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## Kinetics and Tissue Distribution of Niosomal Bleomycin in Tumor Bearing Mice

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*Bleomycin was encapsulated in niosomes and administered to tumor bearing mice. The plasma kinetics and tissue distribution of niosomal bleomycin was studied in tumor bearing mice. The niosome encapsulated bleomycin was cleared from the plasma much more slowly than was the free drug. A significant increase in plasma concentration of bleomycin was achieved in mice when it was administered as niosomes as compared to free drug. The tumor bleomycin level enhanced significantly.*

IMPROVED delivery of anticancer drugs to tumor tissues, appears to be a challenging and achievable effort. Physical approaches for the delivery of anticancer drugs consist of microparticulate drug carriers (liposome, microspheres, nanoparticles), magnetic microcapsules, implantable pump and reservoirs. Some success has been reported in the areas of enhancing efficacy and reducing drug toxicity.<sup>1-5</sup>

Niosomes are vesicles that can entrap solutes and are stable.<sup>6</sup> Niosomes behave *in vivo* like liposomes, prolonging the circulation of entrapped drug and altering its organ distribution and metabolic stability. The effect of non-ionic surfactant vesicle entrapment on the absorption and distribution of the anticancer agent methotrexate (MTX) in mice has been reported by Azmin et al<sup>7</sup>.

The half life of the niosomal doxorubicin was found to be prolonged compared to free solution profiles and hence a potential increase in duration of action of the niosomal drug. Tumor levels of the drug were higher following administration of doxorubicin in niosome and this was reflected in more effective reduction in tumor growth.<sup>9</sup>

Baillie et al<sup>10</sup> compared the efficacy of sodium stibogluconate, an anti-leishmanial drug, after its ad-

ministration to mice in free form, liposomal and niosomal form. High liver and low serum values were obtained by the use of vesicular formulations and it was shown to be more active than free drug against experimental murine leishmaniasis. Bleomycin is an antineoplastic antibiotic which binds to DNA and causes strand scissions. It is used in squamous cell carcinomas including those of the cervix and external genitalia, oesophagus, skin and head and neck in Hodgkin's disease and other lymphomas, and in malignant neoplasms of the testis. A limiting factor in the use of bleomycin as an chemotherapeutic agent is dose limiting pulmonary activity. In the present study, bleomycin was encapsulated in niosomes and the concentration of bleomycin at tumor site and other tissues was monitored. The pharmacokinetic study of niosomal bleomycin was also carried out.

### METHODS & MATERIALS

Bleomycin hydrochloride was dissolved in phosphate buffered saline and calibrated using 0.375 - 30 µg/ml concentrations and the absorbance was measured at 254 nm. Similarly, the standard curve for bleomycin was drawn using 0.375 - 10 µg/ml of pooled tumor sarcoma-180(S-180) bearing mice plasma using HPLC method and UV detection. To 1.0 ml of plasma in a # 10 ml glass stoppered conical centrifuge tube, was added 200 µl of a 20%

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trichloroacetic acid solution. The samples were gently vortexed and then centrifuged at 3000 rpm for 10 min. A 50  $\mu$ l aliquot of the clear supernate was then injected on to the column and peak heights were determined. The HPLC analysis was carried out using a Gilson 305 programmable instrument equipped with the fixed wave length (254 nm) UV detector. A Shimadzu CR-3A integrator was connected to the detector. The C<sub>18</sub> guard column and a C<sub>18</sub> phenomenex 3 micro analytical column were connected to a Rheodyne injector.

The mobile phase consisted of methanol-acetonitrile - 0.0085 M heptane sulfonic acid - acetic acid (30:10:59:1). A flow rate of 2 ml/min was established (2.3 Kpsi). A minimum of 3 injections from each sample of bleomycin were made and the mean of the peak height was used for determination of the calibration curve. The procedure was repeated for 3 times for each data point to statistically validate the calibration curve.

The mice were of BALB/c strain, obtained from the Cancer Research Institute, Bombay, India and reared by brother sister mating. Mice were housed in polypropylene cages containing sterile paddy husk as bedding and maintained under controlled conditions of temperature ( $23 \pm 2^\circ$ ), humidity ( $50 \pm 5\%$ ) and light (10:14H of light and dark respectively). The animals were fed balanced diet water ad libitum. The tumor sarcoma-180 (S-180) was obtained from Cancer Research Institute, Bombay, India in ascites form and was maintained by serial transplantation. Ehrlich ascites was obtained from Amla Cancer Research Centre, Trichur, India in ascites form and was maintained by serial transplantation.

