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Interactions of Solutol HS 15 and Cremophor EL with Plasma Lipoproteins

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Two emulsifying agents, Solutol HS15 and Cremophor EL, were compared with regard to their effects on human plasma lipoproteins *in vitro* and on mouse plasma lipoproteins *in vitro* and *in vivo*. Both agents promoted binding of a hydrophobic photosensitizing agent (C8KC) to a circulating plasma species of low bouyant density. Persistence of this material was greater with Cremophor than with Solutol. Experiments carried out with labeled Solutol indicated that the vehicle itself is a component of this new species. High concentrations of either vehicle ($\geq 0.06\%$) led to decreased electrophoretic mobility of human LDL and HDL *in vitro*. In the mouse, a different effect was observed, resulting in complex changes in electrophoretic mobility of plasma lipoproteins. The plasma half-life of C8KC in the circulation of the mouse was correlated with the persistence of an altered electrophoretic lipoprotein pattern. Since Solutol and C8KC showed similar half-lives, this result suggests that the plasma half-life of the sensitizer is correlated with the persistence of the vehicle. While Solutol and Cremophor were designed to be vehicles for drug formulation, they also influence persistence of some drugs in the circulation.

Keywords: Emulsifiers Photosensitizers Lipoproteins

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INTRODUCTION

Photodynamic therapy is an anti-cancer modality which relies on the selective retention of photosensitizing agents to elicit tumor eradication. Many of the new photosensitizing agents being developed are poorly soluble in water, and require drug-delivery vehicles for their formulation. In a recent study, we found that pharmacokinetics and efficacy of one such photosensitizer, the ketochlorin C8KC (Fig. 1), were affected by the choice of formulation procedure (Woodburn *et al.*, 1994). The use of CRM as the drug-delivery vehicle prolonged C8KC persistence in plasma and tissues; this was associated with enhanced binding of the sensitizer to a plasma fraction of low bouyant density which may represent degraded HDL and/or CRM-bound sensitizer.

CRM is an emulsifying agent prepared from the interaction of ethylene oxide and castor oil, and is used for the formulation of several drugs including cyclosporin (Cohen *et al.*, 1984), taxol (Brown *et al.*, 1991), and hydrophobic photosensitizers (Kessel *et al.*, 1991; Gargo, 1990). The major component of CRM is poly-(oxyethylenglycerol)triricinoleate; the product also contains a variety of hydrophobic and hydrophilic components (Lorenz *et al.*, 1982). HS15 is a similar mixture, prepared from 12-hydroxystearic acid + ethylene oxide, and therefore contains only fully-hydroxystearic acid; minor components include a series of poly-(ethyleneglycol) mono and di-esters and hydroxystearic acid esters. The product also contains a hydrophilic fraction (30%), mainly

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Abbreviations: C8KC, ketochlorin photosensitizer; CRM, Cremophor EL; DMF, N,N-dimethylformamide; HDL, high density lipoprotein; LDL, low density lipoprotein; MP, mesoporphyrin (Fig. 1); PDT, photodynamic therapy; PEG, poly(ethyleneglycol); HS15, Solutol HS15; TX-100, Triton X-100; VLDL, very low density lipoprotein.

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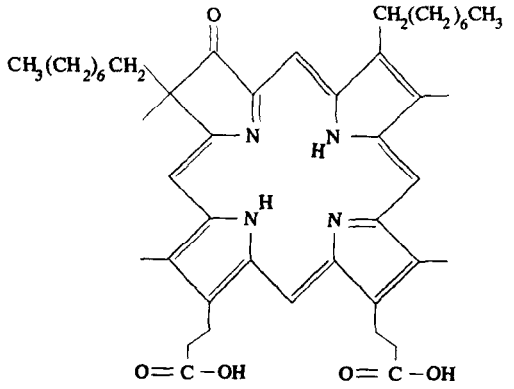

Fig. 1. Structure of the ketochlorin C8KC.

polyethylene glycol*. The availability of radioactive HS15 facilitated this pharmacokinetic and biodistribution study.

In the present study, effects of the choice of solubilizer on the lipoprotein binding of MP and C8KC were assessed. MP binds equally well to plasma proteins and lipoproteins, and has been used as a fluorescent probe for identifying species in plasma fractions after density-gradient ultracentrifugation (Woodburn and Kessel, 1994). The ketochlorin photosensitizer, C8KC, was examined as a model for water-insoluble agents which require special formulation procedures (Woodburn *et al.*, 1994).

