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Report

Preclinical evaluation of alternative pharmaceutical delivery vehicles for paclitaxel

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New solubilizers, including Sorporol 230, Sorporol 120Ex, Aceporol 345-T, Aceporol 460 and Riciporol 335, as potential new delivery vehicles for paclitaxel were investigated, since recent studies have shown that the paclitaxel delivery vehicle Cremophor EL significantly alters the pharmacokinetics of paclitaxel. Cremophor EL and Tween 80 were used as a reference. As in the case of Cremophor EL, alteration of blood distribution of paclitaxel occurred in the presence of all tested vehicles. Also, no differences in the affinity of paclitaxel for the tested solubilizers was found during equilibrium dialysis experiments. The different vehicles could be distinguished by a different rate of esterase-mediated breakdown, which was correlated with the fatty acid content of the solubilizers. The activation of the complement cascade was less pronounced for all solubilizers, except Riciporol 335, compared to Cremophor EL. The strategies presented here provide the possibility to rapidly screen future candidate delivery vehicles with optimal characteristics for use as a solubilizer in clinical formulations of paclitaxel or other poorly water-soluble drugs. [© 2002 Lippincott Williams & Wilkins.]

Key words: Cremophor EL, drug delivery vehicle, paclitaxel, solubilizer.

Introduction

The current paclitaxel formulation vehicle Cremophor EL[1,2] presents a number of serious concerns, including a wide variety of intrinsic toxic side effects, such as peripheral neurotoxicity[3] and anaphylactic hypersensitivity reactions.[4] It has been suggested that the hypersensitivity reactions during paclitaxel therapy are associated with histamine release and complement activation due to Cremophor EL.[5,6]

The plasma pharmacokinetics of paclitaxel has been shown to be non-linear.[7] A substantial number of publications have discussed the role of the pharmaceutical vehicle in the pharmacologic behavior of paclitaxel.[8] Interestingly, studies in mice have demonstrated that in the absence of Cremophor EL, both distribution and elimination of paclitaxel appears to be linear processes.[9] Recently, it was demonstrated that the non-linearity of paclitaxel plasma pharmacokinetics is due to dose- and time-dependent Cremophor EL concentrations in the central compartment. The presence of Cremophor EL in blood as large polar micelles with a highly hydrophobic interior is responsible for entrapment of paclitaxel, leading to significantly lower free-drug fractions of paclitaxel available for cellular partitioning.[10,11] Hence, numerous different alternative dosage forms for paclitaxel, including co-solvents, emulsion systems, micro-encapsulation systems, cyclodextrines and paclitaxel prodrugs, have been evaluated to overcome the pharmaceutical disadvantages of Cremophor EL.[12]

Several candidate delivery vehicles were newly synthesized, based on the chemical structures of Tween 80 and Cremophor EL (Figure 1), with the aim of improving pharmaceutical and pharmacological characteristics of a future paclitaxel solubilizer, as compared to Cremophor EL. These solubilizers were manufactured by ethylene oxide addition and successive fatty acid esterification with polyols (Figure 1). In this paper several *in vitro* experiments are described with the candidate delivery vehicles Aceporol 345-T, Riciporol 335, a purified Cremophor EL

