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Rapid Communication

Solid lipospheres of doxorubicin and idarubicin

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Summary

Solid lipospheres, constituted mainly of stearic acid and egg lecithin, containing amounts of doxorubicin and idarubicin up to 7 and 8.4%, respectively, were prepared. The high incorporation of drug is due to an increase in their lipophilicity as a result of the formation of ion-pairs with monoalkyl phosphate esters.

Anthracycline drugs, glycosidic antibiotics containing an aromatic aglycone linked to an amino sugar, include a variety of molecules with different lipophilicity and are widely employed in clinical use. In particular, doxorubicin is one of the most commonly used drugs against human cancer.

Recently, idarubicin, 4-demethoxydaunorubicin, was introduced in therapy via the oral and parenteral routes. Idarubicin is more lipophilic than doxorubicin: the apparent partition coefficient of doxorubicin in octanol/Mes buffer at pH 6.0 is 0.7 as compared with 0.4 in the case of idarubicin (Pattarino et al., 1989).

In order to enhance the therapeutic efficacy of the anthracyclines, in particular of doxorubicin, several systems, such as liposomes (Crommelin et al., 1990), albumin-heparin microspheres (Cremjers et al., 1990) and nanocapsules (Gasco et al., 1991) with different contents of incorporated drug and of different sizes have been studied.

We have previously reported on the preparation of lipospheres incorporating drugs from o/w microemulsions (Gasco and Morel, 1990; Gasco et al., 1992a, b).

In the present communication, we describe the preparation of lipospheres incorporating doxorubicin and idarubicin. The solid lipospheres were obtained from o/w microemulsions prepared at 65–70°C. The internal phase was constituted of stearic acid (approx. 7.1% w/w), while the continuous phase was distilled water (approx. 71% w/w). Purified egg lecithin (approx. 4.9% w/w) and taurodeoxycholate (approx. 12.8% w/w) were employed as surfactant and cosurfactant, respectively.

Doxorubicin and idarubicin were supplied by Farmitalia (Milan, Italy), stearic acid was obtained from Merck (Darmstadt, Germany) and

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sodium taurodeoxycholate was purchased from Sigma (St. Louis, MO, U.S.A.). Egg lecithin was purified by adsorption chromatography (Hanahan et al., 1951). The monoalkyl phosphates were prepared according to Brown et al. (1955).

Monoalkyl phosphate esters (decyl phosphate and hexadecyl phosphate), doxorubicin or idarubicin (approx. 1.4% w/w) were added to melted stearic acid; the other components were successively added to the mixture at 65–70°C, yielding a clear microemulsion.

The molar ratio of anthracycline to alkyl phosphate was 1:2. The alkyl phosphate percentages varied from about 2 to 2.3% (w/w).

Small differences in the percentages of the components were due to the different molecular weights of alkyl phosphate.

Solid lipospheres were obtained by dispersing the warm microemulsion in distilled cold water (2–3°C) under mechanical stirring. The aqueous liposphere suspension was then washed twice with distilled water by diaultrafiltration (Amicon TCF2A, Grace, Danvers, MA, U.S.A.). After washing, the suspension was freeze-dried with a Modulyo freeze dryer (Edwards Crawley, U.K.).

Sodium monodecyl phosphate and sodium monohexadecyl phosphate were employed as counter ions of the anthracyclines in order to increase their lipophilicity and consequently the extent of their incorporation into the lipospheres.

The apparent partition coefficients between stearic acid and water for doxorubicin and idarubicin were determined at 70°C in order to evaluate the lipophilicity of the anthracycline complexes with monoalkyl phosphates. The drug/alkyl phosphate ratio was always 1:2.

The concentration of drug was determined in the aqueous phase by spectrophotometry (Perkin Elmer Lambda II UV/Vis spectrophotometer).

The average diameters of lipospheres (Table 1) were measured by photon correlation spectroscopy (Zetasizer IIC Malvern, Malvern, U.K.); TEM analysis was performed by staining the liposphere suspension with a 2% solution of osmium tetroxide. The amounts of doxorubicin and idarubicin incorporated into the lipospheres were determined by spectrophotometry.

Experiments were carried out to follow the drug release from doxorubicin and idarubicin lipospheres, respectively. A 'side by side' glass diffusion cell was used. A hydrophilic dialysis membrane separated the donor and receptor compartments. Liposphere suspensions were prepared by dispersing freeze-dried lipospheres in distilled water at a fixed ratio (1:10).

