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Influence of route of administration on the absorption and disposition of α-, γ- and δ-tocotrienols in rats

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Abstract

A study was conducted to evaluate the bioavailability of α-, γ- and δ-tocotrienols administered via oral, intravenous, intramuscular and intraperitoneal routes in rats. Three separate experiments, each conducted according to a two-way crossover design, were carried out to compare intravenous and oral, intramuscular and oral, and intraperitoneal and oral administration. Oral absorption of all three tocotrienols was found to be incomplete. Of the three tocotrienols, α-tocotrienol had the highest oral bioavailability, at about 27.7±9.2%, compared with γ- and δ-tocotrienols, which had values of 9.1±2.4% and 8.5±3.5%, respectively. Such biodiscrimination was also observed in their total clearance rates (estimated from the intravenous data). α-Tocotrienol showed the lowest clearance rate at about 0.16 L kg^{-1} h^{-1}, whereas that of δ- and γ-tocotrienols was quite similar, with values of 0.24 and 0.23 L kg^{-1} h^{-1}, respectively. Interestingly, all three tocotrienols were found to be negligibly absorbed when administered intraperitoneally and intramuscularly. Thus, these two routes of administration should be avoided when evaluating the biological activities of the tocotrienols in whole animal experiments.

Introduction

Vitamin E comprises eight compounds, namely four tocopherols and four tocotrienols, which share similar structural features of a chromanol head and a 16-carbon phytyl chain. Both tocopherols and tocotrienols are designated as α, β, γ and δ, depending on the number and positions of methyl groups on the chromanol ring. The difference between the tocopherols and tocotrienols lies mainly in the former having a saturated phytyl chain, while that of the latter is unsaturated, with three double bonds at 3′, 7′ and 11′ positions (Kamal-Eldin & Appelqvist 1996).

In recent years, tocotrienols have generated much interest as they have been reported to posses certain biological activities that were not observed with the tocopherols, including cholesterol-lowering activity (Qureshi et al 1991, 1995), anticancer and tumour-suppressing activities (Goh et al 1994; Nesaretnam et al 1998), antioxidant properties (Serbinova et al 1992; Kooyenga et al 1997) and anti-aggregation of blood platelets (Mahadevappa et al 1991). Despite the growing interest, there is a paucity of information with regard to their bioavailability and absorption, especially via different routes of administration. We have previously shown that the oral bioavailability of the tocotrienols determined from human studies was markedly increased when taken with food, and their biological half-lives were relatively short, being almost 4- to 5-fold shorter compared with that of α-tocopherol (Yap et al 2001). However, such information is lacking in other animal species even though animals such as rats and guinea-pigs have been used in evaluating the biological activities of the tocotrienols. The present study was conducted to determine the pharmacokinetics and bioavailability of α-, γ- and δ-tocotrienols given via different routes of administration in rats, namely via the oral, intravenous, intramuscular and intraperitoneal routes. In additional, their oral bioavailability was estimated with reference to the intravenous route of administration.

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Materials and Methods

Materials

Tocomin 50%, containing 21.7%, 5.9%, 11.8% and 11.1% of γ-, δ-, α-tocotrienol and α-tocopherol respectively, was obtained from Carotech Pte Ltd (Ipoh, Malaysia). The rest of the Tocomin 50% consisted mainly of palm olein, plant squalene and phyto-sterol complex, with trace amounts of phyto-carotenoid complex and co-enzyme Q10. Tocotrienol standard kit was purchased from Merck (Darmstadt, Germany). Labrasol and Tween 80 were purchased from Gattefossé (Cedex, France) and Sigma (St Louis, MO, USA), respectively. Soybean oil was purchased from Yee Lee Edible Oils Pte Ltd (Ipoh, Malaysia). All other solvents used were either of analytical reagent grade or HPLC grade and were purchased from either Merck or Ajax Chemicals (Auburn, Australia).

Animals

Male Sprague–Dawley rats were obtained from the animal holding of the University of Science Malaysia.