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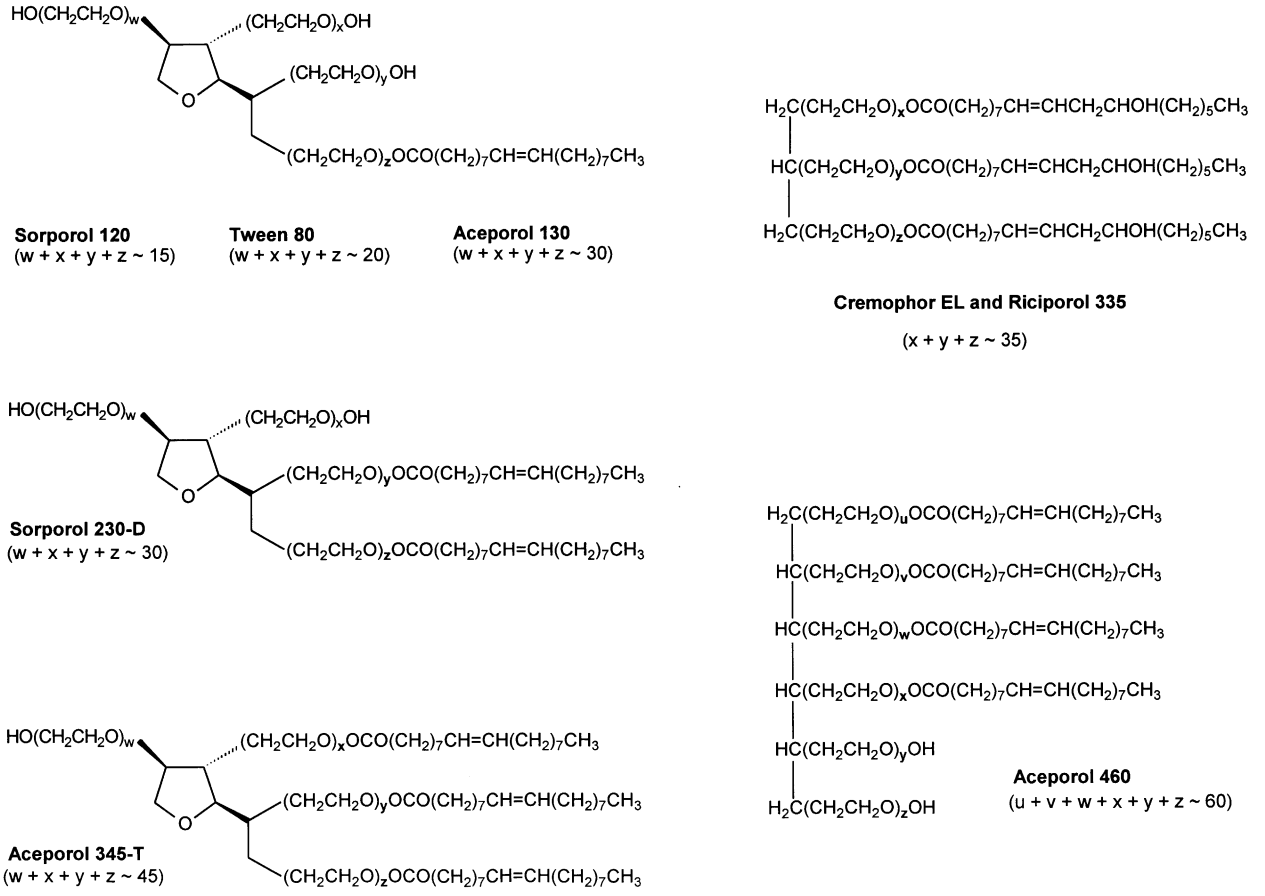

Figure 1. Chemical structures of micellar solubilizers: Sorporol 120 (polyoxyethylene 15 sorbitan monooleate), Tween 80 (polyoxyethylene 20 sorbitan monooleate), Aceporol 130 (polyoxyethylene 30 sorbitan monooleate), Sorporol 230-D (polyoxyethylene 30 sorbitan dioleate), Aceporol 345-T (polyoxyethylene 45 sorbitan trioleate), Cremophor EL, Riciporol 335 (polyoxyethyleneglycerol 35 triricinoleate) and Aceporol 460 (polyoxyethylene 60 sorbitol tetraoleate).

solution from which the polyoxyethyleneglycerol and is monoesters are removed, and Aceporol 460 and two mixtures named Sorporol 230, which is a mixture of 50% Aceporol 130 and 50% Sorporol 230-D, and Sorporol 120Ex, which is composed of 24% Sorporol 120, 36% Sorporol 230-D and 40% Aceporol 345-T. The *in vitro* observations were compared to Cremophor EL and Tween 80, in order to get an insight into the possible role of these new solubilizers as delivery vehicles for paclitaxel or other poorly water soluble drugs.

Materials and methods

Materials

Reference substances and reagents. The reference substances Aceporol 345T, Aceporol 460, Riciporol 335, Sorporol 230, Sorporol 120Ex and paclitaxel powder for the *in vitro* experiments were supplied by Bolak (Seoul, Korea). Cremophor EL, Tween 80, zymosan and porcine liver carboxyl esterase were purchased from Sigma (St Louis, MO). Bristol-Myers Squibb (Woerden, The Netherlands) kindly supplied paclitaxel powder, used for quantification; docetaxel was obtained from Aventis (Vitry-sur-Seine, France), while generally labeled [^{3}H]paclitaxel was purchased from Moravek (Brea, CA). Biosolve (Valkenswaard, The Netherlands) supplied acetonitrile and methanol, while ammonium acetate and formic acid were purchased from Baker (Deventer, The Netherlands). Dimethylsulfoxide (DMSO), *n*-butylchoride and tetrahydrofuran were supplied by Rathburn (Walkerburn, UK), Coomassie brilliant blue by BioRad (Munich, Germany), ethanol by Merck (Darmstadt, Germany), phosphate-buffered saline (PBS) by Oxoid (Basingstoke, UK), scintillation cocktail by Packard Biosciences (Groningen, The Netherlands) and a

SC5b-9 immunoassay kit by Quidel (San Diego, CA). All water used was filtered and deionized using a Milli-Q-UF system (Millipore, Bedford, MA).

