anti-cholinesterase activity may have been due to changes in chain length rather than partition coefficient.

The correlation for CPZ metabolites may indicate that their ability to inhibit cholinesterase is due in part to hydrophobic bonding to the enzyme. The K_i values are relatively high which suggests that the bonding is not very specific.

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J. Pharm. Pharmacol. 1989, 41: 858–860
Communicated August 10, 1989

Incorporation of dyes into low density lipoprotein in the presence of non-ionic surfactants

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Abstract—The interaction of low density lipoprotein (LDL) with two model dyes in the presence or absence of non-ionic surfactants has been studied. The water soluble dye, thymol blue, only weakly interacts with LDL to cause slight increases in particle size and the interaction can be reversed on column chromatography and dialysis. The oil-soluble dye sudan III reacts strongly with LDL to produce marked changes in particle size the complex formed being stable to dialysis and gel exclusion chromatography. These interactions are not affected by the presence of surfactants. The results indicate that even small quantities of lipophilic materials will easily partition into the particle, with or without the aid of surfactants and can have a marked effect on the measured size of the particle.

Several approaches have been made to establish methods for the delivery of cytotoxic agents specifically to neoplastic cells. Such targeting should reduce toxicity due to unwanted effects on normal healthy cells. To achieve this aim drugs have been linked to a variety of carrier molecules (Garnett & Baldwin 1986; Halbert et al 1987) or encapsulated in colloidal carriers (Rogerson et al 1988) to alter the distribution of the active species. One carrier system which has aroused interest is the low density lipoprotein (LDL) particle, a natural component of plasma (Brown & Goldstein 1986) which accumulates in certain tumour cells to a greater degree than normal cells, both in-vitro (Gal et al 1981) and in-vivo (Hynds et al 1984). LDL is the principal cholesterol transport lipoprotein in human plasma and is an almost spherical particle around 22nm in diameter with a molecular weight of approximately $2{\cdot}5 \times 10^6$ Daltons. Each LDL particle contains a non-polar core of cholesterol esters surrounded by a monolayer of phospholipid, unesterified cholesterol and apoprotein B, which is the receptor protein recognized by LDL receptors on cell surfaces (Deckelbaum et al 1977).

Various drugs, such as aclacinomycin (Rudling et al 1983), and chlorambucil (Firestone et al 1984), have been successfully incorporated into LDL using a technique involving freeze drying. The particle is first lyophilized in the presence of saccharides, then extracted with hexane to remove the cholesterol esters and the replacement core added in a volatile solvent which is evaporated before LDL reconstitution with an aqueous buffer.

We have previously shown that the interaction of LDL with surfactants causes the particle to swell and increase in size, a phenomenon that may be useful in assisting the incorporation of cytotoxic drugs into LDL (Tucker & Florence 1983). This work has demonstrated that the Brij (alkyl polyoxyethylene ether) surfactants produce the least alterations to lipoprotein properties whilst producing the greatest increase in size. They may

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therefore increase the ability of LDL to accept drug molecules into the central core. In the present study two dyes were employed as model solutes for incorporation into LDL using the aforementioned surfactants. Sudan III (oil red) was used as an example of a lipid soluble material while the water soluble dye thymol blue was employed for comparison.

Materials and methods

Materials. Brij 35, 78, 96, and 98 were supplied by the Atlas Chemical Industries, Leatherhead, UK. Heparin sodium and Sephadex 200–50 were obtained from Sigma Chemical Company Ltd, Poole, UK. Sudan III (oil red), thymol blue, and all salts (Analar grade) were obtained from BDH, UK. Dialysis tubing type 5-20/32 was supplied by Medicell International, London, UK. Low density lipoprotein was isolated from normal venous blood and assayed by published methods (Halbert et al 1985).

Methods

Photon correlation spectroscopy. A photon correlation spectrometer (Malvern Instruments; Model 7027) with 60 channels was used with a He/Cd laser (Linconix) operating at 441·6 nm with a power output of 10 mW. All samples were thermostated to 25°C and measurements were made at an angle of 90° to the incident beam. Collected data were treated as previously described to obtain the Stoke's radius and polydispersity coefficient (Halbert et al 1985).

