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Enhancement of cyclosporin A solubility by d-alphatocopheryl-polyethylene-glycol-1000 succinate (TPGS)

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Abstract

The aqueous solubility of cyclosporin A (CyA) in the presence of various concentrations of TPGS ranging from 0.01 to 0.50 mM was studied at three temperatures (5, 20, and 37°C). Compared to previously reported solubility data in triple distilled water, solubility in the presence of TPGS was significantly increased at all temperatures. Surface tension and light scattering measurements showed that solubilization in TPGS multimers is the main mechanism responsible for the increased CyA solubility at 20°C and 37°C. In contrast, the increased CyA solubility at 5°C appears to be mediated by other mechanism(s), such as association of TPGS with CyA. These data substantiate the view that the enhanced bioavailability of CyA, when coadministered with TPGS in patients suffering from cholestasis, is due to the increased solubility of CyA in the presence of TPGS.

Key words: Cyclosporin A; d-Alphatocopheryl-polyethylene-glycol-1000 succinate (TPGS); Multimerization; Solubility; Critical micellar concentration; Temperature effect on solubility

1. Introduction

It has been suggested that in patients with severe cholestatic liver disease, the problematic absorption of the lipid-soluble vitamin E can be overcome by administering a water-miscible form of this vitamin, namely, TPGS (Traber et al., 1986). On the other hand, cyclosporin A (CyA) causes cholestasis in a significant proportion of transplant patients (Stone et al., 1987) and many recipients of liver transplantation require massive doses of CyA to achieve therapeutic blood concentrations of the drug. It has been shown (Sokol et al., 1991) that the coadministration of TPGS with CyA in patients with cholestatic liver disease allows a 40–72% reduction of CyA dose within 2 months. A similar effect was recently observed in children after liver transplantation (Boudreaux et al., 1993). In addition, there is some evidence in the literature that this water-soluble form of vitamin E can form micelles (with no reference to a specific temperature) at concentrations as low as 0.04–0.06 mM (Traber et al., 1986). These findings suggest that TPGS (a non-ionic surfactant) functions as a solubilizing agent, and, therefore, as a bile salt substitute in the small intestine when optimal bile flow is absent.

The aim of this study was to elucidate the effect of TPGS on the solubility of CyA at body temperature (37°C). Additionally, due to the unusual solubility behaviour of CyA in aqueous media (Ismailos et al., 1991), solubility studies in the presence of TPGS were also performed at 20 and 5°C.

2. Experimental procedures

Solubility of CyA (Sandoz, Hellas, A.E.B.E.) was studied in aqueous solutions of TPGS (Lot No. AA14-0791, Eastman Chemical Products, Inc., Milan, Italy) using the method described previously (Ismailos et al., 1991). The appropriate amount of TPGS was dissolved in triple distilled water and solutions of 0.01, 0.06, 0.12, and 0.5 mM were prepared. Experiments were performed in a shaking water bath (Julabo SW1, Schwartzwald, Germany) at 5, 20 and 37°C. Samples were collected at 24, 48, 72, 96, and 112 h. The equilibration time was found to be 24 h at 37 and 20°C and 96 h at 5°C. Solubility at each temperature was determined as the

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average value of three to six experiments. Each sample was analyzed in duplicate. The analytical methodology has been described previously (Ismailos et al., 1991). TPGS did not interfere with the assay of CyA. Statistically significant differences in CyA solubility were assessed at the 0.05 level with single-factor factorial ANOVA using the program Statview® SE + Graphics (Abacus Concepts, Berkeley, CA). Significant differences between two sets of data were then assessed with Fisher's PLSD and Scheffe's F-tests.

The surface tension of aqueous TPGS solutions was measured with a du Noüy interfacial tensiometer coupled with a thermostat vessel (Kruss 8600) set at 5 ± 0.1, 20 ± 0.1, and 37 ± 0.1°C. At each temperature the surface tension of triple distilled water was initially measured. In cases where the result deviated from what was theoretically expected (Dean, 1985) a correction factor was calculated and all the subsequent measurements of TPGS solutions were corrected according to this factor. The time allowed for equilibration of TPGS solutions was about 2 h, during which the solution was kept covered with aluminum foil to minimize evaporation (Ray and Nemethy, 1971).

The ability of TPGS to form multimers in water was further assessed with light scattering at 20°C. Light scattering measurements were performed with a Milton Roy® chromatix KMX-6 device at $\lambda = 452$ nm. HPLC grade water was used as solvent. All samples were filtered through a Millipore® filter (0.22 μm, Millipore Filter Corp., Bendford, MA) prior to measurement. The Rayleigh ratios of a total of twelve samples covering TPGS concentrations ranging from 0.01 mM to 0.5 mM were measured.

3. Results and discussion

Fig. 1 shows the ratios of CyA solubility in aqueous TPGS solutions to the solubility of CyA in water (Ismailos et al., 1991) at three different temperatures. In all cases the ratio is significantly higher than one, indicating that the solubility of CyA in the presence of TPGS is significantly increased. However, the magnitude of increase varies with temperature and TPGS concentration.