METHODS

Chemicals and biological products

CRM and TX-100 were obtained from Sigma Chemical Co., St Louis, Mo. HS15 and [^{14}C]HS15 (5.35 mCi/g) were provided by Drs Siegfried Lang and Lee Mores, BASF Corporation. Radioactivity was introduced by using 1,2-[^{14}C]ethyleneoxide in the synthesis of HS15, so that all fractions of the product containing PEG are labeled. MP (Porphyrin Products, Logan, Utah) was dissolved at 1 mg/ml concentrations in 0.1 M NaOH, 20% CRM + 6% propylene glycol in isotonic saline (Garbo, 1990) or 20% HS15.

Pooled normal human plasma was obtained from the blood bank service of the Red Cross. All *in vivo* experiments involved administration of 0.1 ml of 20% (v/v) CRM or HS15 to 20 g female ICR mice by tail-vein injection. Where specified, C8KC or MP were solubilized with these vehicles such that the total dose was 5 mg/kg.

Plasma affinity profiles

Binding of different drugs to plasma proteins and lipoproteins was assessed by density-gradient ultracentrifugation using a modification of a previously published procedure (Sykes *et al.*, 1992). For *in vitro* studies, human (250 μl) or mouse plasma (125 μl) was incubated for 30 min at 37°C with MP or C8KC (8 μg/ml) solubilized as described above. For *in vivo* biodistribution studies, C8KC or MP (1 mg/kg) were administered to female ICR mice by tail vein injection. The method of drug solubilization is described above. Animals were sacrificed after specified times and blood collected using EDTA to prevent coagulation. Erythrocytes were removed by centrifugation (1000 *g*, 5 min, 10°C). Plasmas were used for density-gradient analysis as described below. To determine the total sensitizer level, 20 μl aliquots of plasma samples were mixed with 3 ml of 10 mM Triton X-100 for fluorescence assays (Woodburn *et al.*, 1994).

Density-gradient ultracentrifugation involved dilution of 250 μl human plasma or 125 μl of mouse plasma with 150 mM NaCl to a volume of 750 μl. Solid KBr was then added to bring the density to 1.21 g/ml. A similar analysis of [^{14}C]HS15 was carried out by first mixing the labeled product with 250 μl of isotonic NaCl or control (human) plasma. The resulting solutions contained 0.2% (v/v) HS15, 0.01 μCi.

Gradients were established by layering 750 μl of these 1.21 g/ml preparations over 750 μl of 1.27 g/ml KBr solution, using 13 × 38 mm polyallomer centrifuge tubes (no. 358980, Beckman Instruments Inc., Palo Alto, Calif.). The tubes were then filled with isotonic saline (total volume = 3.9 ml), placed in a TLN-100 "near-vertical" rotor centrifugation was carried out in a Beckman TL-100 table top ultracentrifuge for 60 min at 100,000 rpm (r_{av} = 254,000 *g*). The tubes were fractionated from the top, and 25 fractions collected. Distribution of fluorescent sensitizers into LDL, HDL and albumin fractions was assessed by fluorescence using 400 nm excitation and the appropriate emission wavelength: 620 nm fluorescence for MP, 660 nm for C8KC. Sensitizer recoveries of 93 ± 4% were obtained.

Gel electrophoresis

Experiments carried out *in vitro* involved incubation of human plasma with varying con-

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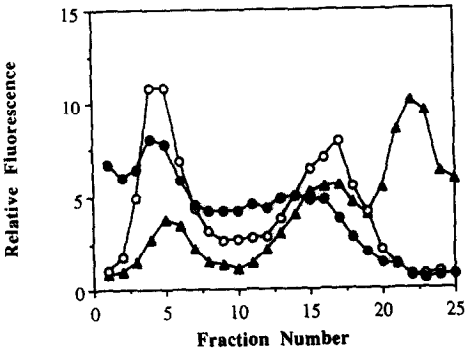

Fig. 2. The effect of the vehicle (0.2%, v/v) on binding of 8 μg/ml C8KC and MP to human plasma proteins *in vitro* as assessed by density-gradient ultracentrifugation: —▲— MP formulated with HS15 (use of NaOH or CRM yielded essentially the same pattern); —○— C8KC formulated with CRM; —●— C8KC formulated with HS15.

centrations of HS15 30 min at37°C. Samples of mouse plasma obtained after administration of HS15 or CRM were also examined. The electrophoretic mobility of plasma lipoproteins was assessed using Paragon lipoprotein agarose gels (Beckman Instrument Co., Fullerton, Calif.). After electrophoresis in 50 mM barbital buffer pH 8.6, the gels were fixed in MeOH/acetic acid mixture and stained with Sudan black. Fractions from density-gradient ultracentrifugation were dialyzed overnight against phosphate-buffered saline (pH 7.2) before electrophoresis, to eliminate gel artifacts caused by high salt concentrations.

