J. Pharm. Pharmacol. 1996, 48: 1128–1132
Received November 6, 1996
Accepted May 21, 1996

Niosomal Plumbagin with Reduced Toxicity and Improved Anticancer Activity in BALB/C Mice

R. A. RAJA NARESH, N. UDUPA AND P. UMA DEVI

College of Pharmaceutical Sciences & Department of Radiobiology, KMC, Manipal, 576 119, India

Abstract

Plumbagin niosomes were prepared using a lipid layer hydration method, and drug entrapment was measured. The acute toxicity studies were conducted following treatment with free and niosomal plumbagin.

The antitumour activity of niosomal plumbagin in a solid tumour (sarcoma-180) and Ehrlich ascites model was evaluated. Niosome-encapsulated plumbagin was less toxic than free drug. The antitumour activity of the drug was also better after encapsulation.

The better anticancer activity can be justified with the help of LD50 survival studies and study of tumour volume doubling time.

Chemotherapy of cancer has withstood several years of criticism and is now an established means of cancer treatment. In such instances, systematic therapy of cancer using chemical agents has been proved to be useful (Carter 1984). The inherent problem of non-specificity of chemotherapeutic agents is a major area of research interest as antineoplastic agents are neither specific nor targeted to cancer cells. Improved delivery of anticancer drugs to tumour tissues thus appears to be a challenging and achievable effort.

Chemical modifications such as altering the partition coefficient, preparation of prodrugs and binding of immunological ligands has met with little success. Physical approaches to the delivery of anticancer drugs consists of microparticulate drug carriers (liposomes, microspheres, nanoparticles) magnetic microcapsules, implantable pumps and reservoirs. Some success has been reported in the areas of enhancing efficacy and reducing drug toxicity (Gabizon et al 1982; Olson et al 1982; Rahman et al 1985). As antitumour agents have a high potential to induce side-effects and toxicity, localization of the drugs to tumour sites would certainly optimize therapy.

Certain carriers like microspheres (Widder et al 1982), nanoparticles (Couvreur et al 1980), liposomes (Alving 1983), cellular carriers like erythrocytes and lymphocytes (Zimmerman et al 1978; Lewis 1984a,b) may be used to ferry the drug to the required site.

Formation of vesicular structure from a mono-alkyl, non-ionic surfactant has been reported (Handjani Vila et al 1979; Baillie et al 1985). These vesicles, termed niosomes, can entrap solutes, are osmotically active and are stable (Baillie et al 1984). In addition, handling and storage of the surfactant requires no special conditions. Niosomes behave in-vivo like liposomes, prolonging the circulation of entrapped drug and altering its organ distribution and metabolic stability (Azmin et al 1985). Inclusion of cholesterol in the preparation of niosomes has been demonstrated to alter the properties of niosomes and markedly decrease efflux-entrapped solute (Baillie et al 1985).

Correspondence: N. Udupa, College of Pharmaceutical Sciences & Department of Radiobiology, KMC, Manipal, 576 119, India.

The effect of non-ionic surfactant vesicle entrapment on the absorption and distribution of the anticancer agent methotrexate in mice has been reported by Azmin et al (1985). Niosomes prepared from non-ionic surfactant, cholesterol and dicetyl phosphate, and containing methotrexate, administered intravenously to mice, maintained the level of methotrexate in the blood.

A large amount of the drug was taken up by the liver after niosomal intravenous administration. There was an indication of increased uptake of methotrexate into the brain, perhaps due to an effect of the niosome components on the permeability of the blood-brain barrier. The metabolic profile of the drug was altered and no adverse effects were observed. Another study, conducted by Rogerson et al (1988), compared the distribution of antitumour efficacy of doxorubicin encapsulated in niosomes with an equivalent dose of the free drug administered to S-180 tumour-bearing mice. The results indicated that the half-life of the niosomal drug was prolonged compared with the half-life in free solution, hence the potential increase in duration of action of the niosomal drug. Tumour levels of the drug were higher following administration of doxorubicin in niosome and this was reflected in more effective reduction in tumour growth. Metabolism of doxorubicin was also altered.