Stock solutions. Stock solutions of the solubilizers were prepared in ethanol at final concentrations of 500 μl/ml. A stock solution of paclitaxel for the *in vitro* experiments, was prepared by dissolving 15.78 mg. paclitaxel in 3.155 ml DMSO, resulting in a solution containing 5 mg/ml paclitaxel. All stock solutions were stored at 4°C. Another stock solution containing 1.0 mg/ml paclitaxel (supplied by Bristol-Myers Squibb) was used for the construction of calibration curves for HPLC analysis.[13]

Methods

Analytical assays. Paclitaxel concentrations were quantified by an HPLC assay described previously,[13] with the flow-rate set at 1–2 ml/min, depending on the experiment.

The quantitation of the solubilizers was based on a colorimetric dye-binding assay described previously for the measurement of Cremophor EL concentrations in human heparinized plasma.[14]

Solubility of paclitaxel in solubilizers. A solution containing 20 mg/ml paclitaxel in ethanol was prepared by dissolving 90.67 mg in 4.5 ml ethanol. Aliquots of 50 and 100 μl were transferred into 4.5-ml glass tubes and evaporated to dryness. To the residues, volumes of, respectively, 100 and 200 μl of the stock solutions of the solubilizers, containing 500 μl/ml solubilizers in ethanol, were added in triplicate. The paclitaxel was dissolved by extensive vortex mixing and ultrasonification. Subsequently, volumes of, respectively, 900 and 800 μl of PBS were added, resulting in samples initially containing approximately 1 mg/ml paclitaxel in the presence of 50 μl/ml solubilizers and 2 mg/ml paclitaxel in the presence of 100 μl/ml solubilizers, respectively. The samples were incubated at ambient temperature for 24 and 48 h. At these timepoints, the samples were centrifuged for 5 min at 2000 *g* and 50 μl of the supernatant was mixed (in triplicate) with 950 μl of ethanol, from which aliquots of 10 μl were injected into the HPLC system, with the flow rate set at 2 ml/min. Calibration curves, in the range of 5–100 μg/ml, were used for the quantitation of paclitaxel in the supernatants of the samples. In addition, after 48-h incubation, the concentrations of the solubilizers were measured, using aliquots of 10 μl of 20-fold dilutions of the samples in water, using the colorimetric dye-binding assay. The responses after 48 h incubation were compared to the responses of the non-incubated solubilizers at concentrations of 2.5 and 5 μl/ml in water.

Blood distribution of paclitaxel in the presence of solubilizers. The effect of different concentrations of solubilizers on blood distribution of paclitaxel was estimated by incubation of 1 ml of freshly obtained human heparinized whole blood with 10 μg/ml paclitaxel in the presence of 0.1, 0.5, 1, 5 and 10 μl/ml of the different solubilizers at 37°C for 5–10 min. Aliquots of 100 μl whole blood and 100 μl plasma, obtained after centrifugation of 400 μl of whole blood for 5 min at 3000 *g*, were transferred into 12-ml glass tube covered with PTFE screw caps. After the addition of 900-μl aliquots of blank plasma and 100 μl of the internal standard docetaxel at a concentration of 50 μg/ml in methanol:water (1:1, v/v), the samples were extracted as described.[13] After the extraction procedure, the residue was dissolved in 200 μl of methanol:water (1:1, v/v) from which an aliquot of 50 μl was injected into the HPLC system, in which the eluent was delivered at a flow rate of 1 ml/min. Calibration curves were prepared at concentrations in the range of 0.25–5 μg/ml. Pools of quality control samples were prepared at concentrations of 10 and 20 μg/ml in plasma and at 10 μg/ml in whole blood, and were processed as described above. Experiments were conducted at least on three separate occasions. In one set of experiments erythrocytes were also collected as described recently,[15] using MESED instruments (Fabre, Kelmis, Belgium). Plasma and other blood components were separated from red blood cells by these instruments, resulting in an aliquot of 102 μl of unwashed packed red blood cells. These cells were further processed as described for plasma and whole blood. After the extraction, aliquots of 100 μl were used for the quantitation of paclitaxel, for which the calibration curve was extended to 0.05 μg/ml.