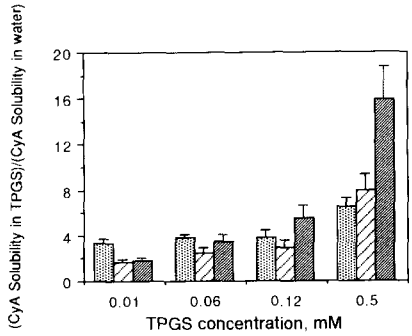

Fig. 1. Means + s.d. of the ratios of the solubility of CyA in aqueous TPGS solutions to the solubility of CyA in water at 37°C (right columns), 20°C (middle columns), and 5°C (left columns). Values for solubility in the absence of TPGS were taken from Ismailos et al. (1991).

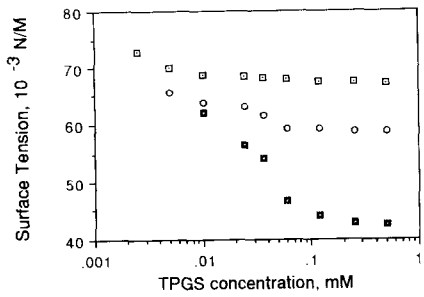

Fig. 2. Surface tension of aqueous TPGS solutions versus TPGS concentration at three different temperatures. Each point is the mean ± s.d. of six determinations. Key: (square with black dot) 5°C; (circle) 20°C; (square with white dot) 37°C.

The ability of TPGS to form multimers was tested with surface tension measurements and the results are graphically depicted in Fig. 2. At 37°C the plot shows two distinct phases indicating a critical micellar concentration (CMC) of about 0.15–0.30 mM. At 20°C, although there is a break in the concentration dependence of surface tension, this is not sharp. In contrast, at 5°C there is a continuous, slow but steady, decrease of the surface tension vs. TPGS concentration.

Light scattering studies were performed in an attempt to gain a better understanding of the multimerization process of TPGS at 20°C. Fig. 3 shows the Rayleigh ratio versus TPGS concentration. It is seen that for concentrations higher than 0.1 mM the plot is linear. In contrast, data points at lower concentrations are very scattered. Although it can be argued that a sociation process (Elias, 1972) between TPGS molecules occurs at concentrations higher than 0.1 mM, the scattering of the data points at low TPGS concentrations does not allow for a solid conclusion on the type of sociation to be drawn (Elias, 1972).

The multimerization ability of TPGS can be compared with the behavior of other non-ionic surfactants. The CMC of triton X-100 decreases as temperature increases (Birdi, 1975). Data from the present study show that micellar formation, based on the sharp break in the concentration dependence of surface tension, occurs

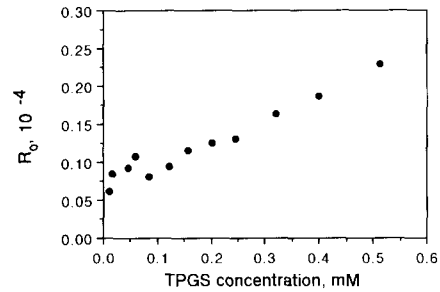

Fig. 3. The Rayleigh ratio, R_0, as a function of TPGS concentrations at 20°C.

only at 37°C. At lower temperatures and for the range of concentrations tested, the type of multimerization (closed, open, or combined) is not clear (at 20°C) or there is no apparent multimerization process (at 5°C).

In view of the surface tension and light scattering data, the increased CyA solubility in TPGS solutions can be discussed as follows:

(a) The increased solubility of CyA in the presence of TPGS at 37 and 20°C is partly due to the multimerization of TPGS. The increase in solubility of CyA reaches a maximum when TPGS concentration increases from 0.12 mM to 0.5 mM (from 33.3 to 96.4 μM and from 67.4 to 215.6 μM, at 37 and 20°C respectively). Apparently, the multimerization of TPGS (which according to the surface tension data occurs at about 0.1–0.3 mM at both temperatures) is the principal reason for these increases. Rough calculations of the relative increase in the solubility of CyA at 37 and 20°C show that the increase of CyA solubility due to solubilization by TPGS is more pronounced at 37 than at 20°C. For example, a 16-fold increase of the solubility in the presence of 0.5 mM TPGS was noted at 37°C (96.4 μM vs. 6.1μM in triple distilled water (Ismailos et al., 1991)) compared with an 8-fold increase at 20°C (215.6 μM vs. 27.3 μM in triple distilled water (Ismailos et al., 1991)). This is in accordance with literature data which show that increase of temperature increases the aggregation number of the micelles of a non-ionic surfactant (Birdi, 1975). Bigger TPGS micelles could accomodate more CyA molecules and, therefore, cause a more pronounced increase in CyA solubility.

The increase of CyA solubility at TPGS concentrations lower than 0.1 mM could be attributed to an association or complexation of TPGS with CyA. However, the exact mechanism(s) cannot be elucidated from the results of the present study.

(b) At 5°C the increase in CyA solubility observed at the lowest TPGS concentration (0.01 mM) was significantly higher than the increase observed at the other two temperatures. At higher TPGS concentrations the increase in CyA solubility is less pronounced compared to the increase at 20 and at 37°C. It seems that the increase of CyA solubility at this temperature is a result of complexation or association between the CyA and the surfactant. However, the mode of interaction needs further investigation.

In conclusion, the presence of TPGS has a dramatic effect on the solubility of CyA with the effect at physiologic temperature being primarily due to solubilization. These data provide a solid explanation for the reduced doses of CyA required to maintain therapeutic drug concentration in the absence of optimal bile flow (i.e. in the absence of efficient bile salt concentrations in the small intestine) when TPGS was coadministered (Sokol et al., 1991; Boudraeux et al., 1993).

4. Acknowledgements

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