Baillie et al (1986), compared the efficacy of sodium stibogluconate, an anti-leishmanial drug, after its administration to mice in the free, liposomal and niosomal form. High liver and low serum values were attained by the use of both vesicular formulations and they were shown to be more active than free drug against experimental murine leishmaniasis.

Plumbagin, a naphthoquinone derivative having the structure 2-methyl-5-hydroxy-1,4-napthoquinone has been obtained from *Plumbagin rosea*, *P. capensis*, *P. europaea*, *P. larpentae*, *P. scandens* and *P. zeylancia*. Plumbagin, when administered orally at a dose of 4 mg kg^{-1} body-weight, induced tumour regression in 3-methyl-4-dimethylaminoazebenzene (3MeDAB)-induced hepatoman Wistar male rats. The rate of glycolysis and gluconeogenesis increased in hepatoma rats, whereas they decreased in plumbagin-treated rats to near normal levels (Parimala & Sachdanandam 1993). Plumbagin

caused regression of experimental animal tumours (Nagarajan & Chandrasekaran 1985; Purushothaman et al 1985). The administration of plumbagin is associated with severe acute toxicity. Many reports in various animal models reveal a consistent ability of liposome encapsulation to ameliorate both acute and chronic toxic side-effects of anticancer drugs. In this paper we report a pilot experiment to explore the potential of plumbagin delivery in non-ionic surfactant vesicles.

Materials and Methods

Drugs

Pure plumbagin (1 mg) was dissolved in minimum quantity of alcohol (1 mL) and made up to volume with buffered saline. Plumbagin solutions of 1–20 μg mL were prepared and the absorbance was measured at 422 nm to prepare a standard curve.

Plumbagin toxicity

The toxicity of various doses of the plumbagin formulation was investigated by determining the apparent LD50 of drug administered via tail vein injection to BALB/c mice (20–25 g). Groups of 10 mice per dose were monitored over 14 days and the deaths were noted. Group 1 (solvent control) was injected intravenously with 0·2 mL of PEG 400 empty niosomes; groups 2–6 were injected intravenously with unencapsulated plumbagin at single doses of 4, 6, 8, 10 and 12 mg kg^{-1}; groups 7–12 were injected intravenously with niosomal plumbagin at single doses of 4, 6, 8, 10, 12 and 14 mg kg^{-1}. Following the injections, the animals were placed in separate cages for close observation. The toxic effect was assessed on the basis of mortality.

Tumour models

The antitumour efficacy of plumbagin was assessed using two tumour models: sarcoma-180 (solid tumour) and Ehrlich ascites. 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 ($23 \pm 2°C$), humidity ($50 \pm 5\%$) and light (10:14 h of light and dark respectively). The animals were fed a balanced diet with water freely available. The tumour sarcoma-180 (S-180) was obtained from the Cancer Research Institute, Bombay, India, in ascites form and was maintained by serial transplantation. Ehrlich ascites was obtained from the Amla Cancer Research Centre, Trichur, India, in ascites form and was maintained by serial transplantation.

For tumour propagation, ascites fluid from the intraperitoneal cavity of the donor animal was aseptically aspirated using a hypodermic syringe with a 18 gauge needle, 7–8 days after tumour inoculation. A small portion of ascites fluid (100 μg) was tested for bacterial contamination. Tumour viability was determined by the tryphan blue dye exclusion test and cells were counted using electronic cell counter (Sysmex F300, Toa Medical Electronics Co., Kobe, Japan). The aspirate was diluted in Dulbecco's modified Eagle's medium (DMEM) to a concentration of 5×10^6 cells mL^{-1} and 0·2 mL of this tumour cell suspension (1×10^6 cells) was injected intraperitoneally to generate ascites tumour. After 7–8 days, the mice were used for the next propagation or transplantation. Ehrlich ascites was propagated and maintained on similar lines. Solid tumours for the experiments were produced by the intradermal inoculation of 5×10^5 viable tumour cells on the dorsum of mice. The tumour volume was calculated after measuring the diameter. Tumours of the size 100 ± 10 mm^3 (10–14 days after inoculation) were taken for investigation.

