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COMMUNICATIONS

Effect of formulation on the bioavailability of retinol, D-α-tocopherol and riboflavine

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Formulation factors influencing vitamin bioavailability have been investigated, with vitamins A, E and B_2, formulated in a new liquid vehicle (Aqua-Biosorb) and encapsulated into soft gelatin capsules, being compared with commercial formulations. There was an enhanced vitamin bioavailability from the new vehicle which appeared to be related to the surfactant system employed and the absence of vegetable oil in the formulation.

There is evidence that fat soluble vitamins are better absorbed from aqueous or emulsified vehicles than from oily preparations. Lewis et al (1947), for example, showed convincingly that vitamin A is absorbed by man, rats, and guinea-pigs more efficiently from an aqueous formulation than from an oily formulation. A similar concept of vitamin formulation affecting absorption has also been proposed for vitamin E (D-α-tocopherol) and supported by the studies of Engelhardt (1977), Von Schmandke & Schmidt (1965) and Akerib & Sterner (1971) while some contradictory evidence has been presented by Kelleher et al (1972).

A variety of commercial vitamin preparations is available. Many of these are formulated as compressed tablets, hardshell gelatin capsules, or soft elastic gelatin (SEG) capsules, having a complex matrix of excipients, fillers and other adjuvants. Little has been done to assess the relative availability of fat- and water-soluble vitamins from these dose forms.

A new liquid base material, Aqua-Biosorb†, into which fat- and water-soluble vitamins can be incorporated and encapsulated into SEG capsules has been developed in our laboratory. The base is water miscible, free from oil and contains a blend of polyhydric alcohols and polyoxyethylene sorbitan fatty acid esters as surfactants. We have compared the absorption of the fat soluble vitamins A and E and the water soluble vitamin B_2 from SEG capsules formulated with the new base with the absorption of the vitamins from various commercially available dosage forms.

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† Trade Mark of R. P. Scherer (Australia) Pty Ltd—patent pending.

Materials and methods

Instrumentation and instrumental conditions. For vitamin A (retinol) and vitamin E (D-α-tocopherol) analysis a Varian Model 5000 liquid chromatograph was coupled on line with a Varichrom multiwavelength uv absorbance detector, a reverse phase column (5 μm Microsorb C18, 15 cm × 4·6 mm i.d.—Rainen Instrument Co., Woburn, MA, USA) and a guard column (10 μm μBondapak C18, 5 cm × 3·9 mm i.d. Waters Associates, Inc.). The column was operated in the isocratic mode and eluted with 95% (v/v) methanol–water (previously degassed) at a flow rate of 2.0 ml min^{-1}. The column eluate was monitored at 292 nm with sensitivity set at 0·05 AUFS and a chart speed 5 cm min^{-1} was used.

For vitamin B_2 (riboflavine) analysis, the column was eluted with 90% (v/v) methanol–water (previously degassed). A Varian Fluorochrome fluorescence detector operated at 350 nm excitation and 450 nm emission was used to monitor the column eluate.

Reagents. Methanol, absolute ethanol and n-hexane were AR grade and had been redistilled. Perchloric acid 70% (AR) was used as received. Standards were DL-α-tocopherol (Sigma Chemical Co. USA), DL-α-tocopherol acetate (Sigma Chemical Co. USA), retinol (Sigma Chemical Co. USA) and riboflavine (Hoffman-La Roche Inc.).

Vitamin analysis in plasma. Vitamin A or E analysis was by a modification of the procedure of De Leenheer et al (1978). To 500 μl of plasma 50 μl of methanol containing DL-α-tocopherol acetate was added as internal standard. The sample was deproteinated with 500 μl of ethanol and extracted with 10 ml of n-hexane by mixing on a vortex mixer for 1 min. After centrifugation (3000 rev min^{-1}, 5 min) the organic phase was evaporated to dryness at 37 °C in a stream of nitrogen. The residue was taken up in 50 μl of methanol and 10 μl injected into the hplc. Quantitation was by measurement of the peak heights of vitamin and the internal standard, calculation of the peak height ratio and reading off the value of unknown vitamin from a standard curve (peak height ratio vs concentration of vitamin standard).

For analysis of vitamin B_2: 500 µl of plasma was deproteinated with 25 µl of 70% perchloric acid. The sample was mixed in a vortex mixer for 1 min. After centrifugation (3000 rev min^{-1}, 5 min) the supernatant was removed and 10 µl was injected into the hplc. Quantitation was by comparison of peak heights to a standard curve prepared daily.

