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Rapid Communication

Pharmacokinetic evaluation of surfactant vesicle-entrapped methotrexate in tumor-bearing mice

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Summary

Methotrexate was encapsulated in niosomes. The entrapment efficiency increased with increase in lipophilicity of surfactant. Unilamellar vesicles prepared with span 60 showed maximum entrapment and its pharmacokinetics in mice transplanted with S-180 tumor was markedly different in comparison to unentrapped methotrexate.

Because of the lack of tumor specificity of systemically administered antitumor drugs, toxic effects are common, often resulting in greater harm to host tissues than to the neoplastic process. The therapeutic index of most antineoplastic agents used in systemic therapy remains marginal, despite efforts to achieve tumor specificity.

Non-ionic surfactant vesicles (niosomes), formed when a mixture of cholesterol and the surfactant is hydrated, can entrap solutes, are osmotically active and stable, and may be similar in terms of their physical properties to liposomes (Baillie et al., 1985). Niosomes like liposomes can be expected to have two beneficial effects: (1) As a vehicle for drug formulations, they may reduce the systemic toxicity of clinically important antineoplastic agents. (2) As carriers for enhanced delivery to specific cells, niosomes may improve the therapeutic index by restricting drug effects to target cells.

Niosomes were prepared by a slight modification of the procedure adopted earlier by Azmin et al. (1985). Surfactant (Tween 80, 60, 40; Span 60, 40, or 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 diethyl ether (10–15 ml) in a round-bottom flask. The solvent was evaporated under reduced pressure at a temperature of about 60 °C using a 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} solution) was added to the flask slowly, while warming the flask at about 50 °C and with intermittant vortexing, until a good dispersion of the mixture was obtained.

The MTX-entrapped niosomes were separated from the unentrapped by dialysis as described by Hardy et al. (1980). The prepared niosomes were

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filled into a glass tube to which a Sigma dialysis membrane was securely attached to one side, and free MTX was dialysed for 30 min each time into 500 ml of 0.9% NaCl (saline). The dialysis of free MTX was always complete after about 10 changes of saline (UV detection at 303 nm), when no MTX was detectable in the 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 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 and were administered intravenously to a group of mice transplanted with Sarcoma-180 (S-180) subcutaneously. To another group of tumor-bearing mice, free MTX was administered. The volume of MTX, both niosome-encapsulated and free, administered was 5 ml kg^{-1}, 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 a sensitive spectrofluorometric method as described by Chakrabarti and Bernstein (1969).

The diameter of the formulated niosomes (as could be observed under the microscope) was found to be in the range of 1.5–13.5 μm with a mean diameter of about 4.5 μm. The niosomes were observed to be mostly spherical in shape with a few being either triangular or slightly elongated. The formulated niosomes were able to entrap 25–50% of the MTX used in the hydration process, with Tween 80-containing niosomes entrapping the least (25.7%) and Span 60-containing niosomes entrapping the maximum MTX (51.7%).

The non-ionic surfactant vesicles containing MTX, prepared with Span 60, markedly altered the pharmacokinetic profile of MTX as compared to free MTX. The plasma profiles of MTX are described by the following equations:

$$C_p^1 = 14.2546e^{-5.4036t} + 0.9632e^{-0.5973t} + 0.3785e^{-0.0098t}$$

$$C_p^2 = 5.2229e^{-8.3160t} + 0.2085e^{-0.6178t} + 0.0358e^{-0.0499t}$$

where C_p^1 is the plasma profile of niosome encapsulated MTX and C_p^2 is that of free MTX. The plasma level was observed to be significantly higher with niosome-entrapped MTX injection as compared to that of free MTX given in the same dose.

The α and β half-lives of MTX given as a solution were 5 min and 1.12 h, respectively, whereas the γ $t_{1/2}$ after 8 h was 13.89 h.

The elimination of MTX from the plasma of mice bearing S-180 was slower when given in the form of niosome-encapsulated MTX, with α and β $t_{1/2}$ of about 8 min and 1.16 respectively, the γ $t_{1/2}$ being about 70.71 h.

The various pharmacokinetic parameters of free MTX and niosome-entrapped MTX in S-180 bearing mice are listed in Table 1.

The modified procedure adopted in preparing niosomes produced consistently unilamellar vesicles without prior sonication. The addition of aqueous MTX solution in portions whilst gently warming the mixture and performing intermittent vortexing could lead to layered 'stripping' of the lipid thin layer, thus forming unilamellar vesicles instead of the typical multilamellar vesicles obtained by hydration with the full quantity of drug solution added in a single portion.

The larger sized vesicles increased the in-vivo terminal $t_{1/2}$ considerably in comparison with that obtained by Azmin et al. (1985). This phenomenon of slower clearance of larger vesicles over smaller ones has been clearly depicted by Allen (1981).

TABLE 1

Parameter	Free MTX	Niosome MTX
AUC (μg h ml^{-1})	1.837	42.985
AUMC (μg h^2 ml^{-1})	16.213	3996.197
MRT (mean residence time) (h)	8.825	92.967
k_{ss} (h^{-1})	0.113	0.011
$t_{1/2}$ (γ) (h)	13.888	70.714
Vd_{ss} (ml)	504.314	227.092
Cl_T (ml h^{-1})	57.139	2.453

The entrapment efficiency has also been observed to be greater for Span 60 and least for Tween 80 containing niosomes. The reason may be attributable to the increased lipophilicity of Span 60. The order of entrapment efficiency increased as the lipophilicity increased.

The increase in mean residence time of the drug administered in the niosomes indicates that sustained release of free drug into circulation could be achieved. This may reduce the toxicity common to antineoplastic agents such as MTX.

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