Ascites fluid from the intraperitoneal cavity of the donor animal was aseptically aspirated using hypodermic syringe with 18 gauge needle, 7-8 days after tumor inoculation. A small portion of ascites fluid (100  $\mu$ l) was tested for bacterial contamination. Tumor viability was determined by the trypan blue dye exclusion test and cells were counted using an

electronic cell counter (Symex F300, Toa Medical Electronics Co., Kobe, Japan). The aspirate was diluted in Dulbecco's modified Eagle's medium (DMEM) to get a concentration of  $5 \times 10^6$  cells/ml and 0.2 ml of this tumor cell suspension ( $1 \times 10^6$ ) was injected intraperitoneally to generate ascites tumor. After 7-8 days, the mice were used for the next propagation or transplantation. Ehrlich ascites was also propagated and maintained in a similar manner.

Solid tumors for the experiments were produced by the intradermal inoculation of  $5 \times 10^4$  viable tumor cells on the dorsum of mice. Once the palpable tumor appears, the tumor diameter in 3 perpendicular planes (D<sub>1</sub>, D<sub>2</sub>, D<sub>3</sub>) were measured thrice a week. The tumor volume (V) was calculated from the standard formula.

$$V = \frac{\pi}{6} (D_1, D_2, D_3)$$

Tumors of the size  $100 \pm \text{mm}^3$  (10-14 days after inoculation) were taken for the experiments.

#### **Preparation of niosomal bleomycin by lipid layer hydration method**

Nonionic surfactants like sorbitan esters (Span 20, 40 and 60), polyoxyethylene sorbitan esters (Tween 20 and 80) Polyoxyethylene ethers (Brij 35 & 78) and polyoxypropylene - polyoxyethylene block co-polymer (PF 108) were chosen for the preparation of niosomes.

Niosomes were prepared by dissolving cholesterol, surfactant and dicetyl phosphate (47.5:47.5:5) (71.25:71.25:5 mg) in chloroform and evaporating the solvent using rotary flash evaporator under low pressure at  $40-50^\circ$ . Niosomes were formed by adding part by part of phosphate buffered saline containing BLM to the dried thin film formed on the walls of the round bottom flask heated to about  $40 - 50^\circ$  on a water bath with gentle agitation. The mixture was intermittently mixed on a vortex to get a good

dispersion of the mixture. Sonic dispersion of the mixture was carried out at 25° using a probe sonicator, 20 KHz, 500 W (Vibra cell, Sonics and Materials Inc. Co., USA) for 30 sec. at one minute intervals for a period of 4 min. After sonication, the suspension was maintained at room temperature for two hours to allow niosomes to form and seal. The BLM entrapment was determined by column chromatography.

### **Plasma Kinetics and tissue distribution profiles of free and niosomal BLM**

Plasma kinetics and tissue distribution studies were carried out in BALB/c mice, bearing sarcoma-180 tumor. Mice weighing 20-25g were distributed in two groups, each group containing 20 animals. The mice were then randomly sorted into 2 groups and treated as follows : Group I - Free BLM, Group II Niosomal BLM.

Both the groups received drug equivalent to 10 mg/kg body weight, intravenously. Following the treatment blood samples were collected from the retro orbit puncture into heparin rinsed haemocrit tubes (from four animals in each group for each point) at pre-determined time intervals, 5, 10, 15, 20, 30, 45 min and 1, 1.5, 2, 3, 5 and 8 h. The blood was allowed to stand for 5 min. immediately after collection and then centrifuged to get the plasma.

Separate group (five mice per group) of mice were sacrificed 4, 8 and 12 h. after injection of free and niosomal BLM and the various tissues - tumor, liver, kidney, lung, spleen, brain, skin and small intestine were removed and assayed for BLM.

To one gram of the tissue (weighed after drying the tissues on a coarse filter paper) one ml of water was added and tip sonicated in an ice bath for half a minute to two minutes depending on the hardness of the tissue in question. Sonication was carried out at maximum frequency tolerable by the tip and 100% duty cycle. The homogenate was vortexed for 5 min.

on a vortex. On vortexing 200 µl of 20% trichloroacetic acid was added to precipitate the proteins. The rest of the steps followed were similar to that adopted for determination of calibration curve for BLM in plasma of S-180 bearing mice.

## **RESULTS & DISCUSSION**

BALB/c mice bearing S-180 tumor were administered free or niosomal bleomycin at 4, 8 and 24 h. post injection. Five mice in each treatment group were sacrificed and the drug levels were determined in specific isolated tissues.