Preparation

For oral, intramuscular and intraperitoneal administration, Tocomin 50% was diluted with soybean oil to give α-, γ- and δ-tocotrienol concentrations of approximately 11.8 mg g^{-1}, 21.7 mg g^{-1} and 5.9 mg g^{-1}, respectively (5 mg mixed tocotrienols). For intravenous administration, Tocomin 50% was emulsified in water using 2% Tween 80 and 14% Labrasol. The emulsion was homogenized at 13 500 rev min^{-1} for 10 min and filtered through a 0.45-μm filter. The first 1 mL of the filtrate was discarded. The mean droplet size of the emulsion, designated the volume median diameter, was determined by a laser diffraction technique using a Malvern Mastersizer S diffraction particle analyser (Malvern Instruments, Worcestershire, UK) and was found to be 0.55 ± 0.05 μm. The final emulsion contained equivalent concentrations of α-, γ- and δ-tocotrienols as the oily solution. The concentration of the tocotrienols in both the oily preparation and the emulsion after filtration was assayed using the HPLC method reported by Yap et al (1999). The volume of both preparations was adjusted to give the desired dose of 5 mg mixed tocotrienols, being approximately 135 μL in volume.

In-vivo absorption studies

The study was approved by the Ethics Committee on Animal Studies, University of Science Malaysia. Three separate experiments were conducted to study the relative absorption of the tocotrienols via different routes of administration; the first was between the intravenous and oral routes, the second was between the intramuscular and oral routes, and the third was between the intraperitoneal and oral routes. Each experiment was conducted using six adult male Sprague–Dawley rats according to a two-way crossover study design with a washout period of 1 week. For each experiment, the rats were randomly divided into two groups of three rats each and administered the tocotrienols according to the sequence shown in Table 1.

Table 1 Sequence of administration following a crossover design.

Study	Group	Sequence of administration	
		1st week	2nd week
Oral versus intravenous	I	Oral	Intravenous
	II	Intravenous	Oral
Oral versus intramuscular	I	Oral	Intramuscular
	II	Intramuscular	Oral
Oral versus intraperitoneal	I	Oral	Intraperitoneal
	II	Intraperitoneal	Oral

All the animals were fasted for 12 h before drug administration and also during the study period, but were allowed free access to water throughout the experiment. For all routes of administration, the dose used was 5 mg of mixed tocotrienols (approx. 1.50, 2.75 and 0.75 mg of α-, γ- and δ-tocotrienol, respectively). The preparation was given orally via oral intubation. In the case of intraperitoneal and intramuscular administrations, the preparations were injected into the peritoneal cavity and the thigh muscle, respectively, using a 27G1/2 needle. For intravenous administration, the emulsion was injected into the tail vein. The animals were then placed in restraining cages and blood samples (approx. 0.5 mL) were collected from the tail vein into heparinized tubes at 0 (before administration), 1, 2, 3, 4, 6, 8, 10, 14 and 22 h after administration. In the case of intravenous administration, two additional blood samples were collected at 15 and 30 min after dosing. The blood samples were then centrifuged for 10 min at 12 800 *g*, and the plasma transferred into new Eppendorf tubes for storage at -20°C until analysis.

Analysis of α-, γ- and δ-tocotrienols

Plasma concentrations of α-, γ- and δ-tocotrienols were determined using a high-performance liquid chromatography (HPLC) method reported previously (Yap et al 1999).

Data analysis

The bioavailability of the tocotrienols after the different routes of administration was assessed using the pharmacokinetic parameters, peak plasma concentration (C_{max}), time to reach peak plasma concentration (t_{max}) and total area under the plasma concentration–time curve ($AUC_{0-\infty}$), which were estimated from the plasma concentration–time data. Both C_{max} and t_{max} were obtained directly from the plasma concentration values (Weiner 1981), while the $AUC_{0-\infty}$ was calculated by adding the area from time zero to the last detectable sampling time t (AUC_{0-t}) and the area from time t to infinity ($AUC_{t-\infty}$). The former was calculated using the trapezoidal formula and the latter by dividing the last measurable plasma drug concentration with the elimination rate constant (k_e). In all cases, the $AUC_{t-\infty}$ was

found to be less than 20% of the $AUC_{0-\infty}$. The k_e was estimated from the terminal slope of the individual plasma concentration–time curves after logarithmic transformation of the plasma concentration and application of linear regression (Gibaldi & Perrier 1982). From the intravenous data, the apparent volume of distribution (Vd) of the three tocotrienols was calculated as dose/($AUC_{0-\infty} \times k_e$), and the total clearance (CL_{tot}) was calculated using the relationship $k_e \times Vd$. In the experiment comparing the oral and intravenous routes, only the extent of bioavailability was compared using the parameter $AUC_{0-\infty}$. The absolute bioavailability was estimated by dividing the $AUC_{0-\infty}$ obtained from the oral administration with that obtained from intravenous administration.