Study design and vitamin preparations. The effects of formulation on the bioavailability of vitamins A, E and B_2 were examined in three studies. Various commercial formulations of vitamin were taken by 10 subjects in open crossover fashion and plasma concentrations of the vitamin monitored. All subjects were 'washed out' for two weeks between each formulation.

Vitamin preparations assessed in each study are shown in Table 1. With the exception of the products formulated with the new base, the other dosage forms were commercially available and within six months of the date of manufacture as stated on the label.

The Aqua-Biosorb SEG capsules were prepared in the laboratory. The composition of the base was (% w/w); polysorbate 80 80, ethanol 10, propylene glycol 10.

Water dispersible SEG capsules (R.P. Scherer (Australia) 'code stock' products) had a composition of the water dispersible base as follows (% w/w): polysorbate 80 20, sorbitan monooleate 1, distilled monoglycerides to 100.

Vitamin A (retinyl palmitate) and vitamin E (D-α-tocopherol) are soluble in both bases. Vitamin B2 (riboflavine) was incorporated as a fine suspension (particle diameter <100 µm).

Table 1. Vitamin preparations assessed for bioavailability of vitamin A, E and B2.

1. *Vitamin A (retinyl palmitate) 50 000 iu per dose*
   (a) Soya bean oil based SEG capsules[a]
   (b) Compressed sugar coated tablets[b]
   (c) Aqua-Biosorb vitamin A SEG capsules
2. *Vitamin E (as -α-tocopherol) 500 iu per dose*
   (a) Soya bean oil based SEG capsules[a]
   (b) Compressed tablets[c]
   (c) Water dispersible SEG capsules[d]
   (d) Aqua-Biosorb vitamin E SEG capsules
3. *Vitamin B2 (riboflavine) 10 mg per dose*
   (a) Multivitamin soya bean oil based SEG capsules[e]
   (b) Multivitamin hard shell capsules[f]
   (c) Multivitamin Aqua-Biosorb B Complex SEG capsules
   (d) Multivitamin water dispersible SEG capsules[d]
   (e) Compressed sugar coated tablets[g]

[a] Vitamin in solution.
[b] Ethnor vitamin A tablets; Ethnor, Australia.
[c] Blackmores Vitamin E tablets; Blackmores, Australia.
[d] 'Code stock'; R. P. Scherer, Australia.
[e] Supradyn; Roche, Australia.
[f] Myadec; Parke Davis, Australia.
[g] Vitaglow Multivitamin mineral tablets; Vitaglow, Australia.

Protocol. For each study, 4 males and 6 females, 22–38 years, 55–82 kg, and in good health, gave written informed consent. No other drugs or vitamin preparations were taken 4 weeks before or during the study.

After an overnight fast, subjects took the vitamin formulation orally with 100 ml of water. For the vitamin B_2 study, three of each dose form (total 30 mg) were taken to obtain measurable plasma concentrations.

Blood samples (10 ml) were withdrawn into heparinized vials from the left antecubital vein before and 2, 4, 6 and 8 h after dosing. Within 1 h the plasma was separated by centrifugation and stored at −20 °C until analysed in duplicate.

Data analysis. A paired Student's *t*-test to compare the commercial vitamin formulations with the Aqua-Biosorb formulations was performed on the calculated mean areas under the blood concentration vs time curves.

The AUC for each subject was calculated using the trapezoidal method after subtraction of baseline (zero time) values from the remaining data points to give a more accurate estimate of the AUC due to the formulation as distinct from that due to endogenous levels of the vitamin.

Results

Vitamin A study. Mean plasma concentrations of total vitamin A (retinol) obtained in ten subjects receiving 3 different formulations, each containing 50 000 iu of vitamin A, showed that throughout the study the new base formulation gave values that were consistently greater than those produced with the tablet and soya oil based formulations (Fig. 1).

A peak in plasma vitamin A concentrations, observed at 4·0 h with each formulation, was most pronounced with the new base formulation where an increase of 39% over predose concentrations was obtained (Table 2). This compares with a 9·5% rise with the oil-filled capsules and a 13% rise with the tablets.

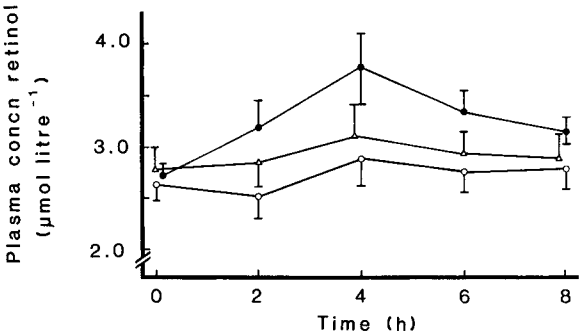

FIG. 1. Mean plasma concentration (±s.e.m., n = 10) of vitamin A (retinol) as a function of time following oral administration of an Aqua-Biosorb vitamin A SEG capsule (●), a sugar coated tablet (△) and a soya bean oil-based SEG capsule (○) each containing 50 000 iu vitamin A per dose.

