DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 19(11), 1331-1342 (1993)

FORMULATION AND EVALUATION OF METHOTREXATE NIOSOMES

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ABSTRACT

Methotrexate was encapsulated in niosomes prepared using Tweens and Spans. The size distribution, entrapment efficiency, pharmacokinetics and effect on tumour remission of mice transplanted with S-180 Sarcoma were evaluated. Niosomes prepared with Span 60 gave promising results.

INTRODUCTION

Nonionic surfactant vesicles (niosomes) which are similar to liposomes can be prepared with cholesterol, surfactant and water. Niosomes may reduce the systemic toxicity of anti-cancer drugs. Niosomes may also improve the therapeutic index of drugs by restricting the effects to target cells.[1]

Niosomes are found to improve therapeutic efficacy of drugs in cancer therapy, parasitic, viral and microbial diseases. Many nonionic surfactants like Tweens and Spans, cationic surfactants like cetrimide, sodium dodecyl sulphate are used with cholesterol to entrap drugs in vesicles.[8]

Liver can act as a depot for many drugs where niosomes containing drugs may be taken up by the liver where they are broken down by lysosomal lipase slowly to release the free drug and reenter the circulation. Niosome is slowly degraded providing a more sustained effect. Niosomes are capable of releasing entrapped drug slowly. Niosomes are found to have selective drug delivery potential for cutaneous application of 5-alpha-dihydrotesterone, triamcinolone and intravenous administration of methotrexate for cancer treatment and sodium stilbogluconate in the treatment of lishmaniasis etc.[8]

Azmin and Florence et al used nonionic hydrophilic surfactant like Tween-80 for making niosomes entrapped with methotrexate and studied the pharmacokinetics of methotrexate after intravenous injection to the mice.[2,8] The tissue distribution of methotrexate was improved after entrapping with niosomes. These vesicles were also found to be osmotically stable. Rogerson et al,[11] Baillie et al[6] and Hunter et al[9] have used niosomes as drug carriers for doxorubicin and sodium stilbogluconate for better targeting property.

Cook and Florence have shown that Tween 80 enhanced the cytotoxic effect of podophyllotoxin derivative of etopside, while Brij 30 at levels, at which it was not in itself cytotoxic, does not.[5] Nonionic surfactants increased both fluidity and permeability of biological membranes. Chitnis et al[7] studied the effect of adriamycin dissolved in Tween 80 and compared its antitumour activity with adriamycin dissolved in water on mice bearing lymphocytic leukemia and found that antitumour activity of adriamycin dissolved in aqueous solution of Tween 80 was higher than that of adriamycin dissolved in water.

Azmin et al[2] made niosomes of smaller size around 120 nm but Rogerson et al[11] made larger vesicles of 800-900 nm diameter. Earlier work in mice had suggested that niosomes may suffer uptake by the reticuloendothelial system, methotrexate and sodium stilbogluconate were found to accumulate in liver following administration as niosomes. The absence of accumulation of drugs in the liver supported the evidence of sustained plasma levels of drugs resulting from slow release of circulating rather than trapped vesicles. The larger niosomes could not accumulate in the liver and spleen and could be filtered out in the passage through the lung capillary network. In the treatment of parasitic infection of liver, spleen and bone marrow, niosomes made with surfactants could be very useful.

The cardiotoxicity of adriamycin may be reduced by administering it as niosomes made with surfactants without the loss of therapeutic efficacy.[10] Surfactants like Tween-80 also increased brain level of methotrexate. Analgesic effect and brain level of D-Kyotorphen was enhanced by Tween-80.[8]

