Indian J Physiol Pharmacol 2003; 47 (3): 270–278

EVALUATION OF INTRAPERITONEAL VINCRISTINE IN MALIGNANT PERITONEAL EFFUSION

K. L. BAIRY+, S. SANATH, G. C. JAGETIA*, S. N. SOMAYAJI**, M. S. VIDYASAGAR*** AND M. S. BALIGA*

*Departments of +Pharmacology, Radiobiology and Radiotherapy***, Kasturba Medical College, Manipal – 576 119

and

**Department of Anatomy, International Centre for Health Science, Manipal – 576 119

(Received on December 24, 2002)

Abstract: The efficacy and safety of intraperitoneal administration of vincristine sulphate was determined in mice bearing Ehrlich ascitic carcinoma. The tumor bearing animals were administered with 0.5 mg/kg body weight (b.wt) of freshly prepared vincristine sulphate intraperitoneally on day 6 after tumor transplantation followed by drug administration once daily 5 days a week consecutively. The observations regarding the survival, alteration in the volume of peritoneal fluid, increase in life span and pathological changes in the liver, kidney, gastrointestinal tract and bone tissues were made. The vincristine sulphate treatment reduced the malignant cell population significantly and there were no significant changes in the histological picture of liver, kidney, bone, except the intestine, where atropy of villi demonstrating nests and cords of uniform small round cells were observed. Our experimental data suggests that intraperitoneal administration of vincristine is beneficial in malignant peritoneal effusion.

Key words: vincristine sulphate, intraperitoneal, malignant peritoneal effusion, median survival time.

INTRODUCTION

Despite seemingly curative surgery, 20–30% of patients of gastrointestinal cancer develop some form or the other of locoregional recurrence (1). Locoregional recurrent cancer causes significant morbidity and may give rise to secondary metastatic diseases, and this group of patients with biologically low–grade disease might, in theory benefit from local treatment, taking the advantage of the presence of a peritoneal plasma barrier (2). Traditionally locoregional cancer recurrence with wide spread peritoneal implantation has been difficult to treat, as most of the patients undergo

+Corresponding Author

palliative procedures or no surgery at all. The same holds true for patients suffering from malignant peritoneal effusion, a disease that remains confined to the peritoneal surfaces during most of their natural history.

An adequate understanding of cancer and its treatment must begin with the appreciation of the fact that cancer is not a single disease, but atleast 100 different diseases each of its own characteristics and natural history (3). Even with single malignancy, major biological differences can exist. Therefore it is important for professionals and researchers to know this and counsel patients as to the nature of cancer, modern methods of therapy and the importance of clinical and experimental research as a way to improve the treatment of future patients (4). For anticancer drug to be effective several features must be present. The drug must reach the cancer cells, sufficiently toxic amount of drug (or its active metabolites) must enter the cells and remain there for a long period of time and the cancer cells must be sensitive to the effect of the drug. All this must occur before resistance to drug therapy develops (5).

Cancer chemotherapy can no longer be conducted on a trial and error basis. It has become a science based largely on the principles derived from animal experiments like log cell kill hypothesis, cell kinetics of normal and malignant cells, mechanism of action of cytotoxic drugs and their effects on cell cycle, pharmacokinetic drug scheduling, drug toxicity, selectivity of cytotoxic drugs for certain histological cell type and drug resistance (6). Of late oncologists are trying out various drugs by intraperitoneal route in malignant peritoneal effusion (7). Recently, a growing number of reports have attracted considerable interest in intraperitoneal chemotherapy following debulking surgery. More over a growing body of experimental evidence also supports use of intraperitoneal chemotherapy for peritoneal carcinomatosis following resection of tumor stage (8).

Vincristine sulphate (VCR), a dimeric alkaloid isolated from the periwinkle plant *Catheranthus roseus*, is used for the treatment of several forms of malignancies (9). It has been reported to possess antitumor activity against experimental animal tumors and exhibits cytotoxic effects both in vivo and in vitro (10). Vinca alkaloids are potent microtubule–inhibiting agents that act by depolymerization of tubulin resulting in the inhibition of mitosis (11). Further, vinca alkaloids have been reported to affect a number of cellular systems like the DNA and RNA synthesis (12), lipid biosynthesis, cyclic neucleotide metabolism (13), glutathione metabolism (14) and calmodulin dependent Ca2+ transport ATPase (15). VCR due to its comparatively mild myelosuppressive action is a standard component of regimens for treating pediatric leukemias and solid tumors and is frequently used in adult lymphoma treatment. Vincristine sulphate has been found to be active against the hematological malignancies, Hodgkin's and non–Hodgkins lymphoma, Wilm's tumor, neuroblastoma, brain tumours, rhabdomyosarcoma, carcinomas of the breast, bladder and the male and female reproductive systems (16). To the best of our knowledge, and literature

search–using web–based search engine there was no reported experimental study no the intraperitoneal use of vincristine therapy in malignant peritoneal effusion. Therefore the present study was planned to evaluate the effect of intraperitoneal vincristine in malignant peritoneal effusion in mice.