RESULTS

Density-gradient studies of drug binding to human plasma components

We previously established that VLDL appears in fractions 1–2, LDL in 4–7, HDL in 14–17 and albumin in 22–24 (Woodburn *et al.*, 1994). There was no significant difference in the affinity of MP for human plasma albumin, HDL or LDL when the drug was solubilized with 10 mM NaOH, CRM or HS15. In contrast, the distribution of C8KC was markedly altered by the choice of solubilizer (Fig. 2). With CRM, we observed a well-defined binding of C8KC to LDL and HDL. Formulation with HS15 resulted in an ill-defined binding pattern to LDL and HDL, the LDL region was extensively broadened and its bouyant density reduced. We also observed C8KC binding to a plasma product with the bouyant density of VLDL (fractions 1–2).

Ultracentrifugation of radioactive HS15 alone resulted in a broad spectrum of radioactivity with a distinct peak in fractions 7–10. There was also a diffuse band of radioactivity in fractions 5–23. In the presence of human plasma, the peak of radioactivity in fractions 7–10 disappeared, and radioactivity was observed in fractions 5–23 (not shown). This effect was not altered when all procedures were carried out at 10°C.

Gel electrophoresis of lipoproteins in vitro

Effects of graded levels of HS15 on lipoprotein components of human plasma is shown in Fig. 3. Electrophoretic mobility of both HDL (the fast-migrating species) and LDL (the slow-migrating species) was reduced as the HS15 concentration was increased. The band which appears at HS15 concentrations of 0.06% (lane 5) or higher and migrates slightly toward the cathode derives from the hydrophobic components of HS15.

Gel electrophoresis was used to analyze the first 8 density-gradient fractions from human plasma treated with 0.2% HS15 + C8KC. These fractions were first dialyzed to remove excess KBr which perturbs the gel patterns. The resulting patterns (Fig. 4) indicate the presence of HS15 in fractions 1–5; this material stains with Sudan black and migrates slightly toward the ⊖ pole. LDL was observed in all fractions, with the highest level in fractions 5 and 6. VLDL (fractions 1–3) migrates slightly faster than LDL.

Murine drug-distribution experiments

Plasma samples were analyzed by gel electrophoresis 1, 1.5, 2 and 3 hr after drug administration (Fig. 5). The control (lane 1) shows the HDL species present in mouse plasma. One hr after HS15 administration, we observed several new lipoprotein species which migrated more slowly than HDL, and one which migrated more rapidly. The electrophoretic perturbation had ended by 3 hr after HS15 administration. The effect of a similar dose of CRM is shown in Fig. 5. The altered pattern persisted for at least 18 hr.

We examined the distribution of C8KC (HS15 formulation) among lipoprotein and protein fractions of mouse plasma *in vivo*. A mixture of 0.1 ml 20% HS15 containing 1 μg of sensitizer was administered, representing 1 g/kg of HS15 and 5 mg/kg of C8KC. One hr after drug administration, when the degree of lipoprotein alteration was greatest (see Fig. 5), we