Preparation of niosomes

A known quantity of plumbagin (10 mL) was dissolved in a minimum quantity of absolute alcohol (300 μg) and the volume was made up to 3 mL with phosphate buffered-saline containing 30% PEG 400. Lipid hydration method (Azmin et al 1985) was used for preparation of plumbagin niosomes. Cholesterol, surfactant (Span 60) and dicetyl phosphate (47·5:47·5:5) (71·2:71·2:2:7 mg) were dissolved in chloroform and the solvent evaporated using a rotary flash evaporator, under low pressure at 40–50°C for preparing niosomes. Niosomes were formed by adding the plumbagin solution slowly to the dried thin film formed on the walls of the round-bottomed flask heated to about 40–50°C on a water bath with gentle agitation. The mixture was intermittently mixed on a vortex. Dispersion of the mixture was carried out at 25°C using a probe sonicator, 20 kHz, 500-W vibra cell (Sonics and Materials Inc. Co. USA) for 30 s at 1-min intervals for a period of 4 min. After sonication, the suspension was maintained at room temperature for 2 h to allow niosomes to form and seal. Unentrapped plumbagin was separated from niosomes by gel filtration on a Sephadex G-50 column.

Assessment of antitumour efficacy

The antitumour efficacy of plumbagin in its free and niosomal forms was evaluated in tumour-bearing mice. Animals were divided into different groups (20 animals per group in the case of Ehrlich ascites and 10 animals per group for solid tumour) as shown in Table 1. In the case of the solid tumour, treatment was started after the tumour size reached 100 ± 10 mm^3. Indicated doses of free plumbagin, niosomal plumbagin in saline and empty niosomes were injected intravenously and the tumour growth was monitored, while mice bearing Ehrlich ascites were treated (intravenously) 48 h after tumour inoculation. The time required to double the tumour volume from 100 to 200 mm^3 was taken as criterion to assess the antitumour efficacy of free and niosomal plumbagin in S-180-bearing mice. For Ehrlich ascites, percent increase in life span (ILS) (animal survival rate) was considered to determine the antitumour efficacy. Mortality at different post treatment days was recorded for each group.

Results and Discussion

The plumbagin niosomes, prepared by lipid hydration using a combination of cholesterol/Span 60/dicetyl phosphate (47·5:47·5:5), were able to entrap 52% of the plumbagin. The mean size of niosomes was 10–15 μm.

Plumbagin toxicity

The toxicity of free and niosomal plumbagin preparations were ascertained by 14-day dose-response survival studies in BALB/c mice. A single dose of 4 mg kg^{-1} of free plumbagin produced no mortality, while 20% animals died within 48 to

Table 1. Treatment of sarcoma-180- (n = 20) and Ehrlich ascites- (n = 20) bearing BALB/c mice with niosomal plumbagin.

Group no.	Treatment	Dose (mg kg^{-1})
1	Control	—
2	Empty niosomes	—
3	FPLM	2
4	FPLM	3
5	FPLM	4
6	FPLM	5
7	FPLM	6
8	NPLM	2
9	NPLM	3
10	NPLM	4
11	NPLM	8
12	NPLM	10

FPLM = free plumbagin; NPLM = niosomal plumbagin.

72 h following an injection of 6 mg kg^{-1} dosage and a sharp rise in mortality was observed at 10 mg kg^{-1}. The animals injected with 10 mg kg^{-1} showed toxic symptoms such as weight loss, diarrhoea, ruffling of hair, drowsiness, lethargy, convulsions, mild tremor in the tail, reduced food intake and poor response to external stimuli. The LD50 for free plumbagin on day 14 was found to be 7·99 mg kg^{-1} body-weight. (Table 2).