TABLE 1

Antracycline lipospheres obtained from o/w microemulsions

Drug	Counterion	Average diameter	% of drug in liposphere
Doxorubicin	decyl phosphate	73.6	6.5
	hexadecyl phosphate	110.4	7.0
Idarubicin	decyl phosphate	180.1	7.8
	hexadecyl phosphate	131.4	8.4

The drug concentration in the receptor solution was evaluated by fluorescence spectrophotometry (Perkin Elmer model 203 fluorescence spectrophotometer).

A previous study (Trotta et al., 1988) was performed on the formation of lipophilic ion pairs of doxorubicin with some biliar salts at a counterion concentration which was maintained below their CMC in order to avoid micelle formation. The permeability coefficients obtained were much lower than that of doxorubicin alone.

In the present work, a phosphate monoester (hexadecyl phosphate), used in pharmacy for the preparation of liposomes, was initially chosen as counterion; successively, another ester was employed (decyl phosphate) to examine the influence of a shorter chain on the lipophilicity of the ion pair.

The molar ratio of drug to counterion was constant (1:2). The microemulsion was stable up to a drug concentration of 20% in the internal phase. The drug content in lipospheres was also rather high after two washings.

On changing the counterion, only small differences in the extent of drug incorporation could be noted (Table 1).

The influence of the counterion was underlined by the apparent partition coefficients between stearic acid and water; indeed a dramatic increase in lipophilicity was observed for doxoru-

TABLE 2

Log apparent partition coefficients (log $P_{app.}$) of doxorubicin and idarubicin between water and stearic acid at 70°C

Drug	Counterion	Log $P_{app.}$ [a]
Doxorubicin	–	−0.25
	decyl phosphate	1.94
	hexadecyl phosphate	2.81
Idarubicin	–	0.66
	decyl phosphate	3.00
	hexadecyl phosphate	3.08

[a] Molar ratio of drug to alkyl phosphate was 1:2.

bicin (> 1000-fold) and for idarubicin (> 300-fold) (Table 2).

The average diameter varied from about 70 to 180 nm and was affected by the amount of incorporated drug.

The washing water contained only minor amounts of the drug, confirming the considerable lipophilicity of the anthracycline-monophosphate ester ion pairs. Possibly TDC, used mainly as cosurfactant, could also play a role as counterion and a mixture of two different ion pairs of the anthracyclines with alkyl monophosphate and TDC, respectively could be present.

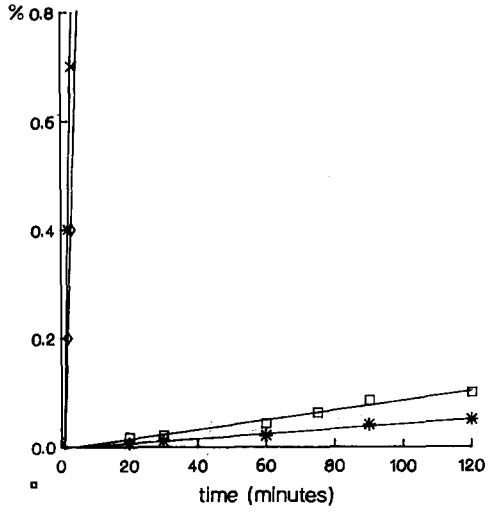

Fig. 1. Release of doxorubicin (◇) and idarubicin (×) from solution; release of doxorubicin (□) and idarubicin (*) from lipospheres (prepared in the presence of hexadecyl phosphate) dispersed in water.

The TEM analysis of lipospheres, irrespective of whether they contained drug, showed that the lipospheres had a narrow size distribution and spherical shape.

The percentage release of doxorubicin and idarubicin vs time from the different samples of lipospheres was compared (Fig. 1) with those from solutions of the two anthracyclines. For both drugs, the release was less than 0.1% after 2 h. The low extent of drug release may be connected with the high lipophilicity of their ion pairs. The kinetics does not follow a biphasic pattern as in the case of albumin-heparin microspheres (Cremjers et al., 1990). In the latter case, the higher initial release may be correlated with the considerable amount of doxorubicin on the microsphere surface. In lipospheres, the drug is randomly distributed; furthermore, the drug concentration at the surface should be lower than that inside as a consequence of washing.

The average diameter of the deeply coloured lipospheres might permit their administration via the intravenous route, thus avoiding the risk of necrosis. A greater therapeutic efficacy should be obtained as a result of the presence of a colloidal system from which the drug can be slowly released.

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