METHODS

Animal care and handling

The animal care and handling was done according to the guidelines set by the World health organization, Geneva, Switzerland and the INSA (Indian National Science Academy, New Delhi, India). Ten to twelve week old female Swiss albino mice weighing 24 to 28 g and healthy Wistar rats of twelves weeks old weighing 150 to 200 g were selected from an inbred colony maintained under the controlled conditions of temperature (23±2°C), humidity (50±5%) and light (10 and 14 h of light and dark, respectively). The animals had free access to the sterile food and water. Three to four animals were housed in polypropylene cage containing sterile paddy husk (procured locally) as bedding throughout the experiment. The institute animal ethical committee approved the experiment.

Experimental design:

The animal were then divided into two groups (n= 8/group). One group acted as control, received 0.01 ml/kg b.wt. of normal saline intraperitoneally, while the other group was treated with 0.5 mg/kg body weight (b.wt) of freshly prepared vincristine sulphate (1 mg dissolved in 10 ml of sterile saline) intraperitoneally, once daily five days a week consecutively and the volume of the drug was kept to 0.01 ml/g b.wt.

Experiment no. 1: Induction of malignant peritoneal effusion

Tumor model: Ehrlich ascitic carcinoma (EAC) (Fig 1–A and 1–B)

The EAC tumor was procured from Cancer Research Institute, Bombay. The ascitic fluid was drawn using an 18–gauge needle into sterile syringe. 100 µl (aliquot) of tumor fluid was tested for microbial contamination. Tumor viability was determined by trypan blue exclusion test and cells were counted using haemocytometer. The ascitic fluid was suitably diluted in DMEM (Dolbecco's modified Eagle's medium) to get a concentration of 10^6 cells/200 µl of tumor cell suspension. This was injected intraperitonially to obtain ascites tumor. The mice were weighed on the day of tumor inoculation and then on alternate days. Drug was administered from 6^{th} day of tumor inoculation. The following parameters were used to evaluate the efficacy and safety of intraperitoneal administration of vincristine sulphate in mice.

Median survival time (MST) and increase in life span (%ILS)

Tumor response was assessed on the basis of Median survival time (MST) and Increase in life span (%ILS).

$$\%ILS = \frac{\text{MST of treated group - MST of control group}}{\text{MST of control group}} \times 100$$

An enhancement of life span by 25% or more

over that of control was considered as effective anti-tumor response.

Volume of peritoneal fluid collection

After five days of drug treatment, animals were sacrificed with cervical dislocation. A small mid line incision was made on the abdomen and cut open. Mice were then placed inverted in a funnel connected to measuring cylinder and volume of peritoneal fluid is collected and measured, and examined cytologically.

Cell viability test (percent of dead malignant cells in peritoneal fluid)

The aspirated EAC were stained with trypan blue, and scored in a hemocytometer (AO Scientific Instruments, Buffalo, NY, USA), under transmitted light microscope (Leitz, Germany) and the viable cells were determined.

Histopathological examination

Kidney, liver, gastrointestinal tract and bone (femur) were isolated and placed in 10% formalin and observed under a light transmitted microscope after staining with haematoxylin and eosin.

Experimental design no. 2: Gastrointestinal transit time

A separate experiment was carried out to study the gastrointestinal transit time in healthy Wistar rats. The animals were allowed to fast by withdrawing the food and water for 18 h. The fasted animals were then divided into two groups (n=8/group). One group acted as control, received 0.01 ml/kg body weight (b.wt.) of freshly prepared vincristine sulphate in sterile saline intraperitoneally, once daily consecutively for five days and the volume of the drug was kept to 0.01 ml/g b.wt. Rats were fed 2ml of test meal by gauge, consisting of 12.5% charcoal in water and few drops of tween 20 on the sixth day (17). Five minutes later, animals were anaesthetized by ether. Laprotomy was done and peritoneal cavity along with peritoneal fluid was examined and evaluated both macroscopically and microscopically. The length of small intestine from pyloric spinter to the ileocaecal junction was measured and distance traveled by the test meal across the length of small intestine was measured and the data are expressed as the percent gastrointestinal transit time.