Statistical analysis

The parameter values of both $AUC_{0-\infty}$ and k_e, obtained from the study comparing oral and intravenous administrations, were analysed using an analysis of variance procedure appropriate for a crossover study design. However, in the comparison of the CL_{tot} values of the three tocotrienols, analysis was performed using an analysis of variance procedure appropriate for a randomized block study design, followed by Tukey's test for pairwise comparison when a statistically significant difference was observed. For the parameter t_{max}, the values obtained for the three tocotrienols were analysed using the Friedman's test. A statistically significant difference was considered at $P < 0.05$.

Results

Figure 1A shows the plasma profiles of the tocotrienols obtained by comparing intravenous and oral administration. It can be seen from the figure that the plasma profiles of the three tocotrienols after intravenous administration appeared to be biphasic in nature, being characterized by an initial rapid decline for about 2 h after dosing, followed by a more gradual decline after this phase. This is clearly demonstrated by plotting the intravenous data on a $\log_{10}$ concentration–time scale, as shown in Figure 1B, suggesting that the pharmacokinetics of the tocotrienols might be better fitted or described by a two-compartment pharmacokinetic model. The initial rapid decline in plasma concentration could be due to redistribution of the compounds from the plasma compartment to other tissues and organ systems. In the case of oral dosing, the plasma concentrations of the tocotrienols showed a rapid increase, reaching a peak at approximately 3 h after dosing, and a gradual decline thereafter, being typical of profiles obtained with extravascular administration. The mean values of the pharmacokinetic parameters of C_{max}, $AUC_{0-\infty}$, t_{max} and k_e obtained from the individual plasma profiles after intravenous and oral dosing are given in Table 2. Of the three compounds, α-tocotrienol achieved the highest mean C_{max} value after oral dosing, followed by γ- and δ-tocotrienols. This was also reflected in the mean $AUC_{0-\infty}$ values; α-tocotrienol had the highest value, while δ-tocotrienol had the lowest. However, the administered doses of the three

