Indian J Physiol Pharmacol 2009; 53 (3) : 265–270

HEPATOTOXIC EFFECTS OF VINCRISTINE : AN EXPERIMENTAL STUDY ON ALBINO RATS

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(Received on January 1, 2009)

Abstract : Vincristine (VCR) is an established drug of choice in treatment of some myelomas, lymphomas and leukemias. Hepatotoxicity is a lesser studied side effect of the drug. Samples of blood and other tissues were collected for morphological, biochemical and histopathological evaluation 2 and 24 hours after single intravenous administration of 1.0 mg/kg of VCR to male Albino Wistar rats. VCR produced weight loss; and elevated serum alkaline phosphatase (515.20±356.22, $P<0.05$), SGPT (192.00±102.62, $P<0.05$), and SGOT (574.20±292.16, $P<0.05$) even after 24 hours of drug administration. Though these changes were most severe during the first 2 hours of VCR administration, they also persisted till 24 hours, which may suggest a possibility of an enterohepatic circulation of the drug or its metabolites. This was complemented with morphological disruption in hepatocytes on light and electron microscopy including Scanning Electron Microscopy and Transmission Electron Microscopy.

Key words : vinca alkaloids hepatotoxicity morphological disruption SGPT SGOT rats

INTRODUCTION

Vinca alkaloids such as vincristine (VCR) and vinblastine are known to disrupt microtubule functions of the cell, especially in the mitotic spindle apparatus leading to arrest of cellular mitotic division in metaphase and apoptosis (1–3). These alkaloids are extensively used intravenously in the treatment of neoplasia. Following intravenous drug administration, it is taken up by hepatocytes. Most of the drug and its metabolites are excreted via hepato-biliary route into the gut to be lost through faeces, producing gut stress (4). Pharmacokinetic studies on VCR have shown its bi-phasic serum decay pattern following rapid intravenous administration which suggests its enterohepatic recirculation and may cause exposure of hepatocytes to the alkaloids for long durations of 6–7 days (5).

Hepatotoxic side effect of VCR is enhanced when the drug is administered concomitantly with abdominal and liver irradiation (6–8). VCR induced hepatic dysfunction might alter the elimination kinetics and increase the drug-exposure of organ augmenting the toxicity of alkaloids.

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In clinical practice, the effect of vinca alkaloids on liver is usually overlooked because of multi drug regime for the treatment of neoplasia. The present experimental study was undertaken to assess the possible susceptibility of hepatic tissue to VCR, in terms of morphological and functional changes.

MATERIALS AND METHODS

The present study was conducted on 18 male Albino Wistar rats weighing 150–200 grams, following approval of Ethics Committee of the Himalayan Institute of Medical Sciences. Rats were caged with 4–5 rats per cage, in a well ventilated room at temperatures ranging between 28°C to 32°C with normal day light. They had free access to freshly prepared diet and water. The animals were divided and caged separately in two groups :

Group 1 : (n=6) as controls.

Group 2 : (n=12) were administered intravenously (iv) with VCR.

Experimental protocol

Injectable aqueous VCR 1 mg/ml weight by volume procured form Cipla, India and refrigerated at 2 to 8°C, was injected in the tail vein in a dose of 1 mg/Kg body weight in Group 2 rats. The controls were injected with normal saline.

Serum and tissue samples for biochemical, morphological and histopathological evaluation were collected after anesthetizing rats by intraperitoneal injection of a mixture of ketamine (50 mg/kg of body weight) and xylazine (6.8 mg/kg of body weight) (21), 2 hours after drug administration from 6 rats randomly selected from Group 2; and 24 hours after drug administration from remaining rats of Group 2 and Group 1. The animal was then sacrificed by exsanguination.

Biochemical studies : Blood sample from left ventricle was collected for estimation of Serum Bilirubin, Alanine Aminotransferase (SGPT), Aspartate Aminotransferase (SGOT) and Serum Alkaline Phosphatase (ALP) on RA-50 semi auto-analyzer using diagnostic reagent kit by DiaSys international (9, 10).