Affinity of paclitaxel for solubilizers. The affinity of paclitaxel for the different solubilizers in PBS was measured using equilibrium dialysis over 24 h as described.[16] A solution containing 1 μg/ml paclitaxel and a tracer amount of [^{3}H]paclitaxel was prepared freshly in PBS. Aliquots of 260 μl were dialyzed against 260 μl of PBS solutions containing 0.1, 1 and 10 μl/ml of the different solubilizers over a dialysis membrane with a 12 000–14 000 molecular weight cut-off (Spectrum Medical, Houston, TX). After a 24-h dialysis period, aliquots of 150 μl of both

sides of the membrane were transferred into separate 2-ml vials, to which 1.9 ml of scintillation cocktail were added. The ^{3}H-labeled paclitaxel was quantified by 20 min counting in a liquid-scintillation counter (Wallac, Turku, Finland). Similar experiments were carried out with 1 μg/ml paclitaxel and a tracer amount of [^{3}H]paclitaxel in human heparinized plasma against 0.1, 1 and 10 μl solubilizers in PBS. All experiments were performed on at least three separate occasions.

In vitro *degradation of solubilizers.* The degradation of the solubilizers at 37°C was initially investigated by incubation of 10 μl/ml of the solubilizers in PBS for 24 h in the presence and absence of 0.1 U/ml. esterase. Aliquots of 10 μl of the solutions were pipetted in triplicate into a flat-bottom 96-well plate at 4 and 24 h after the start of the incubation. The amount of solubilizers during the incubation was compared to the initial amount in the solutions, using the colorimetric-dye binding assay.

The *in vitro* degradation of the solubilizers in human plasma was investigated using a slightly modified method.[17] Aliquots of 25 μl solutions containing 10 μl/ml of the different solubilizers in freshly obtained human heparinized plasma, were incubated at 37°C (15 min and 4 h) and on melting ice (0°C, 4 h), in 12 ml glass tubes. After the incubation, aliquots of 500 μl of acetonitrile were added in triplicate, followed by vortex mixing for 10 s. The samples were stored at −80°C upon further extraction within 3 days. On the day of extraction the samples were thawed and vigorously mixed for 5 min. Aliquots of 2 ml of *n*-butylchloride were added, after which the samples were again mixed for 5 min. Subsequently, the samples were centrifuged for 5 min at 4000 *g*. Volumes of 2 ml of the organic layers were transferred to 4.5-ml glass tubes and evaporated to dryness under nitrogen at 60°C. To the residues, an aliquot of 50 μl of water was added, from which 25 μl was pipetted into a flat-bottom 96-well plate. The amount of solubilizers during the incubation was compared to the initial amount in the solutions using the colorimetric dye-binding assay. Both experiments were conducted at least at three separate occasions.

In vitro *complement activation by solubilizers.* The solubilizer-induced rise of the complement activation marker S protein-bound C5b-9 (SC5b-9) was measured following incubation of human serum with the solubilizers for 45 min at 37°C (40 μl serum + 10 μl solubilizer in PBS) using an ELISA as described recently.[6] PBS served as a negative control, while zymosan was used as a positive control.

Results

Solubility of paclitaxel in the presence of solubilizers

The measured concentration of 50 μl of dried paclitaxel, dissolved in 1 ml ethanol was 0.881 ± 0.0305 mg/ml after 24 h incubation and 0.876 ± 0.0296 mg/ml after 48 h incubation, respectively. Similar results were found for the samples of 100 μl dried paclitaxel, dissolved in 1 ml ethanol with values of 1.86 ± 0.0214 and 1.84 ± 0.0233 mg/ml after 24 and 48 h incubation, respectively. Maximum reachable concentrations of paclitaxel in the solutions containing solubilizers were thus 0.881 and 1.86 mg/ml in the presence of 50 and 100 μl/ml solubilizers, respectively. In one experiment, no visual precipitation of paclitaxel was observed after 24 h incubation in the presence of 100 μl/ml Riciporol 335 and 50 μl/ml Sorporol 120Ex, with paclitaxel concentrations equal to those observed in the control samples in which paclitaxel was dissolved in ethanol. These two single observations were therefore rejected for the calculation of the solubility of paclitaxel. As shown in Figure 2, the solubility of paclitaxel after 24 h was lower in the presence of both 50 and 100 μl/ml Tween 80 and Sorporol 230, as compared to the other solubilizers. After 48 h, the amount of paclitaxel still in solution decreased in the presence of all solubilizers. In the case of Sorporol 230, no accurate sample could be taken for the measurement of paclitaxel after 48 h incubation of the sample containing 2 mg/ml paclitaxel and 100 μl/ml Sorporol 230, due to a gel-like substance of the sample. The measured concentrations of the different solubilizers after 48 h incubation were found in the range of 44.3–58.2 μl/ml for the samples incubated with 50 μl/ml of solubilizers and 94.6–120 μl/ml for the samples containing 100 μl/ml of solubilizers, respectively.