Addition of surfactant and dyes to LDL. To freshly filtered (0·22 μm) LDL solution (1 mL at 2·5 mg mL^{-1} protein concentration) in phosphate buffered saline (PBS) was added filtered (0·22 μm) solutions of dye (0·08 mg), surfactant or surfactant/dye mixtures in PBS. Free sudan III dye was added as a solution in 4 M glacial acetic acid; control experiments demonstrated that the acetic acid did not affect the properties of the LDL.

Gel separation. Mixtures (2 mL at 1·25 mg mL^{-1} protein concentration) of LDL containing surfactant (Brij 78) and dye (0·04 mg mL^{-1}) or dye alone were passed down a Sephadex 200–50 column (2·6 × 15·5 cm) eluted with PBS (0·6 mL min^{-1}). The eluate (4mL fractions) was measured at 254 nm, 510 nm (sudan III λmax) or 400nm (thymol blue λmax) and for protein content by a modified Lowry method (Halbert et al 1985).

Dialysis. LDL solutions (4 mL at 0·6 mg mL^{-1} protein concentration) containing dye (0·02 mg mL^{-1}) or dye/surfactant mixtures were dialysed against PBS (2L) overnight at 4°C. The concentrations of dye and LDL in the original solution were then determined.

Heparin manganese precipitation. A modification of the method of Warnick & Albers (1978) was used: 100 μL of a solution of $MnCl_2$ (1 M containing heparin (0·9 mg mL^{-1})) was added to 1·5 mL (1·7 mg mL^{-1} protein concentration) of LDL solution containing dye or dye/surfactant mixtures, the precipitated LDL was removed by centrifugation (2800 rev min^{-1} at 4°C for 30 min, Mistral 3000 MSE). The concentration of dye remaining in the supernatant was then measured spectrophotometrically.

Results and discussion

The isolated LDL from all the subjects had a measured Stoke's radius of between 11–12·5 nm in agreement with previous studies (DeBlois et al 1973). The diameter of the LDL particles as measured by PCS following treatment with dye, or surfactant or mixtures of dye and surfactant are presented in Fig. 1. As noted

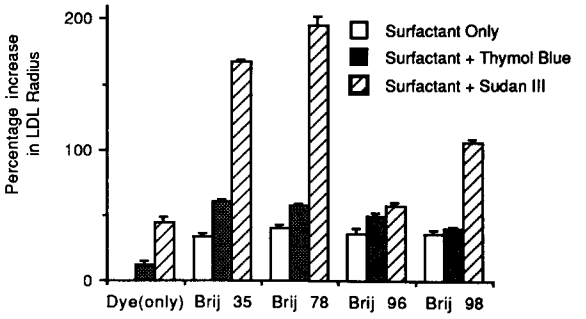

Fig. 1. Measured size changes in LDL on interaction with dyes, surfactants or dye/surfactant mixtures (mean ± s.d., n = 10). Molar ratios of dyes and surfactants to LDL; sudan III 23:1, thymol blue 17:1, Brij 35 430:1, Brij 78 450:1, Brij 96 750:1, Brij 98 450:1.

in previous studies, the interaction of low concentrations of surfactant with LDL causes an increase in the measured size and polydispersity of the sample (Tucker & Florence 1983), indicating that the surfactant is perturbing the structure of the LDL particle. The interaction of the two dyes in the absence of surfactant, however, also causes an increase in the measured size and polydispersity of the LDL. This effect is very much greater for the lipophilic sudan III than for the hydrophilic thymol blue and may reflect the difference in physicochemical properties of the two dyes. The addition of a mixture of dye and surfactant also produces increases in the measured radius and polydispersity of the particles with varying effects dependent on the surfactant and dye combination. The increases measured are also greater than those of the dye and surfactant alone. Mixtures of thymol blue and surfactant appear to produce an additive effect, with the increase equivalent to the summation of the effect of surfactant and thymol blue alone. Mixtures of sudan III and surfactants, on the other hand, appear to have a synergistic effect on LDL diameter and cause increases greater than the individual effects of the two materials, with the exception of Brij 96.