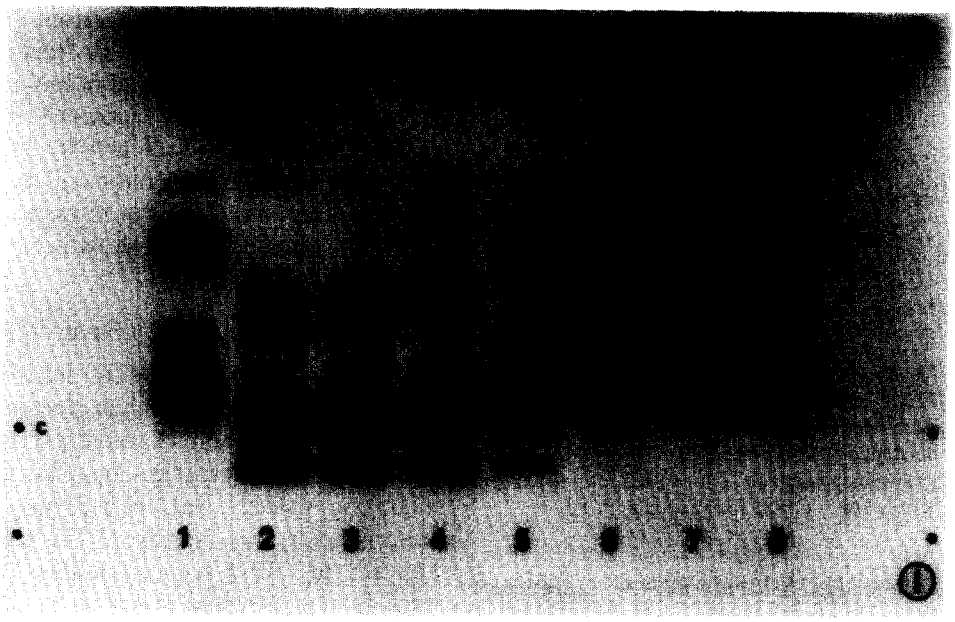

Fig. 3. Electrophoretic patterns of human plasma lipoproteins induced *in vitro* by graded levels of HS15. Lanes 1 and 8, control; HS15 concentrations (v/v): lane 2 = 2%, lane 3 = 0.6%, lane 4 = 0.2%, lane 5 = 0.06%, lane 6 = 0.02% and lane 7 = 0.006%. On these gels, "c" = point of application; HDL is the fastest-moving fraction, followed by VLDL and LDL. Chylomicrons remain at the origin, while free HS15 migrates slightly toward the cathode.

found a bimodal distribution of C8KC to a species of very low buoyant density (fractions 1–3) and to HDL (fractions 13–17). These data are shown in Fig. 6. At later time-points, the fraction of C8KC bound to HDL progressively increased until by 3 hr, only HDL binding was detected. In other experiments, we observed *in vivo* distribution of MP, solubilized with HS15, only to density-gradient fractions 12–16.

In another study, we formulated C8KC with 20% [^{14}C]HS15 and followed the distribution of both fluorescence and label in mouse

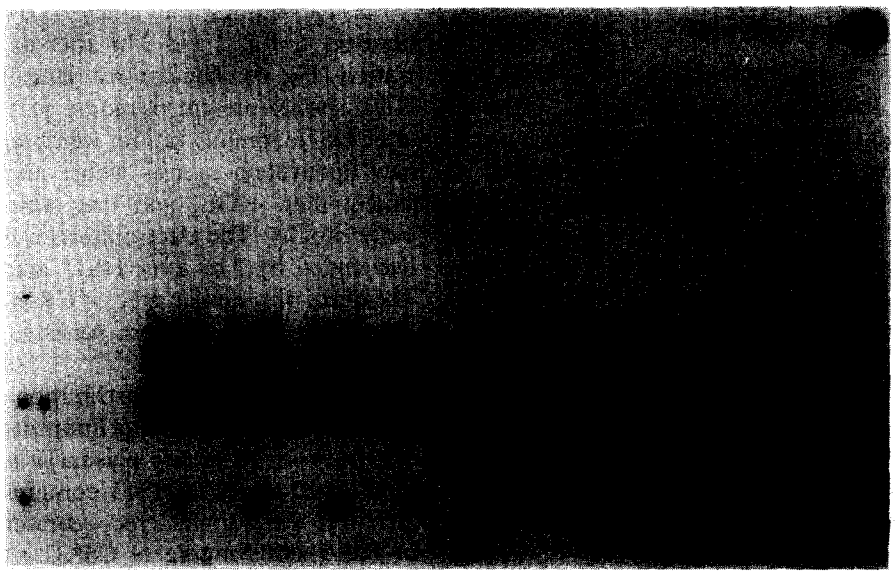

Fig. 4. Electrophoretic patterns of human plasma lipoproteins in fractions separated by density-gradient ultracentrifugation after exposure to 0.2% HS15 *in vitro*. The lanes represent fractions 1–8.