Blood distribution of paclitaxel in the presence of solubilizers

The distribution of paclitaxel in whole blood was constant over time, i.e. no changes of the blood: plasma ratios were observed between 5 and 60 min incubation of 10 μg/ml paclitaxel in heparinized human whole blood in the presence of 5 μl/ml

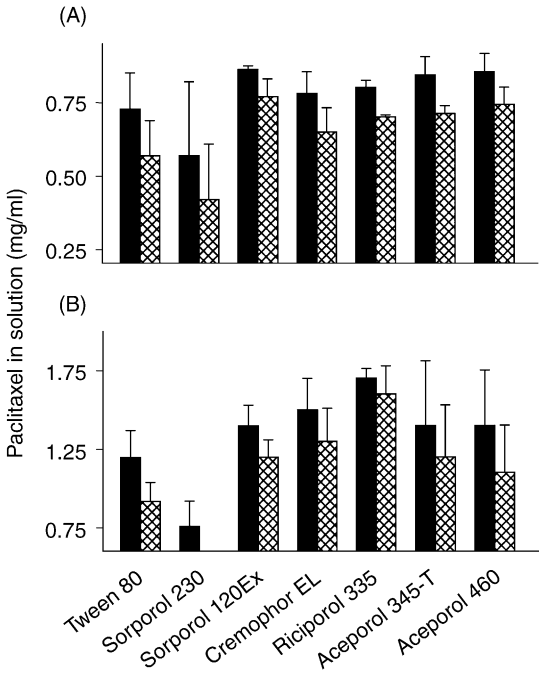

Figure 2. Amount of paclitaxel still in solution after 24 (black bars) and 48 (criss-crossed bars) h at ambient temperature in the presence of 50 (A) and 100 (B) μl/ml of the various solubilizers.

Cremophor EL (data not shown). All other experiments related to the blood distribution of paclitaxel in the presence of the different solubilizers were conducted with incubation times of 5–10 min.

As in the presence of Cremophor EL, addition of all solubilizers, except Sorporol 230, resulted in a decreased blood:plasma ratio, depending on the concentration of the solubilizers (Table 1). This observation is indicative for entrapment of paclitaxel in the plasma compartment in which the solubilizers are located. In contrast, the blood:plasma ratio in the presence of Sorporol 230 increased. During the incubation time all blood samples became hemolytic, with the most pronounced hemolytic effect in presence of Aceporol 345-T and Aceporol 460 at the highest concentrations. In addition to this observation, after centrifugation of the blood samples, major clots were observed in the plasma of the blood sample containing Sorporol 230, while some minor clots were observed in the presence of Aceporol 345-T and Sorporol 120Ex. In the experiments where also red blood cells were harvested, similar blood:plasma ratios were observed as in earlier experiments, with decreased ratios in the presence of the solubilizers, except in the case of Sorporol 230. Surprisingly, the blood:erythrocyte ratios increased in the presence of all tested

Table 1. Blood distribution of paclitaxel in the presence of solubilizers

Solubilizer	Concentration (μl/ml)	Blood:plasma ratio		
		Mean	SD	*n*
None		1.0	0.077	10
Tween 80	0.1	1.1	0.051	3
	0.5	1.0	0.025	3
	1	0.89	0.028	4
	5	0.69	0.009	3
	10	0.62	0.064	7
Sorporol 230	0.1	1.1	0.099	3
	0.5	1.0	0.043	3
	1	1.1	0.068	4
	5	1.3	0.40	3
	10	1.9	0.28	7
Sorporol 120Ex	0.1	1.1	0.067	3
	0.5	1.1	0.044	3
	1	1.1	0.18	4
	5	0.65	0.10	3
	10	0.53	0.064	7
Cremophor EL	0.1	1.1	0.083	3
	0.5	0.95	0.020	3
	1	0.87	0.027	4
	5	0.68	0.027	6
	10	0.66	0.083	6
Riciporol 335	0.1	1.0	0.10	3
	0.5	0.96	0.029	3
	1	1.00	0.19	4
	5	0.73	0.039	3
	10	0.60	0.035	7
Aceporol 345-T	0.1	0.96	0.16	3
	0.5	1.0	0.063	3
	1	0.95	0.062	4
	5	0.63	0.056	3
	10	0.56	0.063	6
Aceporol 460	0.1	1.0	0.059	3
	0.5	0.94	0.049	3
	1	0.83	0.025	4
	5	0.70	0.096	3
	10	0.59	0.045	6

solubilizers, including Sorporol 230, with the ratios ranging from 3.3 ± 0.073 to 4.9 ± 0.25 in the presence, compared to a ratio of 1.0 ± 0.034 in the absence of the solubilizers. Paclitaxel is thus not driven into the red blood cells by Sorporol 230, as suggested by the increased blood:plasma ratio, but is most likely located in the clots formed on top of the plasma after centrifugation.