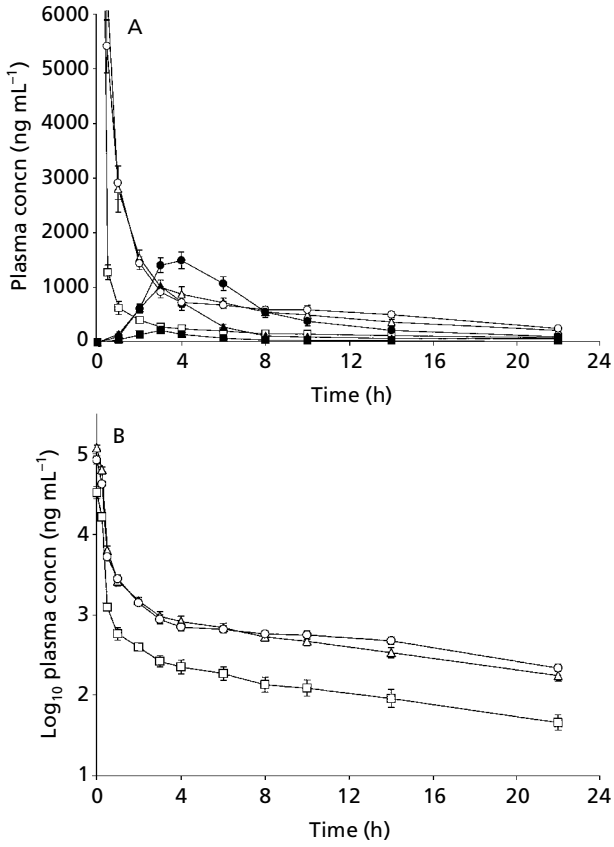

Figure 1 A. Plasma concentration–time profiles (mean±s.e.m., n = 6) of α-, γ- and δ-tocotrienols after intravenous and oral administration of a single dose of 5 mg mixed tocotrienols (approx. 1.50, 2.75 and 0.75 mg of α-, γ- and δ-tocotrienol, respectively). ○, intravenous α-tocotrienol; ●, oral α-tocotrienol; △, intravenous γ-tocotrienol; ▲, oral γ-tocotrienol; □, intravenous δ-tocotrienol; ■, oral δ-tocotrienol. B. $\log_{10}$ plasma concentration–time profiles (mean±s.e.m., n = 6) of α-, γ- and δ-tocotrienols after intravenous administration of a single dose of 5 mg mixed tocotrienols (approx. 1.50, 2.75 and 0.75 mg of α-, γ- and δ-tocotrienol, respectively). ○, α-tocotrienol; △, γ-tocotrienol; □, δ-tocotrienol.

compounds were not in the same rank order. While the dose of α-tocotrienol was almost 2-fold lower than that of γ-tocotrienol, its mean C_{max} and $AUC_{0-\infty}$ values were markedly higher than those of the latter, suggesting that the oral bioavailability and/or disposition of the two compounds was different.

Referring to Table 2, it can be seen that the $AUC_{0-\infty}$ values obtained from intravenous dosing were significantly higher than those obtained from oral dosing ($P < 0.01$), suggesting that oral absorption was not complete. As shown in Table 2, α-tocotrienol had an estimated absolute bioavailability of approximately 27.7%, whereas the absolute bioavailability of γ-tocotrienol was only about 9.1%. In the case of δ-tocotrienol, its estimated absolute bioavailability was also quite low, being similar to that of γ-tocotrienol, with a value of only about 8.5%.

Notwithstanding the above discrepancy, however, the t_{max} values of all three compounds were quite comparable

Table 2 Pharmacokinetic parameters of α-, γ- and δ-tocotrienols after oral and intravenous administration of 5 mg mixed tocotrienols (1.50, 2.75 and 0.75 mg of α-, γ- and δ-tocotrienol, respectively).

Parameter	α-Tocotrienol		γ-Tocotrienol		δ-Tocotrienol	
	Oral	Intravenous	Oral	Intravenous	Oral	Intravenous
C_{max} (ng mL^{-1})	1614.4±347.4	–	1023.2±262.3	–	223.1±80.4	–
t_{max} (h)	3.3±0.5	–	3.0±0.0	–	2.8±0.4	–
Vd (L kg^{-1})	–	1.68±0.28	–	2.64±1.05	–	2.77±1.14
k_e (h^{-1})	0.128±0.043	0.093±0.009	0.125±0.050	0.098±0.032	0.139±0.068	0.094±0.026
$AUC_{0-\infty}$ (h ng mL^{-1})	10580.0±2741.5	39254.4±5880.6*	4360.7±1083.9	48216.1±5312.1*	1038.9±269.4	13188.7±3773.7*
CL_{tot} (L kg^{-1} h^{-1})	–	0.155±0.021†	–	0.231±0.026	–	0.242±0.064
Bioavailability (%)	27.7±9.2		9.1±2.4		8.5±3.5	

*$P < 0.01$, significantly different compared with oral dosing. †$P < 0.01$, significantly different compared with δ- and γ-tocotrienols.

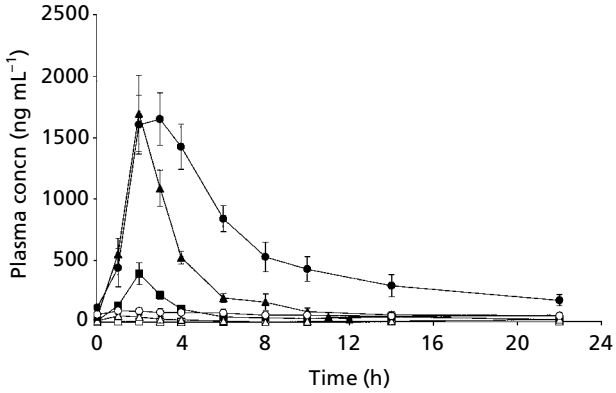

Figure 2 Plasma concentration–time profiles (mean±s.e.m., n = 6) of α-, γ- and δ-tocotrienols after intramuscular and oral administration of a single dose of 5 mg mixed tocotrienols (approx. 1.50, 2.75 and 0.75 mg of α-, γ- and δ-tocotrienol, respectively). ○, intramuscular α-tocotrienol; ●, oral α-tocotrienol; △, intramuscular γ-tocotrienol; ▲, oral γ-tocotrienol; □, intramuscular δ-tocotrienol; ■, oral δ-tocotrienol.