Morphological studies

Liver sample : Tissue sections from left lobe of liver were immediately processed separately for histological study under Light and Electron microscope.

For Light microscopy : 4 samples were randomly selected, 2 from controls and 2 from 24 hours post intra-venous VCR administration. Sample were fixed in a solution of formaline (10%) and stained with Hematoxylin & Eosin to be examined under light microscope at 40 to 100X magnification.

For Electron microscopy : Samples of liver tissues were fixed according to the protocol and were transported in phosphate buffer to All India Institute of Medical Sciences, New Delhi, to be evaluated under Scanning & Transmission Electron Microscopy (SEM and TEM).

Statistical analysis

Statistical analysis was performed with One way analysis variance (ANOVA) followed by Student's "t" test. The results were expressed in mean ± SD.

RESULTS

The results are summarized in Table I and Table II. After 24 hrs of IV dose of VCR, the animal showed toxic features with reduction in general activity and decreased appetite. On opening the abdomen, hyperemia of liver and mesentery, friable small intestine and stomach; with distention of stomach in some cases were present. After 2 hrs of drug administration, liver section in light microscopy (40X), showed some disruption of normal architecture with increased amount of kupffer cell infiltration and after 24 hrs marked disruption of arrangement of cells with loss of sinusoidal spaces and ballooning of the hepatocytes at all the regions of tissue was seen (Fig. 1 and 2).

SEM studies also suggested disruption with indistinct boundaries of hepatocytes giving a diffused and homogenous appearance. The normal shape of the hepatocyte was not visible and there was marked anisocytosis.

TEM sections (Table II) following 2 hrs showed mild degree of condensation of the nuclear and cytoplasmic material and distinct double layered membrane boundaries of the organelle were seen. Following 24 hrs a greater damage of the organelles were recognizable. There were definite breaks in the double layered structure of the membrane surrounding the organelles and the nuclear material appeared to be clumped which suggested homogenization. The mean size of the cell showed very highly significant difference from the control (P<0.001).

Hepatic function assay of both the groups (Table I) showed statistically significant changes in enzyme levels between the control group and the samples taken after 24 hrs of drug injection, the SGOT and SGPT being statistically highly significant (P value <0.001).

TABLE I: Hepatic functional assay of control rats and VCR administered rats (n=18).

	Control (n=6)	*iv 2 hours (n=6)*	*iv 24 hours (n=6)*	*Analysis of varience (P value)*	
				Ctrl vs iv	*2 hrs vs 24 hrs iv*
S. Bil (mg/dl)	0.42±0.20	0.65±0.22*	0.58±0.19*	0.023*	0.482
SGPT (IU/L)	42.17±7.31	374.67±262.42***	192.00±102.62***	0.0144**	0.108
SGOT (IU/L)	118.5±13.61	4148.67±4292.25**	574.20±292.16***	0.022*	0.0085**
S.Alk.P (IU/L)	183.50±36.43	529.17±360.28**	515.20±356.22**	0.0031**	0.911

Values are expressed as means±SD.
*P<0.05; **P<0.01; ***P<0.001.
P values are comparisons between controls and the effect of iv VCR.

Abbreviations:
S.Bil: Serum bilirubin.
SGPT - serum glutamate alanine transaminase.
S.Alk.P: Serum alkaline phosphatase.
SGOT - Serum glutamate oxaloacetate transaminase.

TABLE II: Morphometric analysis (by T.E.M.) (n=18).

	Cell size (nm)	*Nucleus size (nm)*	*Organelle size (nm)*	*Vacuole size (nm)*
Controls	8663.315±361.245	6210.285±806.31	974.275±49.27	2119.03±682.53
iv VCR	16356.825±909.118***	6631.8025±959.60	838.1±165.15	560.295±138.88

Values are expressed as means±SD.
*P<0.05; **P<0.01; ***P<0.001.
P values are comparisons between controls and the effect of iv vincristine.