Since we did not find major differences between the different solubilizers in the overall blood distribution of paclitaxel, except for Sorporol 230, we hypothesized that prolonged incubation might give increased insight in possible *in vitro* degradation of the solubilizers, resulting in a change in the blood:plasma ratios. Indeed we found increased blood:plasma ratios in the presence of Aceporol 345-T, Sorporol 120Ex and Tween 80 during the

prolonged incubation, with the most pronounced effect for Aceporol 345-T. This effect was likely not caused by the degradation of the solubilizers, but by gross cellular damage of erythrocytes during the incubation with these solubilizers. This effect, as observed after centrifugation of the samples, leads to lower hematocrit values, and thus higher blood: plasma ratios, since the volume of the plasma compartment is increasing, resulting in lower concentrations (data not shown).

Affinity of paclitaxel for solubilizers

No major differences between the solubilizers were found in the percentages of paclitaxel initially dissolved in PBS, dialyzed towards the solubilizers, with 59–61% at the site of the solubilizers at concentrations of 0.10 μl/ml, while these percentages ranged from 79 to 84% and from 93 to 94% with solubilizer concentrations of 1 and 10 μl/ml, respectively. Also no major differences between the solubilizers were noted in the percentage of paclitaxel dialyzed from plasma towards the solubilizers dissolved in PBS, with percentages at the site of the solubilizers ranging from 9.9 to 11%, from 20 to 26% and from 32 to 38%, with solubilizer concentrations of 0.1, 1 and 10 μl/ml, respectively.

In vitro degradation of solubilizers

The utility of the colorimetric dye-binding assay with Coomassie brilliant blue G-250 for the different solubilizers was investigated by examination of the linearity of the curves constructed using dilutions of non-incubated solubilizers in water. Linear relationships, with regression coefficients of at least 0.997, were found between the added amount of any of the tested solubilizer and the response, making this technique usable for the estimation of the *in vitro* degradation of all the solubilizers, with no differences noted between the responses after 15 min and 1 h (data not shown).

None of the solubilizers showed a decrease in response during 24 h incubation in PBS at 37°C, while in the presence of esterase, the responses decreased for all solubilizers, except in the case of Aceporol 460 (Figure 3A). The most pronounced decrease in response in the presence of esterase was observed with Tween 80 and Sorporol 120Ex, with approximately a 50% reduction in color intensity. In addition to the observed decreased responses, the samples incubated at 37°C with Sorporol 230 and esterase showed a dissolution of the solubilizer, while a slightly blurred sample was observed in the presence of Aceporol 345-T.

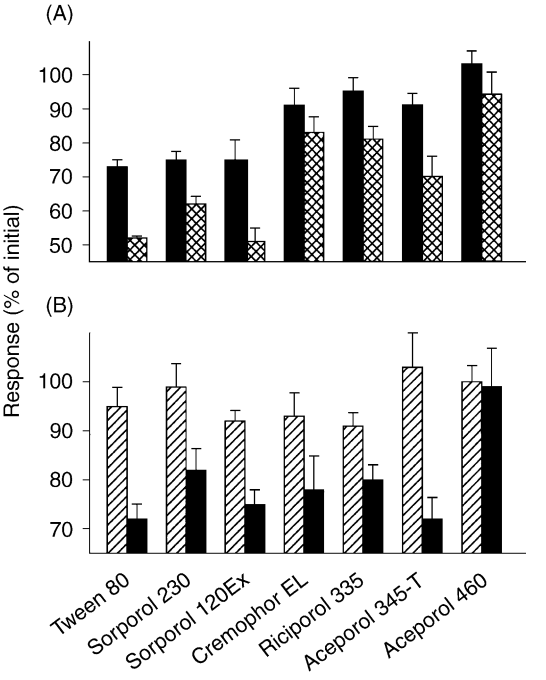

Figure 3. *In vitro* degradation of solubilizers at 37°C in the presence of 0.1 U/ml esterase in PBS (A) and in freshly obtained human heparinized plasma (B). Incubation periods of 15 min (hatched bars), 4 h (black bars) and 24 h (criss-crossed bars).