Statistical analysis

Data of the volume of peritoneal fluid, Cell Viability and G.I.T motility was analyzed using Student 't' test and Median Survival Time by Mann Whitney U test using SPSS statistical package.

RESULTS

Experimental no. 1: Ehrlich ascitic carcinoma model response

The median survival time in control animals was 15 days and the survival range was 12-16 days while the vincristine sulphate treatment increased the median survival time up to 22 days and survival range up to 16-24 days. This increase in the median survival time between the two groups was statistically significant. The increase of life span in the vincristine sulphate treated group was 46.66 % (Table I). The vincristine

TABLE I: Effect of vincristine sulphate on MST, %ILS, Volume of peritoneal fluid and percent dead malignant cells in EAC model.

Group	*Amount*	*MST (Days) (Survival range)*	*%ILS*	*Volume of peritoneal fluid Mean ± SE*	*Dead Malignant cells Mean ± SE*
Control (normal saline)	0.01ml/g b.wt	15 (12–16)	–	2.40± 0.11	11.37±0.9
Vincristine sulphate (in Normal saline)	0.01ml/g b.wt	22 (16–24)	46.66	1.38± 0.14a	29.69±0.14b

N= 8/group; a=P<0.0001Vs control' b=P<0.001Vs control
MST–Median Survival Time.
%ILS–Percentage increase in life span.
EAC–Ehrlich Ascitic Carcinoma model.

sulphate treatment clearly decreased the volume of malignant peritoneal fluid by 1.74 folds and the statistical significance was found to be a = P<0.0001 when compared to normal saline treated control (Table I). The results show tat vincristine treated mice increased the percent of dead cells by 2.61 fold which was statistically significant b = P<0.001 when compared to normal saline treated controls (Table I).

TABLE II: Effect of vincristine sulphate on gastrointestinal transit time in rats.

Group (N=8/group)	*Distnace traveled by charcoal meal (cms) Mean±SE*
Control (Normal saline)	23.31±1.58
Vincristine Sulphate (in Normal saline)	19.84±1.19*

*Not significant

Histopathologica examination

Liver : Histopathological examination of mice liver in malignant peritoneal effusion treated with normal salin showed plates of highly differentiated, large neoplastic hepatocytes (with prominent nucleoli and finely granular cytoplasm)

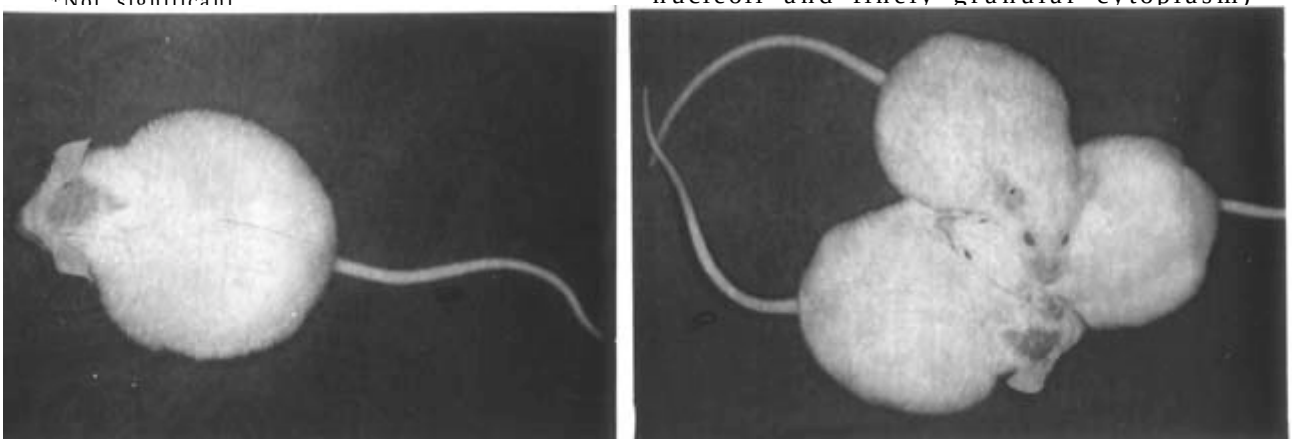

Fig. 1–A and 1–B: Photograph of Swiss Albino Mice bearing Ehrlich Ascitic Carcinoma.

without discernible hepatic architecture (Fig. 2-A). But mice liver in malignant peritoneal effusion treated with vincristine sulphate showed variable differentiation and less neoplastic hepatocytes. A large blood vessel was also evident in center (Fig. 2-B).