Fig. 5. Effect of HS15 on the electrophoretic patterns of mouse plasma lipoproteins. Lane 1 = control. Lanes 2–5, at intervals after administration of 0.1 ml of 20% HS15: lane 2 = 1 hr, lane 3 = 1.5 hr, lane 4 = 2 hr, lane 5 = 3 hr. Lane 8 = 18 hr after CRM administration.

plasma fractions after drug administration (C8KC = 5 mg/kg, HS15 = 1 g/kg). After 1 hr, both radioactivity and C8KC fluorescence were detected in early fractions (1–4). A distinct peak of C8KC fluorescence was observed in fractions 14–17, corresponding to the HDL region, while HS15 radioactivity was found in a diffuse band extending from fractions 13–24 (Fig. 7). Table 1 shows the clearance of C8KC and [^{14}C]HS15 from mouse plasma *in vivo* over 6 hr. Both agents were lost from the circulation with a half-lives of approx. 1.7 hr.

DISCUSSION

Previous investigations involving use of CRM (Garbo, 1990; Kessel *et al.*, 1991) had suggested CRM-induced promotion of the binding of photosensitizers to LDL. This phenomenon could promote sensitizer uptake by neoplastic tissues which contain elevated levels of LDL receptors (Maziere *et al.*, 1991). The first suggestion of an alternative explanation was provided by Kongshaug *et al.* (1991) who found that CRM altered the structure of

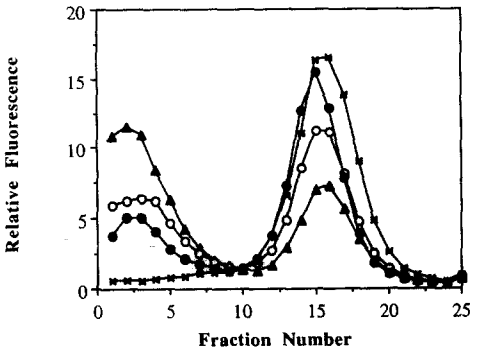

Fig. 6. Density-gradient analysis of mouse plasma after administration of C8KC formulated with HS15. Samples were obtained after 1 hr (▲), 90 min (○), 2 hr (●) and 3 hr (×).

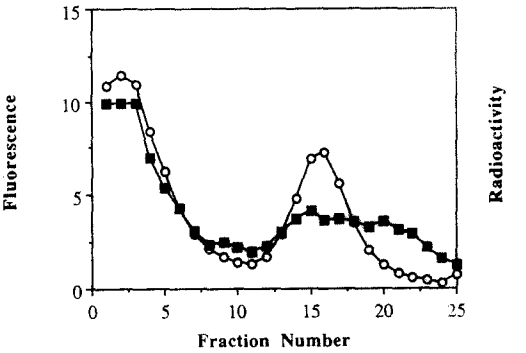

Fig. 7. Density-gradient analysis of mouse plasma 1 hr after administration of C8KC formulated with [^{14}C]HS15. C8KC levels were monitored by fluorescence (—○—), HS15 by radioactivity (—■—).

HDL. Based on information that the LDL level in the mouse is approx. 10% of the HDL value (Chapman, 1986), we carried out additional studies (Woodburn and Kessel, 1994) that showed CRM-induced modifications of both HDL and LDL. The bouyant density of HDL was markedly reduced and electrophoretic mobility of both species was decreased.

Human plasma was useful for additional studies since readily detectable levels of VLDL, LDL and HDL are present, and there are good techniques for resolving these species by agarose gel electrophoresis and density-gradient ultracentrifugation. Use of CRM as the drug-delivery vehicle resulted in the appearance of C8KC in two (human) plasma fractions which could be separated by density-gradient ultracentrifugation; peak drug levels were found in fractions 4–5 and 15–17. With HS15, a more diffuse pattern was obtained (Fig. 2).

Administration of CRM to a mouse (Woodburn *et al.*, 1994) led to the initial appearance of C8KC in density-gradient "light" fractions (4–7) and "heavy" fractions (13–16). With HS15, the distribution of C8KC to the "heavy" fractions was observed, but the drug was initially bound to a "very-light" plasma species, fractions 1–4 (Fig. 7). Binding of drug to these plasma components was not detectable after 3 hr. While the radioactive label allowed us to assess HS15 distribution, it must be remembered that the specific activity of each HS15 component will be a function of the number of poly(ethyleneglycol) residues. Both C8KC fluorescence and HS15 radioactivity were detected in the "very light" plasma component(s) (Fig. 7) suggesting a C8KC:HS15 complex or a ternary complex involving drug, vehicle and plasma components. We cannot rule out the possibility that unesterified radioactive PEG was preferentially incorporated into these products.