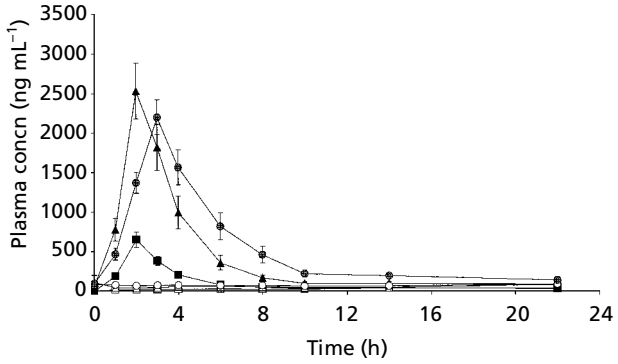

Figure 3 Plasma concentration–time profiles (mean±s.e.m., n = 6) of α-, γ- and δ-tocotrienols after intraperitoneal and oral administration of a single dose of 5 mg mixed tocotrienols (approx. 1.50, 2.75 and 0.75 mg of α-, γ- and δ-tocotrienol, respectively). ○, intraperitoneal α-tocotrienol; ●, oral α-tocotrienol; △, intraperitoneal γ-tocotrienol; ▲, oral γ-tocotrienol; □, intraperitoneal δ-tocotrienol; ■, oral δ-tocotrienol.

($P > 0.05$), with a mean value of about 3 h, indicating that their rate of absorption was similar. It can also be noted from Table 2 that the estimated elimination rate constant of the three tocotrienols was quite similar. Moreover, for all three compounds, the k_e values estimated from the oral administration data were similar to those estimated from the intravenous administration data ($P > 0.05$). It is interesting to note that the apparent Vd of the three compounds (calculated from the intravenous data) was also different. While both γ- and δ-tocotrienols had quite similar Vd values of about 3.0 L kg^{-1}, that of α-tocotrienol tended to be smaller, with a value of about 1.7 L kg^{-1}. Multiplying the Vd values with the respective k_e values, showed that δ- and γ-tocotrienols had similar CL values of about 0.24 L kg^{-1} h^{-1} and 0.23 L kg^{-1} h^{-1}, respectively, whereas that of α-tocotrienol was significantly smaller ($P < 0.01$), with a value of 0.16 L kg^{-1} h^{-1}.

Figure 2 shows the plasma concentrations of the tocotrienols obtained by comparing intramuscular and oral administration. It can be seen from the figure that the tocotrienols were negligibly absorbed when given intramuscularly as evidenced by the low plasma concentrations obtained, which were essentially comparable with the baseline values measured before administration of the compounds. In the case of oral administration, the plasma concentration profiles of all three compounds were similar to the profiles obtained orally in the previous experiment comparing intravenous and oral administration.

The plasma concentration profiles obtained by comparing intraperitoneal and oral administration are shown in Figure 3. As for intramuscular administration, the tocotrienols were found to be negligibly absorbed when given intraperitoneally; the plasma concentrations obtained were essentially comparable with the baseline concentrations. On the other hand, the plasma concentrations obtained after oral administration appeared to be slightly higher than those in the two previous experiments. This discrepancy may be attributed to the smaller bodyweight of the rats used in this experiment compared with the previous

experiments. The mean weight of the rats used in the intraperitoneal versus oral study was 221±18.5 g, whereas the mean bodyweight of the rats used in the first and second study was higher, being 280.0±22.9 g and 277.3±26.4 g, respectively.