In contrast, 4 to 8 mg kg^{-1} of niosomal plumbagin showed no mortality. On administration of 10 mg kg^{-1} of niosomal plumbagin, 20% of the animals died within 48 to 72 h. With 12 mg kg^{-1} niosomal plumbagin there was a sharp rise in mortality and development of toxic symptoms such as ruffling of hair, mild tremor in the tail, weight loss and reduction in food intake. The LD50 for niosomal plumbagin (Table 2) on day 14 was found to be 9·34 mg kg^{-1} body-weight.

Antitumour efficacy

The niosome containing only PEG 400 did not have any effect on the ascites tumour growth. All the animals developed tumour and died within 16 to 22 days which was similar to untreated control. The lower dose, 2 mg kg^{-1}, of free plumbagin produced a non significant increase in % ILS compared with control, but the response increased with increase in drug dose. Doses of 3 to 5 mg kg^{-1} of free plumbagin gave a similar effect and produced 10 to 15% of survival at 120 days. There was an increase in % ILS for 4 and 5 mg kg^{-1} compared with 3 mg kg^{-1} free plumbagin. The maximum effect however was seen at 6 mg kg^{-1}, with 20% of survival at 120 days.

In the case of niosomal plumbagin, 2 mg kg^{-1} did not show any effect on tumour growth, but a dose of 3 mg kg^{-1} produced a significant increase in % ILS compared with 3 mg kg^{-1} of free plumbagin. Niosomal plumbagin at 4 to 6 mg kg^{-1} gave almost similar effect, but 7 and 10 mg kg^{-1} showed an increase in MST and ILS % compared with 6 mg kg^{-1} niosomal plumbagin. Though a dose of

Table 2. Acute intravenous toxicity (14 days) of free and niosomal plumbagin in BALB/c mice.

	Free plumbagin						Niosomal plumbagin			
Group	Probit value	Dose (mg kg^{-1})	Log dose	Mortality (total)	Mortality (%)	Corrected (%)	Probit Value	Mortality (total)	Mortality (%)	Corrected (%)
1	3·04	4	0·60	0/10	0·00	2·50	3·04	0/10	0.00	2.50
2	3.04	6	0·78	1/10	10·00	10·00	3·72	0/10	0·00	2·50
3	3·72	8	0·90	3/10	30·00	30·00	4·48	1/10	10·00	10·00
4	4·48	10	1·00	7/10	70·00	70·00	5·52	3/10	30·00	30·00
5	6·28	12	1·07	10/10	100·00	97·50	6·96	9/10	90·00	90·00
6	6·98	14	1·14	—	—	—	—	10/10	100·00	97·50

Corrected formula: for the 0% mortality = 100 × (0·25/n); for the 100% mortality = 100 × (n − (0.25/n)). LD50: 7·99 mg (kg mouse weight)$^{-1}$ for free plumbagin and 9·34 mg (kg mouse weight)$^{-1}$ for niosomal plumbagin.

Table 3. Antitumour activity of single modality treatment with free plumbagin and niosomal plumbagin in BLAB/c mice bearing Ehrlich ascites cells.

Sample	Dose (mg kg^{-1})	Survival on day 120	Time (days) (mean)	ILS (%)	N/F[a]
Control	—	—	18·00	—	—
Empty niosomes	—	—	18·00	—	—
Free plumbagin	2	—	19·80	10·00	—
	3	10	21·60	20·00	—
	4	15	24·80	37·77	—
	5	15	25·80	43·33	—
Niosomal plumbagin	2	10	20·60	14·44	1·04
	3	25	24·80	37·77[c]	1·15[b]
	4	35	29·60	64·44[c]	1·19[b]
	6	35	34·40	91·11[c]	1·29[b]
	8	50	40·00	122·22[b]	—
	10	40	42·60	136·66[b]	—

Number of animals used per group = 20. [a]niosomal/free mean survival time; [b]significant at $P < 0.05$ level; [c]significantly different ($P < 0.05$) from the respective controls.

Table 4. Tumour volume doubling time of S-180 after treatment with free plumbagin or niosomal plumbagin.