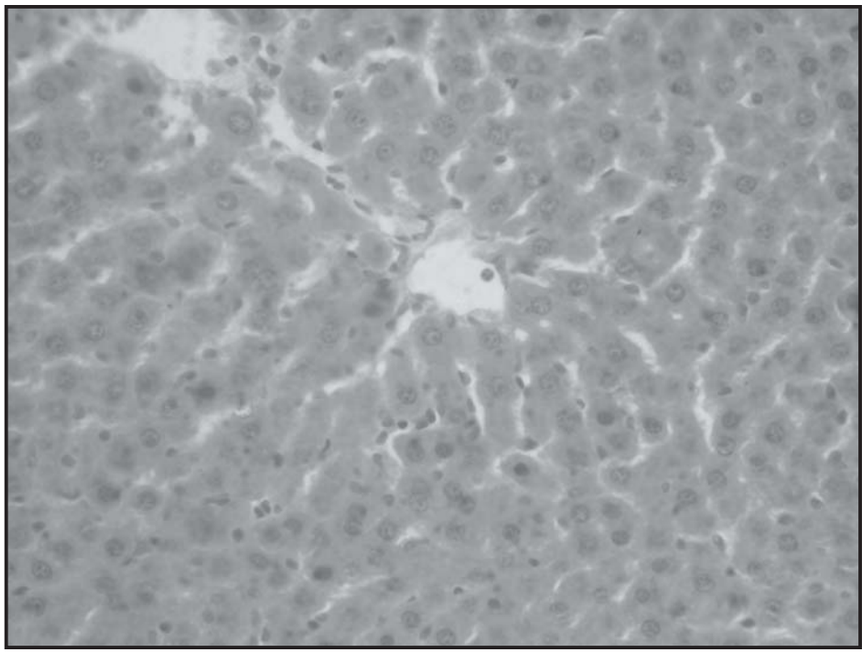

Fig. 1: Light Microscopy in control group.

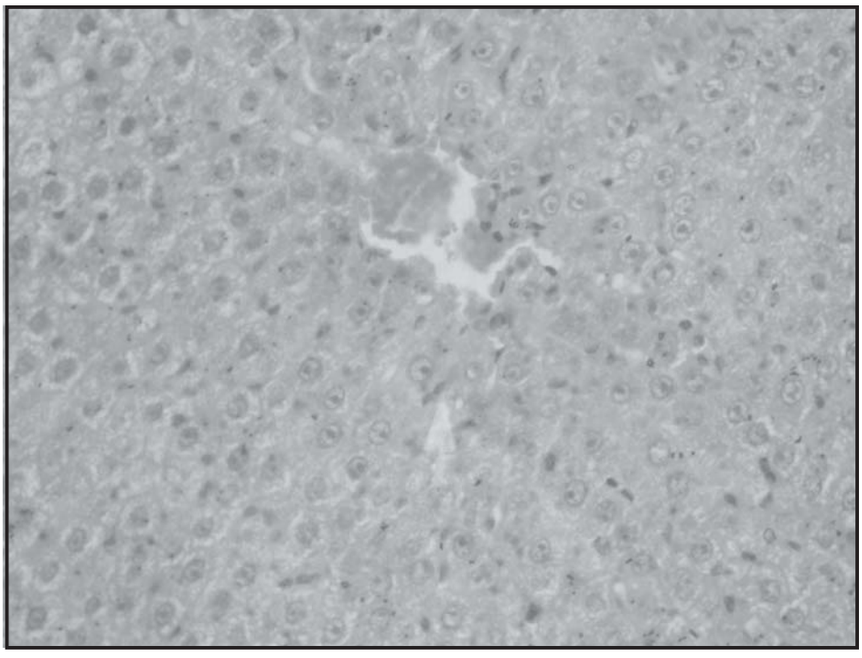

Fig. 2: Light Microscopy in drug group: 24 hours after VCR.