Table 2. Mean predose and peak concentrations[a] of vitamin A (retinol) in plasma following oral administration of three different formulations containing 50 000 iu vitamin A (n = 10).

Formulation	Mean predose ± s.e.m.	Mean peak ± s.e.m.	% Increase[b]
Aqua-Biosorb Vit A	2·71 ± 0·12	3·76 ± 0·34	39·0
Soya oil SEG	2·63 ± 0·15	2·88 ± 0·25	9·5
Tablet	2·76 ± 0·23	3·13 ± 0·28	13·4

[a] Concentrations expressed as μmol litre^{-1}.
[b] % Increase calculated using the equation: 100 × (mean peak-mean predose)/mean predose.

Total absorption of vitamin A, as determined from the AUC, was also greater from the Aqua-Biosorb formulation, representing some 3 fold increase on the tablet and oil-filled SEG capsule formulations (Table 3(1)). Some subjects, however, appeared to be better able to absorb vitamin A than others and a large intersubject variation in the AUC was evident with all three formulations.

Vitamin E study. The results of the vitamin E study showed the Aqua-Biosorb formulation to give a plasma concentration of the vitamin at 8·0 h of 24·38 μmol litre^{-1} (s.e.m. ± 0·87) which represents a 74% increase over mean fasting vitamin E values (14·04 μmol litre^{-1}, s.e.m. ± 1.05) (Fig. 2).

Table 3. Mean of areas under the plasma concentration/time curves from zero time to 8·0 h (AUC_0^8) for all formulations. Bioavailability relative to Aqua-Biosorb formulations and probability factors (Ho: $u_1 = u_2$, Student's *t*), determined from a comparison of the mean area calculated for Aqua-Biosorb formulations with mean areas calculated for all other formulations in the same vitamin class are also shown.

	AUC_0^{∞}	Significance *P*	Relative bioavailability[b] %
(1) *Vitamin A 50 000 iu per dose*			
(a) Soya oil SEG	1·5 ± 1·5[a]	<0·025	31·9
(b) Tablet	1·7 ± 1·1	<0·025	36·2
(c) Aqua-Biosorb vitamin A	4·7 ± 2·8		100
(2) *Vitamin E 5000 iu per dose*			
(a) Soya oil SEG	10·9 ± 15·2[a]	<0·005	29·2
(b) Tablet	4·5 ± 3·5	<0·0005	12·1
(c) Water dispersible	4·6 ± 6·4	<0·0005	12·3
(d) Aqua-Biosorb vitamin E	37·3 ± 7·9		100
(3) *Vitamin B2 30 mg (3 × 10 mg)*			
(a) Soya oil SEG	13·7 ± 8·5[c]	<0·0005	4·8
(b) Hardshell capsule	16·4 ± 12·7	<0·0005	5·8
(c) Tablets	46·2 ± 21·6	<0·0005	16·3
(d) Water dispersible SEG	9·5 ± 9·7	<0·0005	3·4
(e) Aqua-Biosorb Multi-vitamin B Complex	282·8 ± 96·1		100

[a] Mean ± s.e.m. (n = 10) expressed as μmol litre^{-1} h^{-1}.
[b] Bioavailability relative to Aqua-biosorb calculated as (AUC_0^8/AUC_0^8 (Aqua-Biosorb)) × 100.
[c] Mean ± s.e.m. (n = 10) expressed as μg dl^{-1} h^{-1}.

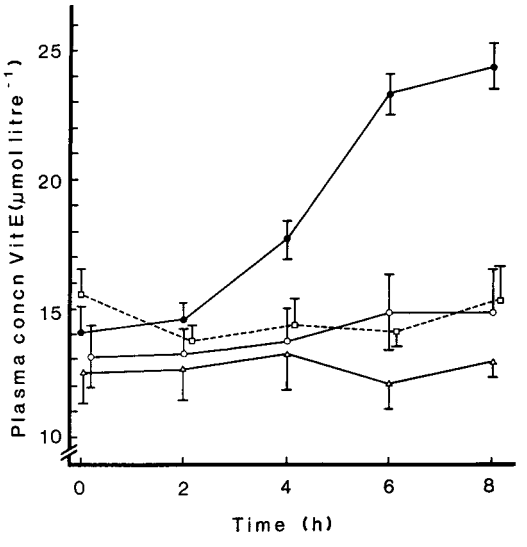

FIG. 1. Mean plasma concentration (±s.e.m., n = 10) of vitamin A (retinol) as a function of time following oral oral administration of a single Aqua-Biosorb vitamin E SEG capsule (●), a tablet (△), a water dispersible SEG capsule (□) and a soya bean oil-based SEG capsule (○), each containing 500 iu D-α-tocopherol per dose.