Kidney : Similarly cross section of mice kidney in control group showed well differentiated renal cortical carcinoma, characteristic large clear cells with inflammatory cells (Fig. 3-A), whereas vincristine sulphate treated slide showed moderately differentiated cells with less inflammatory cells (Fig. 3-B).

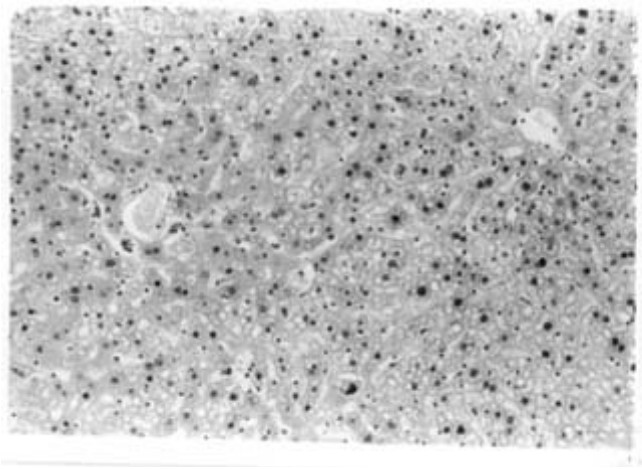

Fig. 2–A : Photomicrograph of mice liver in EAC treated with normal saline showing plates of highly differentiated, large neoplastic hepatocytes (prominent nucleoli and finely granular cytoplasm) without discernible hepatic architecture.

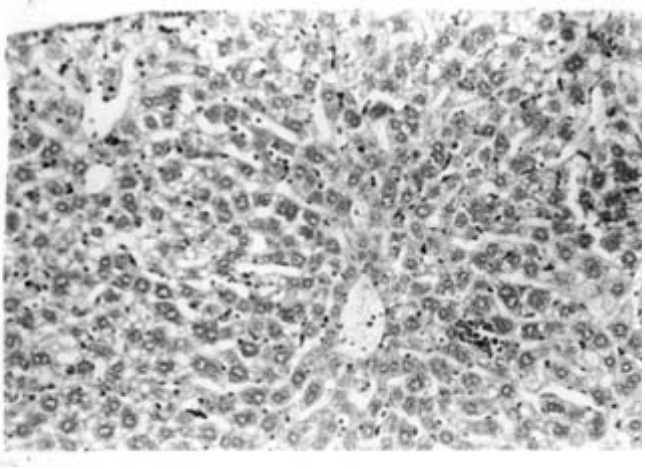

Fig. 2–B : Photomicrograph of mice liver in EAC treated with vincristine showing plates of variable differentiation and less neoplastic hepatocytes. A large blood vessel is also evident in center.

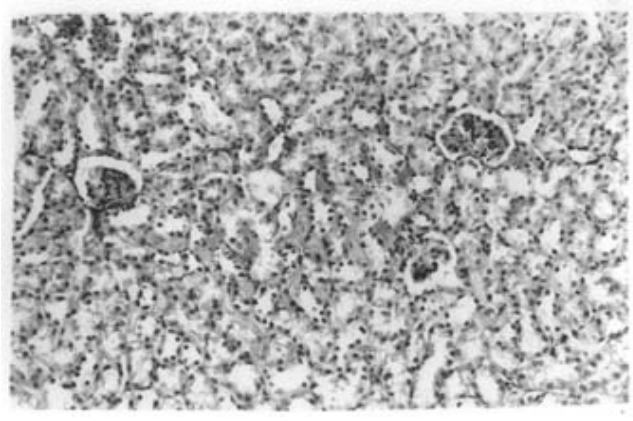

Fig. 3–A : Photomicrograph of mice kidney in EAC treated with normal saline showing well differentiated, renal cortical carcinoma, and characteristic large clear with inflammatory cells.