Group no.	Treatment	VDT (days)
1	Control	[b]3·14 ± 0·10
2	Empty niosomes	3·20 ± 0·12
3	2 mg kg^{-1} FPLM	[b]3·58 ± 0·14
4	3 mg kg^{-1} FPLM	[b]4·48 ± 0·26
5	4 mg kg^{-1} FPLM	[b]5·06 ± 0·32
6	6 mg kg^{-1} FPLM	[b]5·86 ± 0·36
7	2 mg kg^{-1} NPLM	[a,c,b]3·88 ± 0·16
8	3 mg kg^{-1} NPLM	[c,b]6·64 ± 0·28
9	4 mg kg^{-1} NPLM	[c,b]7·12 ± 0·38
10	6 mg kg^{-1} NPLM	[c,b]7·64 ± 0·42
11	8 mg kg^{-1} NPLM	[b]8·82 ± 0·28
12	10 mg kg^{-1} NPLM	[b]10·14 ± 0·30

FPLM = free plumbagin; NPLM = niosomal plumbagin. VDT is the tumour volume doubling time presented as mean ± s.e.m. Number of animals used per group = 10. All the groups except Group 2 are significant ($P < 0·05$) when compared with control. [a]significantly different at $P < 0·05$ when compared with Group 7. [b]significantly different at $P < 0·05$ from their respective controls. [c]significantly different at $P < 0·05$ when compared with Group 7, 8, 9 and 10.

10 mg kg^{-1} of niosomal plumbagin showed a slight increase in MST, the percent survival at 120 days decreased to be 40%, indicating that the maximum tolerated dose of NPLM in this system was 10 mg kg^{-1} (Table 3).

There was no spontaneous regression of the tumour in the untreated control animals and all the animals died of disease between 20 and 40 days. The tumours in empty niosome control animals developed to a measurable size by 6 to 8 days after tumour transplantation and the growth curve was exponential. Both the untreated and empty niosome-treated control groups showed identical tumour growth and there was no significant difference in the volume doubling time (Table 4).

Both free plumbagin and niosomal plumbagin at all the dose levels tried, increased volume doubling time significantly ($P < 0·05$) when compared with the control animals. The effect of plumbagin on the tumour growth weight was dose-dependent as evidenced by the increase in tumour volume doubling time values. Increasing the dose of free plumbagin from 2 to 3 mg kg^{-1} increased the tumour volume doubling time significantly. Increasing the dose from 3 to 6 mg kg^{-1} did not show any marked increase in the volume doubling time of animals treated with free plumbagin.

In contrast, animals treated with niosomal plumbagin displayed significantly increased tumour volume doubling times at all dose levels except 2 mg kg^{-1}. Niosomal plumbagin significantly increased ($P < 0·05$) tumour volume doubling time even at a dose level of 3 mg kg^{-1}. Moreover, it produced a significant increase in tumour volume doubling time compared with 2, 3, 4 and 6 mg kg^{-1} free plumbagin. Increasing the niosomal plumbagin dose from 3 to 4 mg kg^{-1} or from 4 to 6 mg kg^{-1} or from 6 to 8 mg kg^{-1} did not produce significant increases in the tumour volume doubling time. The body weight of mice did not vary during the one-week observation. An increase in body weight was observed in mice treated with 2 mg kg^{-1} of free plumbagin and 2 and 3 mg kg^{-1} niosomal plumbagin. Increasing the dose of free plumbagin resulted in a progressive loss of body weight. In contrast, the animals treated with niosomal plumbagin appeared normal with minimal body weight changes (Table 5).

Table 5. Percentage change in body weight 7 days after injection of different doses of free plumbagin or niosomal plumbagin.

Dose (mg kg^{-1})	Percentage change in body weight	
	FPLM	NPLM
2	+ 1·2	+ 1·9
3	− 4·9	+ 2·2
4	− 5·2	− 1·8
5	− 5·9	—
6	− 7·2	− 4·2
8	0·0	− 6·6
10	0·0	− 8·9

FPLM = free plumbagin; NPLM = niosomal plumbagin. Number of animals used per group = 10. The data are presented as mean values.