In fresh human heparinized plasma, all solubilizers were stable when incubated for 4 h on melting ice, while at 37°C a decrease in responses was observed, except for Aceporol 460 for which no difference in the response during incubation was observed (Figure 3B). The most pronounced decrease in response was observed with Tween 80, Sorporol 120Ex and Aceporol 345-T. In addition, the plasma sample containing Sorporol 230 became blurred after incubation at 37°C.

In vitro complement activation by solubilizers

The results of the complement activation are summarized in Table 2. As shown, Cremophor EL and Riciporol 335 resulted in the most pronounced rise in one of the markers of complement activation, SC5b-9. The fact that Cremophor EL and Riciporol 335 were equally potent activators as the yeast cell extract, zymosan, shows that they are potent complement activators. The other solubilizers also caused

Table 2. Complement activation by solubilizers

Solubilizer	SC5b-9 (μg/ml)		
	Mean	SD	*n*
PBS	4.38	0.522	3
Zymosan	17.0	0.146	3
Tween 80	11.7	0.224	3
Sorporol 230	11.1	1.22	3
Sorporol 120Ex	12.3	0.399	3
Cremophor EL	17.9	0.844	3
Riciporol 335	16.4	0.227	3
Aceporol 345-T	10.7	0.529	3
Aceporol 460	10.6	1.53	3

a rise of this marker, compared to the negative control, although less than Cremophor EL and Riciporol 335.

Discussion

Paclitaxel, a water-insoluble anticancer agent, has a non-linear pharmacokinetic profile, as a result of micellar encapsulation, due to its formulation vehicle Cremophor EL.[10,11] Novel paclitaxel delivery vehicles ideally should not influence the blood distribution of paclitaxel, while at least equal solubility[1] is required and no acute hypersensitivity reactions should occur.[5,6]

The choice of 50 and 100 μl/ml solubilizers in the solubility experiments is based on the assumption of a clinical formulation of paclitaxel in these vehicles as Taxol, i.e. 6 mg of paclitaxel in Cremophor EL:ethanol (1:1, v/v), resulting in a Cremophor EL concentration of 50.0 μl/ml in the infusate of a patient receiving 300 mg of paclitaxel in a total volume of 500 ml.[11] From the tested potentially new paclitaxel delivery vehicles, lower solubility of paclitaxel, compared to Cremophor EL, was observed in the case of Tween 80 and Sorporol 230, after 24 and 48 h incubation periods at ambient temperature. The solubility of paclitaxel in Sorporol 120Ex, Riciporol 335, Aceporol 345-T and Aceporol 460 was identical to the solubility in Cremophor EL, under the applied conditions. These results indicate that besides Tween 80, also Sorporol 230 seems not suitable as a delivery vehicle for paclitaxel. On the other hand, prodrugs of paclitaxel are currently under preclinical[12] and clinical evaluation,[18–21] so Sorporol 230 might be a potential delivery vehicle for paclitaxel prodrugs, depending on the *in vitro* findings of Sorporol 230 with respect to lack of influencing the blood distribution of paclitaxel and/or esterase mediated degradation of the solubilizer itself.

All delivery vehicles influenced the blood distribution of paclitaxel, with no clear differences compared to Cremophor EL, except for Sorporol 230, which showed an opposite effect on the blood distribution. This phenomenon is most likely caused by entrapment of paclitaxel in Sorporol 230, which formed a gel-like substance after centrifugation on top of the plasma compartment, since no sequestration of paclitaxel was observed in the red blood cells. All solubilizers, including Cremophor EL, are toxic for the red blood cells in these *in vitro* experiments, resulting in hemolysis, even after the short incubation periods of 5–10 min. During prolonged incubation periods, the red blood cells degraded most extensively in the presence of Tween 80, Sorporol 120Ex and Aceporol 345-T. In addition, in the presence of Sorporol 120Ex and Aceporol 345-T, clots were also noted in the plasma compartment after centrifugation. Although the results of these *in vitro* blood distribution experiments in combination with the affinity experiments and solubility of paclitaxel do not provide a direct rationale for continuing the development of any of the potentially new delivery vehicles, they might be of interest if degradation by esterases of the solubilizers occurs, as has been described for Tween 80.[17] Tween 80 also causes a shift in the blood distribution of paclitaxel in favor of the plasma compartment and was also found to be responsible for the hemolysis of the red blood cells *in vitro* in the experiments described.