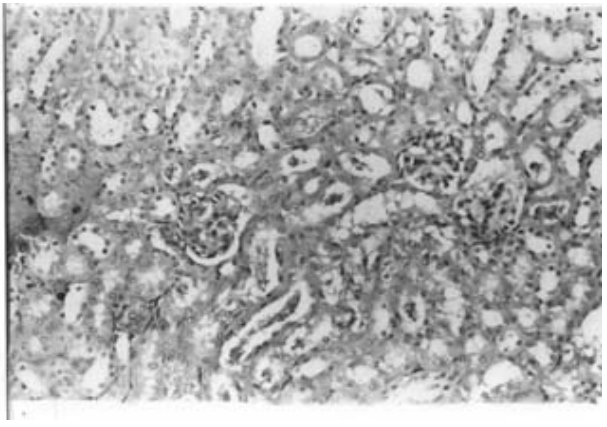

Fig. 3–B : Photomicrograph of mice kidney in EAC treated with vincristine showing plates of moderate differentiation and less inflammatory cells.

Bone (femur) : The histological section of mice bone in both the groups did not show any major histological changes (Fig. 4-A and 4-B). In both slides there was a characteristic mosaic pattern of lammellar bone units.

Gastrointestinal tract (small intestine): Intestinal biopsy specimen of control group showed highly differentiated mucinous epithelium, dysplastic changes with nuclei lying near the surface and neoplastic cells (smaller than normal acinar cells, but have large nuclei) (Fig. 5-A). The however vincristine sulphate treated group showed atrophy of villi, nests and cords of uniform small, round cells (Fig. 5-B).

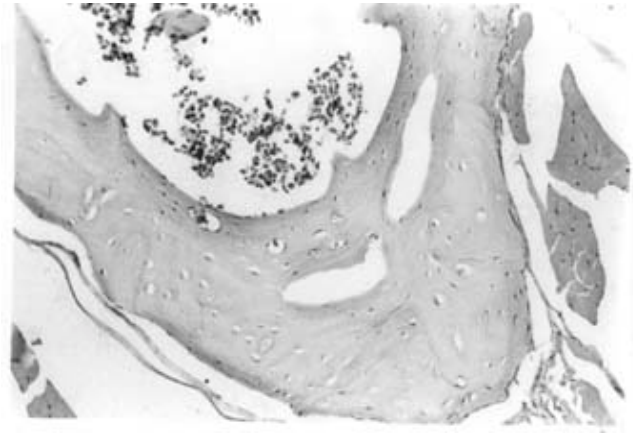

Fig. 4–A: Photomicrograph of mice bone in EAC treated with normal saline showing characteristic mosaic pattern of lammellar bone units.

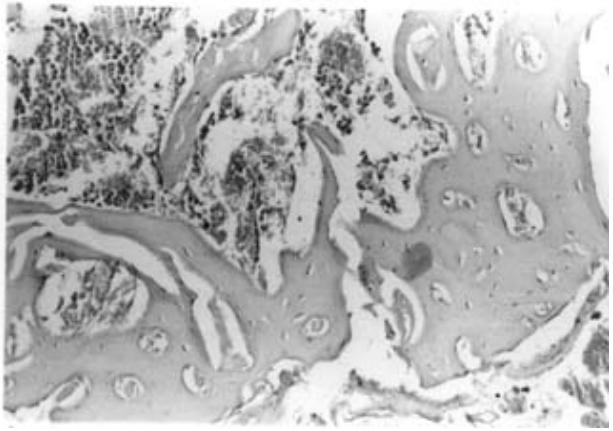

Fig. 4–B: Photomicrograph of mice bone in EAC treated with vincristine did not show any major histological changes. Note: characteristic mosaic pattern of lammellar bone units.

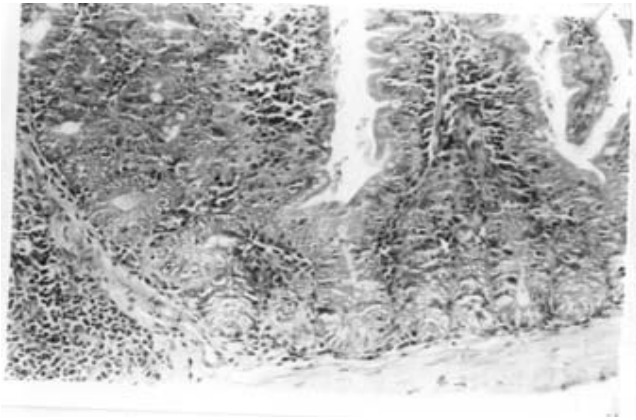

Fig. 5–A: Photomicrograph of mice small intestine in EAC treated with vincristine showing highly differentiated mucinous epithelium, dysplastic changes with nuclei lying near the surface and neoplastic cells. (smaller than normal acinar cells but have large nuclei).