### **Liver**

At 4 h about 0.44 µg/g of the drug was localised in the liver following FBLM injection. The drug level decreased to about 0.24 µg/g at the end of 24 h. Niosomes encapsulation had the effect of enhancing the total amount of drug accumulation of the liver. Though the drug level at 24 h relatively decreased, this level was still greater than that of free bleomycin at any point during the study period.

### **Kidney**

Significant accumulation of drug in kidney was observed in the Free bleomycin treated group, when compared to that of Niosomal bleomycin treated group. From concentration of 1.2 µg/g at 4 h FBIM concentration decreased to 0.38 µg/g by the end of 24 h. Whereas in case of niosomal bleomycin only 0.58 µg/g was achieved at 4 h which declined to 0.38 µg/g at the end of 24 h.

### **Spleen**

The comparative drug concentration in spleen at 4, 8 and 24 h after injection of free bleomycin and niosomal bleomycin were determined. Spleen displayed maximum bleomycin concentration at all the points studied following niosomal administration. Following bleomycin administration, the bleomycin

**Table - 1: Tissue disposition of Free BLM and Niosomal BLM in BALB/c mice ( $\mu\text{g/g}$  tissue  $\pm$  S.D.)**

Tissue	4h		8h		24h	
	FBLM	NBLM	FBLM	NBLM	FBLM	NBLM
Liver	0.44 $\pm$ 0.23	0.86 $\pm$ 0.21	0.32 $\pm$ 0.14	0.70 $\pm$ 0.24	0.24 $\pm$ 0.06	0.49 $\pm$ 0.18
Kidney	1.20 $\pm$ 0.31	0.58 $\pm$ 0.20	0.72 $\pm$ 0.22	0.49 $\pm$ 0.18	0.38 $\pm$ 0.18	0.42 $\pm$ 0.11
Spleen	0.42 $\pm$ 0.12	1.46 $\pm$ 0.29	0.38 $\pm$ 0.16	1.23 $\pm$ 0.31	0.36 $\pm$ 0.16	0.95 $\pm$ 0.24
Lung	1.34 $\pm$ 0.31	0.61 $\pm$ 0.22	0.86 $\pm$ 0.28	0.49 $\pm$ 0.18	0.38 $\pm$ 0.21	0.38 $\pm$ 0.12
Brain	0.22 $\pm$ 0.03	—	0.16 $\pm$ 0.02	—	—	—
Skin	0.14 $\pm$ 0.01	—	—	—	—	—
Solid tumor	0.56 $\pm$ 0.22	0.82 $\pm$ 0.23	0.62 $\pm$ 0.19	0.96 $\pm$ 0.29	0.59 $\pm$ 0.16	1.28 $\pm$ 0.31
Small intestine	0.38 $\pm$ 0.12	0.19 $\pm$ 0.02	0.30 $\pm$ 0.11	—	0.22 $\pm$ 0.06	—

Number of animals used per time interval : 5

disposition in the spleen was gradual from 4 h. through 24 h post injection.

### Lung

Accumulation of Bleomycin following free bleomycin treatment was found to be 1.34, 0.86, 0.38  $\mu\text{g/g}$  of tissue at 4, 8 and 24 h. respectively. Drug levels in lungs decreased after injection of niosomal bleomycin treatment. But the fall in drug content was relatively sustained and the amount present at 24 h was the same that of free bleomycin treated group.

### Brain

Accumulation of drug in the brain of free bleomycin was found to be 0.22 and 0.16  $\mu\text{g/g}$  of tissue at 4 and 8 h. The drug concentration decreased to an undetectable level at the end of 24 h. Similarly, with niosomal bleomycin treatment, the drug concentration could not be detected.

### Tumor

After 4 h post treatment, tumor bleomycin level for mice receiving free drug was about 0.56  $\mu\text{g/g}$  tissue. The drug concentration remained almost the

same at the end of 24 h. Administration of vesicle entrapped bleomycin, however, resulted in an uptake pattern which demonstrated net tumor accumulation of 0.82  $\mu\text{g/g}$  at 4 h, which is 1.46 times greater than that for free bleomycin treatment group. This treatment increased to 2 fold at 24 h. Although by 24 h tumor associated bleomycin had decreased to 1.28  $\mu\text{g/g}$  for the niosomal bleomycin treated group, this level was still greater than that for free drug at any point of time during the study period.