Table 1. Plasma pharmacokinetics of C8KC and [^{14}C]Solutol HS15

Time (hr)	C8KC (μg/ml)	Solutol	
		counts/min/ml	mg/ml
1	43 ± 9	35,400 ± 2100	6.9 ± 0.41
1.5	32 ± 4		
2	30 ± 3	24,940 ± 1890	4.9 ± 0.37
3	18 ± 3		
6	4.6 ± 1.4	3150 ± 265	0.61 ± 0.052

Drug levels in ICR mice after injection of 5 mg kg^{-1} sensitizer in 20% HS15 as a function of time. Data represent mean ± SD of three determinations.

Additional information was provided by experiments involving gel electrophoresis. Using human plasma *in vitro*, we found that CRM or HS15 levels $\geqslant$0.06% led to decreased electrophoretic mobility of both HDL and LDL (Fig. 5, Woodburn *et al.*, 1994). Administration of CRM to the mouse led to a different effect: the electrophoretic pattern of lipoprotein species showed a marked dispersal for 18 hr with CRM (Fig. 7), >3 hr with HS15 (Fig. 5). The persistence of C8KC in plasma is correlated with this effect. The plasma half-life using a CRM vehicle was 12–13 hr; using HS15, 2.5 hr. The finding (Table 1) that HS15 and C8KC exhibit similar plasma half-lives suggests a possible interpretation of these results. We propose that the persistence of the drug-delivery vehicle is an important determinant of the persistence of the sensitizer. This implies that CRM or HS15 do not merely serve as vehicles for both drug formulation, but also play a role in the subsequent pharmacokinetic behavior of drugs in the circulation.

In order to examine the possibility that relatively minor components of CRM and HS15 are responsible for the behaviour of these products, we have recently begun a study involving fractionation of each agent, in order to provide a more detailed explanation for the pharmacologic effects of CRM vs HS15.

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REFERENCES

Brown T., Havlin K., Weiss G., Cognola J., Koeller J., Kuhn J., Rizzo J., Craig J., Phillips J. and Von Hoff D. (1991) A phase I trial of taxol given by a 6-hour intravenous infusion. *J. clin Oncol.* **9**, 1261–1267.

Chapman M. J. (1986) Comparative analysis of mammalian plasma lipoproteins. *Meth. Enzymol.* **128**, 70–143.

Cohen D. J., Loertscher R., Rubin M. F., Tilney N. L., Carpenter C. B. and Strom T. B. (1984) Cyclosporin: a new immunosuppressive agent for organ transplantation. *Am. Int. Med.* **101**, 667–682.

Garbo G. M. (1990) The use of liposomes, emulsions or inclusion complexes may potentiate *in vivo* effects of SnET2. *Proc. SPIE* **1203**, 118–125.

Kessel D., Morgan A. and Garbo G. M. (1991) Sites and efficacy of photodamage by tin etiopurpurin *in vitro* using different delivery systems. *Photochem. Photobiol.* **54**, 193–196.

Kongshaug M., Cheng L. S., Moan J. and Rimington C.

(1991) Interaction of Cremophor EL with human plasma. *Int. J. Biochem.* **23,** 473–478.

Lorenz W., Schmal A., Schult H., Lang S., Ohman Ch., Weber D., Kapp B., Lüben L. and Doenicke A. (1982) Histamine release and hypotensive reactions in dogs by solubilizing agents and fatty acids: analysis of various components in cremophor EL and development of a compound with reduced toxicity. *Agents Actions* **12,** 64–80.

Maziere J. C., Morliere P. and Santus R. (1991) The role of the low density lipoprotein receptor pathway in the delivery of lipophilic photosensitizers in the photodynamic therapy of tumours. *J. Photochem. Photobiol. B: Biol.* **8,** 351–360.

Sykes E., Meany M., Schulz V. and Kessel D. (1992) Separation of plasma lipoproteins on a table-top ultracentrifuge. *Clin chim. Acta* **205,** 137–144.

Woodburn K. and Kessel D. (1994) The alteration of plasma lipoproteins by Cremophor EL. *J. Photochem. Photobiol. B: Biol.* **22,** 197–201.

Woodburn K., Chang C. K., Henderson B. and Kessel D. (1994) Biodistribution and PDT efficacy of a ketochlorin photosensitizer as a function of the delivery vehicle. *Photochem. Photobiol.* **60,** 154–159.