Liposomes have the ability to alter the in-vivo drug distribution which has many therapeutic advantages (Gregoriadis & Florence 1993). Using this modality, it could be possible to link selective drug delivery to local treatment of tumours and potentiate the drug effects. The present results show that free plumbagin is tolerated at a dose of 6 mg kg^{-1} without any acute side-effects. But above this dose there is a sharp increase in toxicity. It was observed that animals which survive the acute lethal effect up to three days are able to recover and continue to survive at 14 days. The toxic effect of plumbagin on chick embryo fibroblasts was studied by Santhakumari et al (1980) who observed that plumbagin at lower concentrations behaves like a spindle poison, while at higher concentrations it exhibits radiomimetic nucleotoxic and cytotoxic effects. On the contrary, niosomal plumbagin was found to be safer than free plumbagin, as evidenced by significant increase in LD50 of niosomal plumbagin compared with the free substance. The dose-response survival curve in mice also indicated a positive reduction in mortality, which is an index for reduction in toxicity, following niosomal plumbagin administration. Apart from an adverse effect on mortality, morbidity was also affected by lethal doses of free plumbagin by way of ruffling of hair, decrease in food and water intake and weight loss. This may have been due to a high degree of cell kill of rapidly dividing gastrointestinal mucosal cells. The aforementioned adverse effects were not observed after niosomal plumbagin administration, thus demonstrating a better safety profile for the niosomal formulation.

The antitumour activity of niosome-encapsulated plumbagin was compared with that of the free drug. A maximum ILS of 136·66% was achieved with 10 mg kg^{-1} niosomal plumbagin while with 6 mg kg^{-1} free plumbagin it was only 47·77%. Niosomal plumbagin at 6 mg kg^{-1} showed an almost 2-fold increase in % ILS. This results from enhanced antitumour activity over free plumbagin at equivalent doses as well as ease of injection of elevated doses. Further, niosomal/free mean survival time values as high as 1·29 were achieved. Moreover, niosomal plumbagin at doses of 3, 4, 6 and 8 mg kg^{-1} significantly increased survival rate when compared with the respective controls.

With free and niosomal plumbagin a linear dose-dependent increase in the tumour response was observed. Niosomal plumbagin showed increased cytotoxic effect as revealed by the increased tumour volume doubling time. This finding

suggests a marked effect on cell-growth kinetics. Similar findings were reported with doxorubicin-loaded niosomes in this tumour model (Rogerson et al 1988). Rahman et al (1985) reported the therapeutic effectiveness of free and encapsulated doxorubicin in murine P 388 leukemia. They observed a significant increase in life span for doxorubicin-loaded liposomes when compared with free drug. Even methotrexate, when encapsulated in niosomes or liposomes, was found to be more effective compared with free drug in experimental tumour models.

Earlier studies have demonstrated that the distribution and accumulation patterns were similar with liposomal and niosomal encapsulation, despite the chemical differences between these carriers (Baillie et al 1986). Sodium stibogluconate, an antileishmanial agent was administered to mice in free, liposomal and niosomal forms. Both the microencapsulated formulations studied had similar efficacy indicating that the chemical differences (non-ionic surfactants and phospholipids) between these carrier systems are relatively unimportant. In the present study, the antitumour efficacy and life span of niosomal plumbagin was substantially increased when compared to free plumbagin. An increased LD50 observed with encapsulated plumbagin over free plumbagin clearly indicates the potential of niosomes as drug carriers for plumbagin.