METHODS AND MATERIALS

Niosomes were prepared by slight modification of the procedure adopted earlier by Azmin et al.[2] Surfactant (Tween 80, 60, 40 or Span 60, 40, 20) (71.25 mg), cholesterol (71.25 mg) and dicetyl phosphate (7 mg) to give a ratio of 47.5 : 47.5 : 5 were used as the lipid ingredients. These ingredients were dissolved in about 15 ml of diethyl ether in a round bottom flask. The solvent was evaporated under reduced pressure using rotary evaporator. The rotating flask was positioned about 1.5 cm above a boiling water bath, thus depositing a thin layer of the solid mixture on the wall of the flask. Methotrexate (MTX) (5 ml of a 10 mg ml^{-1}. solutions) was added to flask slowly, while warming the flask at about 50^{o}C and with intermittent vortexing, until a good dispersion of the mixture was obtained. The MTX-entrapped niosomes were separated from the unentrapped drug by dialysis as discussed by Hardy et al.[3]

The prepared niosomes were filled into a glass tube to which a sigma dialysis membrane was securely attached to one side and free MTX was dialysed for 30 minutes each time into 500 ml of 0.9% NaCl (Saline). The dialysis of free MTX was always complete after about 10 changes of saline (U.V. detection of 303 nm) when no MTX was detectable in recipient solution. Since the initial amount of MTX used for formulation of niosomes was 50 mg the difference between this and the amount dialysed would yield the amount of MTX entrapped in the niosomes. Measurement of the niosome size was made by using a microscope.

For pharmacokinetic studies, the niosomes prepared with Span 60 were diluted with saline to a suitable concentration after dialysis and were administered intravenously to a group of mice transplanted with Sarcoma S-180 subcutaneously next day. To another group of tumour bearing mice free MTX was administered. The volume of MTX, both niosome encapsulated and free administration was 5 ml $kg,^{-1}$ free equivalent to 2.72 $mg/kg.^{-1}$ Blood samples were withdrawn at predetermined time intervals from the orbit of the eye using hematocrit capillaries. A group of three mice was used at each time point. The serum was separated by centrifugation and the amount of MTX was determined by spectrofluorimetric method as described by Chakrabarti and Bernstein.[4]

Long term effects of niosome encapsulated MTX was studied on mice bearing S-180 tumour and also compared with the control groups of mice bearing tumour with the administration of plain MTX and without. Three sets of mice (four mice in each set) were used for these studies. One set was injected with normal saline (5 ml kg^{-1}) while the other two sets were injected with free MTX and MTX entrapped niosome respectively at the dose of 2.72 mg kg^{-1} and the tumour of each mice was observed for nearly a month.

RESULTS AND DISCUSSION

Size, shape and entrapment of methotrexate in niosomes

Larger unilamellar niosomes were formed after the hydration process of the lipid thin layer. The diameter of niosomes was found to be in the range of 1.5 to 13.5 mcm with the mean diameter being 4.5 mcm (with the exception of Span 40 containing niosomes which were slightly larger) (Table 1).

The niosomes were mostly spherical in shape and few being either triangular or elongated. The niosomes were able to entrap 25 to 50% of the MTX in the hydration process, with niosomes having Tween 80 entrapping the least (25.7%) and niosomes having Span 60 entrapping the maximum MTX (51.7%) as shown in Table 2.

TABLE 1 : CALCULATION OF STATISTICAL DIAMETERS OF FORMULATED NIOSOMES

Mean Size (diameter range of niosome (mcm)	Number of niosomes in each size range						Percentage of niosomes in each size range
	SPAN 60	SPAN 40	SPAN 20	TWEEN 80	TWEEN 60	TWEEN 40	
01.50	16.00	19.00	20.00	31.00	29.00	25.00	23.33
04.50	34.00	27.00	32.00	36.00	38.00	40.00	34.50
07.50	29.00	36.00	25.00	15.00	17.00	19.00	23.50
10.50	14.00	15.00	18.00	09.00	11.00	09.00	12.67
13.50	07.00	03.00	05.00	09.00	05.00	07.00	06.00