Discussion

Differences among the three tocotrienols lie mainly in the number of methyl groups in the chromanol ring of the molecules. α-Tocotrienol has three methyl groups, γ-tocotrienol has two and δ-tocotrienol has one. It appears that such differences also lead to differences in their bioavailability and disposition. α-Tocotrienol not only showed the highest oral bioavailability compared with the other two, but also had the lowest apparent Vd and CL rate. Similar preferential absorption of α-tocotrienol over δ- and γ-tocotrienols has also been observed by Qureshi et al (1991) in pigs. In our previous study (Yap et al 2001), conducted to evaluate the oral bioavailability of the tocotrienols under different food status using human volunteers, the observed plasma concentrations as well as the $AUC_{0-\infty}$ values of α-tocotrienol were similar to those of γ-tocotrienol, even though the dose of the former was approximately half that of the latter. Moreover, from studies using lymphatic cannulated rats, Ikeda et al (1996) demonstrated that α-tocotrienol was preferentially absorbed compared with γ- and δ-tocotrienols, which may explain its higher oral bioavailability over the other two compounds observed in our study. They suggested that the mechanism of preferential absorption could be due to differences in their micellar solubility, affinity for intestinal brush border membranes, transport in enterocytes or their incorporation into chylomicrons or a combination of these processes, which in turn might be related to differences in the lipophilicity of the molecules. Since α-tocotrienol has three methyl groups compared with two in γ-tocotrienol and one in δ-tocotrienol, the lipophilicity of the three molecules would be expected to be different, with α-tocotrienol having the highest lipophilicity, followed by γ- and δ-tocotrienols. This was reflected in the elution time of the three compounds during analysis using reversed phase chromatography, where the elution is influenced by the lipophilicity of the molecules. The δ-tocotrienol, being least lipophilic, has the fastest elution time, followed by γ- and α-tocotrienols. Since the lipophilicity of a molecule can affect its passage across biological membranes and transport into the lymphatic system, the higher bioavailability obtained with α-tocotrienol might in part be related to its higher lipophilicity compared with γ- and δ-tocotrienols.

Such biodiscrimination has also been reported with the tocopherols. For example, plasma and tissue concentrations of α-tocopherol were observed to be 2–3 times higher than those of γ-tocopherol, even though the diet of the subjects studied contained more γ than α-tocopherol (Bieri & Evarts 1973). This discrepancy was attributed to the presence of α-tocopherol transfer protein, which has high selectivity in regulating the secretion of α-tocopherol from the liver as well as in maintaining its plasma concentrations (Kayden & Traber 1993). Hosomi et al (1997) demonstrated that the methyl group at position 5 of the chromanol ring, which is found in α-tocopherol (and also in α-tocotrienol), was important for recognition by this regulatory protein. However, it is not known if the α-tocopherol transfer protein has similar effects on the disposition of α-tocotrienol in-vivo. Ikeda et al (1996) have also suggested that a carrier protein specific for α-tocotrienol might be present in the intestinal cells.

From the intramuscular and intraperitoneal studies, it was found that the tocotrienols were essentially negligibly absorbed when given as an oily injection via these two routes of administration. Negligible absorption was similarly observed when the intramuscular administration was repeated using the tocotrienol emulsion (instead of an oily injection) that was utilized in the earlier intravenous study (data not shown). The negligible absorption via these two routes could be owing to a lack of partitioning of the compounds out of the lipidic vehicle for absorption in the peritoneal compartment or muscle. It is known that intraluminal processing of orally administered lipophilic drugs contained in a lipidic vehicle, which results in the formation of mixed micelles with bile salts, is essential for their absorption (MacGregor et al 1997). Such processing is absent in intramuscular and intraperitoneal administrations, which may explain the negligible absorption of the tocotrienols administered via these two routes. Therefore, these two modes of dosing should be avoided when conducting studies to evaluate the biological activities of the tocotrienols in whole animal experiments. In a study conducted to evaluate the effects of α-tocopherol and tocotrienols on HMG-CoA reductase activity in hamsters, in which the compounds were given intraperitoneally, Khor & Ng (1999) reported that α-tocopherol, but not the tocotrienols, could be determined in the serum and liver. This could be due to negligible absorption of the tocotrienols when given via this route. In another study in which the tocotrienols were also given intraperitoneally to hamsters, Khor et al (2000) reported that the inhibitory effect of the tocotrienols on the HMG-CoA reductase activity tended to decline with increase in the dose level. This somewhat inverse activity versus dose relationship might be an artefact of the study, as it was shown in our study that the tocotrienols were essentially not absorbed when given via the intraperitoneal route.

In conclusion, absorption of tocotrienols was found to be low and incomplete via the oral route. There appeared to be biodiscrimination in the absorption and disposition among the three homologues. In addition, they were found to be negligibly absorbed when administered intraperitoneally and intramuscularly.

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