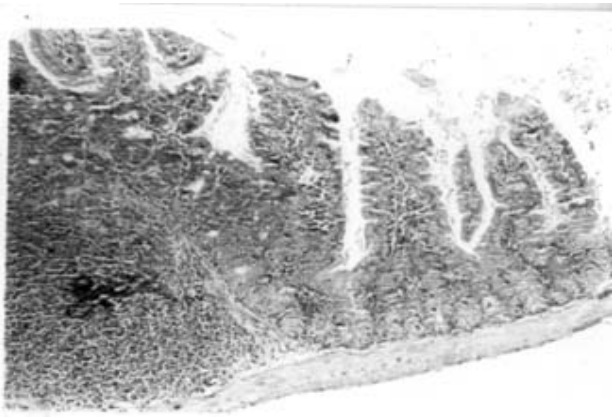

Fig. 5–B: Photomicrograph of mice small intestine in EAC treated with vincristine showing atrophy of villi, demonstrates nests and cords of small uniform, round cells.

Experimental no. 2: Gastrointestinal transit time

Our study suggests that intraperitoneal administration of vincristine sulphate in normal rats did not affect the gastrointestinal transit significantly when compared to control (Table II).

DISCUSSION

The present study was aimed to evaluate the safety and efficacy of vincristine sulphate in malignant peritoneal effusion when administered intraperitoneally. Malignant peritoneal effusion was induced by EAC tumor cell inoculation. Though EAC model does not simulate exactly to any of the known human cancerous condition, it can be considered as valuable animal model for dispersed type of carcinoma.

The present study demonstrates that vincristine sulphate increased death of malignant cells, thereby reducing the volume of peritoneal fluid and consequently increasing the MST of EAC bearing mice. This may be due to the achievement of optimum and uniform concentration of vincristine sulphate in the peritoneal cavity because of intraperitoneal than the intravenous administration. Thus vincristine sulphate might have bound to malignant cells and inhibited cell proliferation by altering the dynamics of tubulin addition. This would have caused arrest of dividing compartment and thereby reducing the tumor growth. As a result there was a constant decrease in the volume of peritoneal fluid and increase the MST of tumor bearing mice. This contention is supported by the study of Bleyer et al who have shown a precise transport mechanism of this agent from peritoneal cavity into malignant cells, where the amount of drug binding increased with increasing length of cell exposure to vincristine in peritoneal cavity (18). However there may be possibility of false positive results in control group animals that received intraperitoneal normal saline in EAC bearing mice.

This beneficial effect was also further supported by histopathological examination of EAC bearing mice liver, kidney and gastrointestinal tract, which showed reduced number of neoplastic cells in vincristine sulphate treated group than the non-drug treated group. This may be because of increased intracellular concentration of vincristine sulphate following intraperitoneal therapy. The vincristine sulphate may have accumulated differentially in some malignant cells and acted on several process that appear unrelated to microtubules including RNA, DNA, lipid biosynthesis, cyclic nucleotide, glutathione metabolism and calmodulin dependant ATP ase activity (11, 12, 13, 14, 15).

Though there are reports of bone marrow suppression and paralytic ileus or adynamic ileuys following intravenous vincristine therapy (19), however no such effect was observed when vincristine sulphate was administered intraperitoneally in our study. The vincristine sulphate also did not affect the gastrointestinal transit time in rats significantly when administered intraperitoneally. This may be because; vincristine sulphate may not have been absorbed from peritoneal cavity and might have just local action on malignant cells in peritoneal cavity. Delivery of chemotherapy into peritoneal cavity has been investigated in-patients with malignant ascities (20). The major advantages of intraperitoneal chemotherapy are pharmacological, where higher concentration of some of the drugs

can be delivered to the tumor than could be safely administered by other routes. The intraperitoneal administration also enhances the cytotoxicity by direct drug contact with the cells in the peritoneal cavity than the other routes of administration. The use of very high concentrations and clinical trials has already established safety and efficacy of this method of drug delivery.