Mixtures of dyes and surfactants when subjected to gel exclusion chromatography also exhibit different properties (Fig. 2). Thymol blue-Brij 78-LDL mixtures on chromatography separate into their constituents, sudan III however co-elutes with the LDL. The presence or absence of Brij 78 in the original sample does not affect this result. A similar picture is obtained on dialysis (Table 1) when 40% of the thymol blue is lost but the sudan III remains with the LDL. Precipitation of the LDL with heparin manganese provides further confirmation of the interaction between the LDL and the sudan III. Forty percent of the sudan III co-precipitates with the LDL but only 12 percent of the thymol blue (Table 1).

The results demonstrate that the interaction of sudan III and surfactants with LDL appears to be synergistic and produces a large increase in particle size. The increase caused by the addition of the sudan III alone is greater than that expected due to a simple increase in LDL volume with increasing number of entrapped dye molecules (based on the molar ratio of sudan III to LDL and the calculated molar volume of sudan III (Bondi 1964)). This result indicates that the sudan III may have the ability to perturb the structure of the particle, a feature that has been noted before in the loading of LDL (Vitols et al 1985) and microemulsions (Halbert et al 1984) with drugs. The interaction of LDL and sudan III appears to be strong as the dye remains with the LDL on dialysis, chromatography or precipitation. The results indicate that the dye is probably partitioning into the central core of the particle. It is interesting that only 40 percent of the sudan III precipitates with the LDL on addition of heparin/manganese reagent. This result may indicate that the

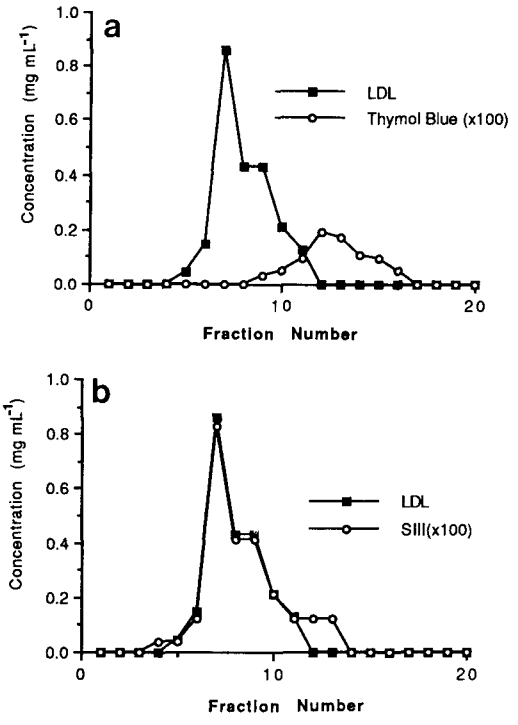

Fig. 2. Gel chromatography of Brij 78, dye and LDL mixtures. Molar ratios of dyes and surfactant to LDL; thymol blue 17:1, sudan III 23:1, Brij 78 450:1.

Table 1. Interaction of thymol blue and sudan III with low density lipoprotein in the presence of Brij 78.

	Measured dye:LDL Molar ratios	
	Dialysis	Precipitation
Thymol blue+surfactant	10	2
Thymol blue only	10	2
Sudan III+surfactant	23	9
Sudan III only	23	9

Starting molar ratios of dye, Brij 78 and LDL, sudan III 23:1, thymol blue 17:1, Brij 78 450:1.

precipitation method leaves residual traces of phospholipid and/or surfactant micelles in the supernatant that can solubilize the dye and reduce LDL incorporation in the precipitate. Further investigations would be required to support this hypothesis. The hydrophilic thymol blue produces smaller changes that appear to be reversible and related to surface binding effects. On dialysis, for example, only 40 percent of the Thymol Blue is lost and most apparently remains bound to the LDL. The hydrophilic nature of the dye will restrict its ability to penetrate into the core of the LDL particle and hence produce dramatic increases in size.

The results indicate that surfactants may have a role to play in the loading of LDL with lipid soluble materials. However, increases in size and changes in structure may be too great and not always reversible, to warrant the use of this method. Also the results indicate that size measurement should be included in LDL loading experiments as a means of assessing the integrity of the particle after interaction with lipophilic materials, as the incorporation of small amounts can produce large size increases.

We thank Professor Shepherd, Department of Clinical Pathology, Glasgow Royal Infirmary for help with the LDL isolation and the Garfield Weston Foundation and SERC for funding J. Eley.

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