine	15	0.29±0.01	0.35±0.02	0.31±0.03
Vitamin C	50	0.31±0.01	0.44±0.01	0.40±0.01
Vin+Vitamin C	40	0.30±0.02	0.39±0.03	0.35±0.02

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

In this study, significant decrease in body weight at the end of the experimental period following the administration of vincristine in mice has been observed. It may be attributed to the effect of insecticide on gastrointestinal tract resulting in decreased appetite and absorption of nutrients from gut (Venkateshwarlu et al. 1997) or might be due to direct toxicity of vincristine. Mice that received Vitamin C along with vincristine showed increase in body weight as compared to vincristine alone treated rats which indicate that Vitamin C has appetite inducer and anti-stress effect. This finding is in agreement with observation that Vitamin C treatment caused increase weight gain in rats pretreated with arsenic (El-Demerdash, Yousef, and Radwan 2009).

VINCRISTINE treatment caused significant decrease in TLC in rats as compared to control groups. Similarly, vincristine given orally at 55.4 and 22.2 mg kgÀ1 body wt per day for 28 days has been reported to significantly decrease the absolute TLC in rats (Institoris et al. 1999). No significant decrease in TLC was observed in rats that received vincristine along with Vitamin C as compared to vincristine alone treated animals.

The serum total protein, albumin, and globulin values were found to be significantly reduced in the vincristine-treated group as compared to control groups. These results are in agreement with those of Yousef et al. (1999) on rabbits, Yousef et al. (1998) on sheep, and Baligar and Kaliwal (2001) on rats. Rivarola and

Balegno (1991) reported that the reduction in plasma protein, particularly albumin, in animals treated with pesticides could be attributed to changes in protein and free amino acid metabolism and their synthesis in the liver. The decrease in serum protein also may be due to loss of protein either by reduced protein synthesis or increased proteolytic activity or degradation (Shakoori et al. 1990). In addition, the decrease in serum protein as observed in this study could be attributed in part to the damaging effect of vincristine on liver cells. Reversal of these metabolic alterations in rats has been achieved when Vitamin C was administered along with vincristine.

In this study, a significant decline in antibody titer in VINCRISTINE-treated group was observed suggesting vincristine-induced immunosuppression. Similarly, Stelzer and Gordon (1984) showed that VINCRISTINE inhibited the proliferation of mouse T and B cells at the 1-5 Â 10À5 M concentration range. As for the humoral immune response, Desi, Dobronyi, and Varga (1986) reported a decreased S. typhimurium antibody titer of the rats with vincristine treatment. Increased antibody titer in rats that received curcumin along with vincristine than vincristine alone was observed.

In vivo cell-mediated immune response was assessed by DTH reaction using ovalbumin. After secondary sensitization, a significant decrease in skin thickness in vincristine-treated group as compared to control suggested deleterious effect of vincristine on cell mediated immunity. Tamang et al. (1988) reported in vivo immunosuppressive effect of vincristine in mice and goats. Their results revealed significant depression of cell mediated immunity in both the species, and humoral immune response in goats. Vincristine given in feed at a concentration of 100 ppm for 8 weeks was also found to cause significant depression in DTH reaction in broiler chickens (Khurana, Chauhan, and Mahipal 1999). A dose-dependent decrease in DTH has been reported in rats following a two-month oral treatment with 20 and 40 mg kgÀ1 of vincristine (Varshneya et al. 1992), and also in rabbits fed 300, 150, and 75 mg kgÀ1 of vincristine for 7 weeks (Desi, Dobronyi, and Varga 1986). In this study, we observed a significant increase in the ear thickness in rats treated with Vitamin C plus vincristine as compared to vincristine alone. Our findings are indicative of increase in cell mediated immunity and effective modulation of vincristine-induced immunotoxicity by curcumin. Immunomodulatory action of curcumin could be attributed to its effect on various facets of the immune response, including its effect on lymphoid cell populations, antigen presentation, humoral and cell-mediated immunity, and cytokine production (Gautam, GAO, and Dulchavsky 2007). Recently, Varalakshmi et al. (2008) showed that curcumin enhanced the mitogen and antigen induced proliferation potential of T cells and also produced immunomodulatory

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