There is no evidence of carcinogenecity of mutagenecity following intraperitoneal vincristine sulphate injection in mice and rats (21). Therefore intraperitoneal administration of vincristine sulphate could be an acceptable method for treating malignant peritoneal effusion. Clinical studies for best methods of administration and effectiveness for vinca alkaloids on malignant peritoneal effusion are ongoing and the results are awaited (22). Our study may be additional evidence as far as safety and efficacy are concerned with regards to intraperitoneal vincristine sulphate therapy for malignant peritoneal effusion.

REFERENCES

1. Abulafi AM, Williams NS. Local recurrence of colorectal cancer: the problem, mechanisms, management and adjuvant therapy. *British J Surg* 1994; 81: 7–19.
2. Swedish Rectal Cancer Trial. Improved survival with preoperative radiotherapy in resectable rectal cancer. *N Engl J Med* 1997; 336: 980–987.
3. Devita VM, Heliman S, Rosenberg SA. Principles and practice of Oncology. Ed 3, Philadlphia, JB Lippincott 1989; 276–288.
4. Cadmen EC, Durivage HJ. In Harrison's Principles of internal medicine. Eds 12. Newyork, McGraw-Hill. 1990; 1587–1599.
5. Chabner BA, Collins JM. In Cancer chemotherapy, Principles and Practice, Eds Philadelphia, JB lippincott 1990; 341–355.
6. Salman SE, Apple M. In "Review of Medical Pharmacology". Eds Meyer FH, Jowetz, Goldfien A Lange Medical Publication. California 1972; 448.
7. Markmann M. Intraperitoneal chemotherapy in management of colon cancer. *Semin Oncol* 1999; 26: 536–539.
8. Spratt JS, Adcock RA, Muskovin M Sherril W, McKeown J. Clinical delivery system for intraperitoneal hyperthermic chemotherapy. *Cancer Res* 1980; 40: 256–260.
9. Avila. Structure based design of novel anti cancer agents. *J Life Science* 1997; 50: 327–334.
10. Johnson IS, Armstrong JG, Gorman M, Burnett JP (jr). The Vinca Alkaloids, A new class of oncolytic agents. *Cancer Res* 1968; 23: 1390–1427.
11. Jordon MA, Thrower D, Wilson L. Mechanism of inhibition of cell proliferation by vinca alkaloids. *Cancer Res* 15 1991; 51: 2212–2222.
12. Creasey WA. Modifications in biochemical pathways produced by the vinca alkalodis. *Cancer Chemotherapy Res* 1988; 52: 501–507.
13. Sheppard JR. Effects of vinca alkaloids on cyclic AMP metabolism of mouse splenic lymphocytes. *Contrib Oncol* 1980; 6: 27–36.
14. Bernstam VA, Gray RH, Bernstein IA. Effect of microtubule disrupting drugs on protein and RNA synthesis in Physarum polycephalum amoebae. *Arch Microbiol* 1980; 128: 34–40.
15. Gietzenk, Wutrich A, Mansard A, Bader H. Effect of Vincaalkalodids on calmodulin–dependant calcium transport ATP ase. *Contrib Oncol* 1990; 6: 16–26.
16. Dorr RT, Fritz WL. Cellular chemotherapy consideration in Cancer chemotherapy Hand Book. Eds Henry – Klimpton publishers, London 1980; 3–18.
17. Manara L, Biancheltti A, Ferrti P, Tavani A. Inhibition of gastrointestinal transit by morphine in rats results primaily from direct action on gut opiod sites. *J Pharmacol Exp Ther* 1986; 237: 945–949.
18. Bleyer A, Goldenberg GJ, Geran RL. Alteration in drug transport of vinca almaloids. *The Journal of Organic Chemistry* 1992; 63: 8586–8588.
19. Maulik SK, Seth SD. In Antineoplastic agents. Text book of Pharmacology. 2nd edition, Chapter 13 1999; 674–675.
20. Loggie BW, Perini M, Fleming RA, Russel GB, Geisinger K. Treatment and prevention of malignant ascities associated with disseminated intraperitoneal malignancies by aggressive combined modality therapy. *Ann Surgery* 1997: 63: 137–143.
21. Summary of Data Reported and Evaluation on vincristine sulfate. 1981 vol: 26. p.365.
22. Canellos, George P. Chemotherapy of Advanced Hodgkin"s Disease with MOPP, BVD, or MOPP alternating with ABVD. New England. *Journal of Medicine* 1992; 327: 1478–1484.