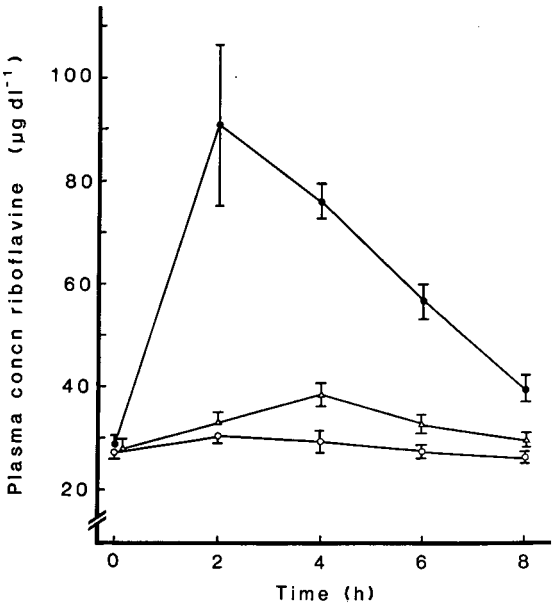

FIG. 3. Mean plasma concentration (±s.e.m., n = 10) of vitamin B2 (riboflavine) as a function of time following oral administration of Aqua-Biosorb B Complex SEG capsules (●) sugar-coated tablets (△) and hard shell capsules (○). Total riboflavine ingested in each case was 30 mg.

Plasma concentrations of vitamin E rose only marginally following the tablet and soya oil based formulations and in some subjects a drop below fasting levels was recorded with the water-dispersible formulation.

In Table 3(2) a comparison of the mean AUC obtained with the various vitamin E formulations shows the vitamin to be significantly more bioavailable from the Aqua-Biosorb formulation.

Vitamin B_2 study. Absorption of vitamin B_2 into the general circulation was significantly greater from the Aqua-Biosorb B Complex formulation than from any of the other formulations. This is evident from the plasma concentration vs time profiles shown in Fig. 3 and the AUC data presented in Table 3(3).

The water-dispersible formulation, the hardshell capsule formulation and the soya bean oil-based SEG capsule formulation performed similarly in that plasma concentrations of vitamin B_2 did not rise to values significantly different from fasting. (Values ranging from $26{\cdot}3 \pm 1{\cdot}0$ to $30{\cdot}4 \pm 1{\cdot}6\ \mu g\ dl^{-1}$ mean ± s.e.m., n = 10.)

Discussion

The bioavailability of vitamins A, E and B_2 when formulated in a new base, Aqua-Biosorb, and encapsulated into SEG capsules was enhanced over conventional dosage forms. Two possible reasons for this are: (i) the surfactant system enhanced absorption and (ii) the fact that the new base contains no vegetable oils.

Surfactants are known to enhance intestinal absorption depending on their concentration and physicochemical properties (Florence & Gillan 1975; Egan et al 1976; Slinde & Flatmark 1976). There are two general mechanisms by which surfactants may enhance absorption (Blanchard 1978).

(i) By increasing the solubility and dissolution rate, more material goes into solution and becomes available for absorption. This is probably the mechanism underlying the increase in vitamin B_2 bioavailability from the Aqua-Biosorb base. Although the vitamin is classed as water-soluble, its solubility in water is low (1 g in 3000–15 000 ml, Merck Index 1983).

(ii) The ability of surfactants to penetrate and disrupt the normal structure of biological membranes commonly results in increased membrane permeability. Such an increase to fat soluble vitamins may explain their enhanced absorption from formulations containing surfactants as opposed to oil-based formulations, which depend on bile and the formation of micelles in which fat soluble vitamins can be taken into mucosal cells. However, the absorption of vitamin E from the water-dispersible formulation, which also contains a surfactant system, was significantly less than from the soya bean oil-based SEG capsules and little different from the tablet formulation. The reasons for this are not clear but may be related to the concentration of surfactants and the content of vegetable oil in the water-dispersible formulation. Vegetable oil may withhold fat soluble vitamins from direct absorption so that the bile-dependent mechanism is the route for absorption.

It appears that the Aqua-Biosorb base delivers vitamins A, E and B2 more effectively, and more in accord with the doses ingested, compared with the other dosage forms studied.

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