The *in vitro* degradation experiments of the solubilizers by esterase leads to a clear differentiation between the potentially new paclitaxel delivery vehicles. In Figure 4, the relationship between the fatty acid mole and response to the initial color intensity after 24 h incubation at 37°C in PBS in the presence of 0.10 U/ml esterase, i.e. a measure of intact delivery vehicles, is presented. As shown, a clear relationship is found between the fatty acids and the breakdown of the solubilizers. The most pronounced breakdown was observed in the case of Tween 80, which only has a content of 1 mol fatty acid, while no degradation was observed in the case of Aceporol 460, which has a content of 4 mol fatty acids. The triangles in Figure 4 indicate the mixtures Aceporol 230 and Sorporol 120Ex, which are both composed of different solubilizers. Since the native color intensity of the different tested vehicles was different (data not shown), the response after incubation relative to the initial value might be confounding in the case of the mixtures, since not all the components of the mixtures might be degraded

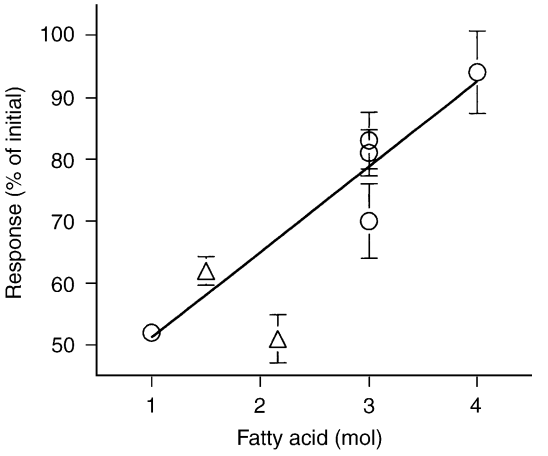

Figure 4. Relationship between fatty acid content of the different solubilizers and their *in vitro* degradation after 24-h incubation at 37°C in PBS in the presence of 0.1 U/ml esterase (triangles, not used for the calculation of the line, indicate the tested mixtures Aceporol 230 and Sorporol 120Ex, with on average fatty acid contents of 1.5 and 2.16 mol, respectively).

to the same extent. Since the applied method is aspecific, i.e. the formed degradation products might also result in a response in the colorimetric dye-binding assay, conclusions regarding the differences in esterase-mediated breakdown of Cremophor EL, Riciporol 335 and Aceporol 345-T cannot be drawn.

Finally, the *in vitro* complement activation by the solubilizers was tested by incubation of serum samples with the vehicles, since one of the well known side effects of paclitaxel therapy is an acute hypersensitivity reaction due to Cremophor EL-mediated complement activation.[5,6] The ideal delivery vehicle for paclitaxel, and other water-insoluble drugs, should thus not activate the complement cascade, which was tested by *in vitro* incubation of freshly obtained human serum with the vehicles. All of the tested vehicles resulted in an activation of the complement system as measured by the rise in SC5b-9. However, the rise in SC5b-9 was lower than observed in the presence of Cremophor EL and Riciporol 335.

Conclusions

Riciporol 335, the purified variant of Cremophor EL, does not improve the pharmaceutical and pharmacological characteristics of paclitaxel, and will thus not be better than Cremophor EL as a paclitaxel solubilizer. Sorporol 230 is unattractive as paclitaxel solubilizer with respect to the low solubility of paclitaxel, but might be of interest for other water-insoluble drugs or prodrugs of paclitaxel, since a relatively extensive esterase-mediated *in vitro* breakdown has been observed. Aceporol 460 will not improve the non-linear pharmacokinetics of paclitaxel, but in relation to side effects might be a better vehicle than Cremophor EL as a delivery vehicle for paclitaxel or paclitaxel prodrugs. Among the tested new paclitaxel delivery vehicles, Aceporol 345-T is one of the most promising vehicles. The solubility and blood distribution of paclitaxel are not different from that in the case of Cremophor EL; however, the *in vitro* degradation by esterases might be faster, while the complement activation is less. However, the *in vitro* hemolytic effect was more pronounced than in the case of Cremophor EL, while clots were noted also in the plasma compartment after centrifugation. Further *in vivo* experiments with Aceporol 345-T are warranted to investigate the possible toxic effects of this solubilizer on red blood cells.

The strategies presented here not only provide the possibility to rapidly screen potential candidate delivery vehicles with optimal characteristics for use as future paclitaxel solubilizers, but might also be used as methodologies for preclinical evaluation of potential (pharmacokinetic) effects by drug-delivery vehicles for any other water-insoluble drug. In addition to the *in vitro* experiments presented here, a comparative study of paclitaxel pharmacokinetics in *in vivo* models is needed to further characterize the novel solubilizers as potential new delivery vehicles for paclitaxel or other water-insoluble drugs.

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