### Small intestine

There was accumulation of bleomycin in small intestine after treatment with free and niosomal bleomycin. A considerable amount of the drug still remained 8 h after free bleomycin administration and about 22  $\mu\text{g/g}$  was found even after 24 h. After encapsulation, a significant decrease in the drug concentration was observed in the small intestine 4 h after injection. The drug concentration further declined with time and fell below the detectable range.

### Plasma Kinetics

Ohnuma et al (1974)<sup>11</sup> found that bleomycin was cleared rapidly in the initial phase followed by

a slow phase plasma drug clearance. A similar pattern of drug elimination was observed in the present study. However, when the drug was administered in the niosomal form the pharmacokinetics of bleomycin was markedly altered. The plasma levels of niosomal bleomycin treated animals were higher for almost the entire course of study, than when the free bleomycin was given alone confirming the sustained release characteristics of niosomes.

Plasma kinetics Free Bleomycin

$AUC_{0-8} = 9.10$

$AUC_{0-\infty} = 9.84$

$t_{1/2} \text{ (hr)} = 3.65$

$K_{el} \text{ (}\beta\text{)} = 0.19$

Plasma kinetics Niosomal Bleomycin

$AUC_{0-8} = 94.63$

$AUC_{0-\infty} = 96.26$

$t_{1/2} \text{ (hr)} = 0.29$

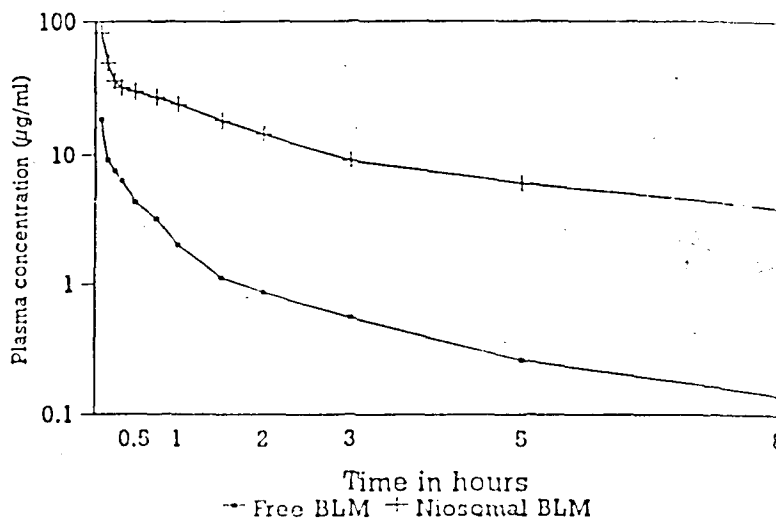
$K_{el} \text{ (}\beta\text{)} = 2.36$

After treatment with free bleomycin, a small but significant amount of drug was detected in the small intestine, but maximum amount was found in the lung and kidney. The favourite site of accumulation for encapsulated drug are liver and spleen. After encapsulation a significant increase in liver and spleen bleomycin levels were noted, while there was almost a two fold decrease in the lung and small intestine. The decrease in small intestine and lung drug level was reflected in the absence of gastrointestinal and pulmonary toxicities after niosomal bleomycin administration.

In most instances, encapsulation of drugs in vesicular drug carriers results in enhanced accumulation in reticulo-endothelial cell rich tissues such as liver and spleen. The same phenomenon was also observed in the present study after encapsulating bleomycin within niosomes. Encapsulation also resulted in reduced accumulation in gut and kidney.

Tumor bleomycin concentration was increased by 60% after encapsulation. Even after 24 h niosomal bleomycin levels were significantly higher, the tumor drug levels were more than that of free drug at any point during the study periods. These results suggested that administration of bleomycin loaded

Plasma clearance of BLM following i.v. injection of FBLM and NBLM in BALB/c mice bearing sarcoma-180 tumor



niosomes increase the life span of tumor bearing mice and decreased the rate of proliferation of the sarcoma. Sustained release of bleomycin from vesicle depot site such as liver or spleen, could possibly account for the observed pattern of blood bleomycin levels. The prolonged drug levels in plasma with niosomal bleomycin indicate a lower rate of clearance, as well as decrease in non-specific binding and a selective sequestration of the drug in particular tissues.

## CONCLUSION

Subsequent to intravenous administration, the niosome encapsulated bleomycin was cleared from the plasma much more slowly than was the free drug. A markedly enhanced plasma concentration of drug was achieved in mice when it was administered in niosomes compared to free bleomycin.

Encapsulation caused a marked alteration in the tissue disposition of the injected drug. Thus the tumor bleomycin level was enhanced significantly.

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