References

Alving, C. R. (1983) Delivery of liposome-encapsulated drugs to macrophages. Pharmacol. Ther. 22: 407–424

Azmin, M. N., Florence, A. T., Handjani Vila, R. M., Stewart, J. F., Vanlerberghe, G., Whittaker, J. S. (1985) The effect of non ionic surfactant vesicle (niosome) entrapment on the absorption and distribution of methotrexate in mice. J. Pharm. Pharmacol. 37: 237–242

Baillie, A. J., Florence, A. T., Hume, L. R., Muirhead, G. T., Rogerson, A. (1984) The effect of cholesterol on single chain, non-ionic surfactant vesicles. J. Pharm. Pharmacol (Suppl.) 36: 488P

Baillie, A. J., Florence, A. T., Hume, L. R., Muirhead, G. T., Rogerson, A. (1985) The preparation and properties of niosomes-non-ionic surfactant vesicles. J. Pharm. Pharmacol. 37: 863–868

Baillie, A. J., Coombs, G. H., Dolan, T. F., Laurio, J. (1986) Non ionic surfactant vesicle, niosomes, as delivery system for the anti-leishmanial drug, sodium stibogluconate. J. Pharm. Pharmacol. 38: 502–505

Carter, S. K. (1984) Some thoughts on resistance to cancer chemotherapy. Cancer Treat Rev. II (Suppl. A): 3–7

Couvreur, P., Kante, B., Lenaerts, V., Scaitteur, V., Ronald M., Speiser, P. (1980) Tissue distribution of antitumor drug associated with polyalkyl cyano acrylate nanoparticles. J. Pharm. Sci. 69: 199–202

Gabizon, A., Dagan, A., Goren, D., Barenholz, Y., Fuks, Z. (1982) Liposomes as in vivo carriers of adriamycin; reduced cardiac uptake and preserved antitumor activity in mice. Cancer Res. 42: 4734–4739

Gregoriadis, G., Florence, A. T. (1993) Liposomes in drug delivery, clinical, diagnostic and ophthalmic potential. Drugs 45: 15–28

Handjani-Vila, R. M., Ribier, A., Rondot, B., Vanerberghe, G. (1979) Dispersion of lamellar phases of non-ionic lipids in cosmetic products. Int. J. Cosmetic Sci. 1: 303–314

Lewis, D. A. (1984a) Red blood cells for drug delivery. Pharm. J. 233: 384–385

Lewis, D. A. (1984b) Therapeutic possibilities of drugs encapsulated in erythrocytes. Int. J. Pharm. 22: 137–146

Nagarajan, B., Chandrasekaran, B. (1985) Plant extracts echitamine and plumbagin possess antitumor activity. Abstracts of papers presented. Satellite symposium on traditional medicine as adjunct to Asian congress of Pharmacology.

Olson, F., Mayhew, E., Maslow, D., Rustom, Y., Szoke, F. (1982) Characterization toxicity and therapeutic efficacy of adriamycin encapsulated in liposomes. Eur. J. Clin. Oncol. 18: 167–176

Parimala, R., Sachdanandam, P. (1993) Effect of plumbagin on some glucose metabolizing enzymes studied in rats in experimental hepatoma. Mol. Cell. Biochem. 18: 125–159

Purushothaman, K. K., Mohana, K., Susan, T. (1985) Biological profile of plumbagin. Bull. Med. Ethno-Bot. Res. 6: 177

Rahman, A., White, G., More, N., Schein, P. S. (1985) Pharmacological, toxicological and therapeutic evaluation in mice of doxorubicin entrapped in cardiolipin liposomes. Cancer Res. 45: 796–803

Rogerson, A., Cummings, J., Willmott, N., Florence, A.T. (1988) The distribution of doxorubicin in mice following administration in niosomes. J. Pharm. Pharmacol. 40: 337–342

Santhakumari, G., Saralamma, P. G., Radhakrishnan, N. (1980) Effect of plumbagin on cell growth and mitosis, Indian J. Exp. Biol. 18: 215–218

Widder, K. J., Senyei, A. E., Sears, B. (1982) Experimental methods in cancer therapeutics. J. Pharm. Sci. 71: 379–387

Zimmerman, U., Pilwat, G., Esser, B. (1978) The effect of encapsulation in red blood cells on the distribution of methotrexate in mice. J. Clin. Chem. Biochem. 16: 135–147