DISCUSSION

Vinca alkaloids introduced in 1960's are extensively used as anti-cancer agents by virtue of their property to arrest cell division in metaphase. Many toxic effects of Vinca alkaloids are now well known including neurological and those on the gastro-intestinal tract. The dangers of prescribing these anti cancer agents to patients with impaired liver function are always emphasized but hepato-toxicity of these drugs is often overlooked (11–15).

Pharmacokinetic studies on VCR have demonstrated biliary excretion as the major route of elimination. Nearly 60–70% of the administered drug gets excreted through bile into the gut lumen before 2–4 hours of injecting the drug. In some studies delayed faecal elimination of VCR has been attributed to some degree of paralytic ileus. Entero hepatic circulation and subsequent elimination may also account for its prolonged elimination (16, 17).

In the present study, light microscopy and electron microscopy (EM) shows that VCR produces alterations in the morphological features of hepatocytes within 24 hours of drug administration which can be interpreted as liver damage.

The observations obtained from EM suggest that the VCR not only affect the cell division of hepatocytes, they also grossly disorganize the architectural pattern of tissue including elastic and fibrous tissue components.

The histological picture supports the gross observations. Similar patchy necrosis in liver on autopsy of patients on therapeutic dose of VCR was found by Coasta et al, but surprisingly the hepatic function tests were in normal limits (7). Steadman, in a study on mouse, following single intra-peritoneal dose of VCR did not reveal hepatic necrosis, but enzyme levels were raised (18). James et al reported centrilobular haemorrhagic necrosis following Catharanthus extract on intra-gastric administration (19).

In the present study a significant increase in mean serum bilirubin levels was observed only in 2 hrs post i/v drug administration. Similar results were reported by Nagi et al in their study on humans, following i/v VCR challenge (8). In contrast, levels were within normal limits as reported by Coasta et al (7).

Also, a highly significant rise in the SGPT levels was seen following 2 hrs of drug administration than as seen following 24 hrs. James et al in their study on mammals found a significant increase in SGPT following higher dose of the extract for 9 consecutive days (19). However, the histopathological findings confirmed the morphological disruption in the liver architecture as seen in the present study. The study by Steadman on mice reported a significant increase in SGPT levels following VCR but in higher dose to the present study (18). Study by Nagi et al also reported similar increase in SGPT levels in human subjects following VCR challenge which returned to normal by 48th day.

The present study also reported an increase in the levels of SGOT. The same was also observed by Capel et al in their study on rats following weekly dose of VCR (20). Study by Steadman and Harrison on mice, following single dose of VCR observed a increase in levels which was seen from first day and remained high till day three (18). However studies on human subjects revealed inconsistent findings as reported by Coasta et al (7). A rise in the levels of SGOT to two to six times the elevation in SGOT values was reported by Nagi et al (8)

following i/v VCR challenge on lung cancer patients.

The levels of SAlk.P were consistently raised following drug administration in this study. Similar studies attributed this rise to spill over of the enzyme following VCR toxicity of gastro intestinal epithelium and cholestasis (8, 18, 19).

Conclusion

The serum enzyme markers and corresponding histological examination reflects liver damage. The result suggests a significant damage in hepatic lobules following VCR administration. The damage seems more severe by 24 hrs of iv drug administration which may suggest a longer stay of the drug in the gut and prolonged activity of the same and its metabolites.

Studies with additional drug dosage for longer duration of time interval will be required before the overall predictive reliability of its hepatotoxic effect can be expressed quantitatively

Further studies for longer duration and different routes of administration are necessary to substantiate the toxic effect of VCR on hepatic cells.

ACKNOWLEDGMENTS

Authors are grateful to Himalayan Institute of Medical sciences, Dehradun and Electron Microscope Division, Department of Anatomy, All India Institute of Medical Sciences, New Delhi for providing facility to conduct the study. The authors are grateful to Professor RK Sharma and Dr. Archana for their contribution during the study.

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