TABLE 2 : CALCULATION OF ENTRAPMENT OF MTX IN FORMULATED NIOSOMES

Surfactant used in niosome preparation	Amount of MTX dialysed (mg)			Average concentration of MTX dialysed into saline ± SD (n-1) (mg)	Percentage coefficient of variation (%CV)	Percentage of MTX entrapped in niosome
Span 60	21.60	24.75	26.05	24.13 ± 2.29	9.48	51.70
Span 40	26.55	24.95	27.05	26.18 ± 1.10	4.20	47.60
Span 20	23.50	26.85	22.60	24.32 ± 2.24	9.21	51.40
Tween 80	41.35	35.50	34.55	37.13 ± 3.68	9.92	25.70
Tween 60	30.65	29.35	28.10	29.37 ± 1.28	4.34	41.30
Tween 40	26.25	27.05	25.95	26.42 ± 0.57	2.15	47.20

• Total amount of MTX in niosomes used for dialysis studies : 50 mg.

TABLE 3 : PHARMACOKINETIC DATA

Parameter	Free MTX	Niosome MTX
AUC (ug hr ml^{-1})	1.837 ± 0.2	42.985 ± 3.8
AUMC (ug hr^2 ml^{-1})	16.213 ± 1.3	3996.197 ± 182.2
MRT (Mean Residence Time hr.)	8.825 ± 0.6	92.967 ± 8.3
Kss (hr^{-1})	0.113 ± 0.01	0.011 ± 0.002
t 1/2 (Y) (hr)	13.888 ± 1.2	70.714 ± 4.1
Vd ss (ml)	504.314 ± 32.0	227.092 ± 18.0
Cl_T (ml hr^{-1})	57.139 ± 4.6	2.453 ± 0.3

By Mann Whitney method, significance of difference in Pharmacokinetic Data of MTX entrapped niosomes compared to free MTX : $P < 0.05$.

Effect of Span 60 containing niosomes on the distribution of methotrexate in S-180 tumour bearing mice after intravenous injection

The nonionic vesicles prepared with Span 60 markedly altered the pharmacokinetic profile of MTX (Table 3). The plasma levels of MTX was significantly higher with MTX entrapped niosome injection than free MTX in the form of solution in normal saline. The elimination of MTX from the plasma of mice bearing S-180 tumour was slower when given as niosome. A notable increase in the area under the MTX concentration time curve and mean resident time of MTX could be noticed after injection of MTX entrapped niosome as compared to free MTX injection (Table 3). The apparent volume of MTX distribution also decreased with niosomes compared to that of free MTX injection.

Long term effects of MTX encapsulated niosomes in mice bearing transplanted S-180 tumour

In the control group of mice tumour was viable and after 1 month necrosis was observed. The extent of tumour viability was less with treated group of mice with free MTX injection. Total regression of tumour with completely healed tumour was observed in the treated group of mice with MTX encapsulated injection.

Thus it was evident that surfactant vesicles made with Span 60 containing MTX was more effective for tumour regression compared to plain MTX injection.

CONCLUSION

The larger vesicles increased the in vivo terminal $t_{1/2}$ significantly. Such an increase in $t_{1/2}$ could be attributed to the inability of niosomes to leave circulation through fenestrated vessels thus prolonging their sojourn in the circulation. The order of entrapment efficiency increases as the lipophilicity increases; as observed in case of Span 60 vesicles compared to Tween 80 vesicles. Niosomes could maintain the MTX level in the blood for prolonged period after intravenous injection. The increase in mean retention time of the drug administered as niosomes indicates that an sustained release of drug to circulation could be achieved. This could reduce the toxicity of anticancer drugs and niosomes could be very useful drug delivery systems for better cancer therapy.

ACKNOWLEDGEMENTS

The authors are very thankful to M/s C.S.I.R. New Delhi and M/s Dr. T.M.A. Pai Research Foundation for sponsoring this research project and M/s Cynamid for supplying gift sample of methotrexate.

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