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Comparisons between Absorption of Vitamin E in Patients with Chronic Pancreatitis and Healthy Controls: The Bioavailability of Vitamin E

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FUNAKOSHI, A., KIMURA, T., SHINOZAKI, H. and IBAYASHI, H. *Comparisons between Absorption of Vitamin E in Patients with Chronic Pancreatitis and Healthy Controls : The Bioavailability of Vitamin E.* Tohoku J. exp. Med., 1986, **148** (4), 393-401 —— The fasting serum levels of α-tocopherol were determined by high-pressure liquid chromatography in 13 patients with chronic pancreatitis of whom 7 were positive for pancreatic calcification (CCP) and 6, negative (NCP) and 10 healthy subjects. The fasting serum levels of α-tocopherol were significantly lower in patients with chronic pancreatitis (7.2 ± 1.1 μg/ml for CCP and 7.9 ± 0.6 for NCP) than in healthy subjects (11.3 ± 0.7 μg/ml). Vitamin E absorption was determined in those with chronic pancreatitis and in healthy subjects after postprandial oral administration of 400 mg of vitamin E, using soft capsules which contained tocopherol nicotinate along with an appropriate amount of a suspension of an ester of fatty acids with glycerol and middle chain triacylglycerol. The mean absorption of vitamin E was 12.7 ± 2.0 μg/ml • hr for healthy subjects, 9.1 ± 3.1 μg/ml • hr for CCP and 13.0 ± 2.7 μg/ml • hr for NCP, respectively. There was no significant difference in vitamin E absorption between patients with chronic pancreatitis and healthy subjects. Further, the rate of hydrolysis of tocopherol nicotinate did not significantly differ between healthy subjects and patients with chronic pancreatitis. It is of interest to note that vitamin E absorption in patients with chronic pancreatitis was increased by the postprandial use of an oily suspension type preparation of tocopherol nicotinate. ——— chronic pancreatitis ; vitamin E ; bioavailability

It was formerly considered that vitamin E deficiency in man was rare, but in recent years it has been made clear that not only premature infants but also children and adults with vitamin E deficiency may develop neurological or muscular disorders such as areflexia, cerebellar ataxia, pigmentary retinopathy, ophthalmoplegia, and paresthesia. As these symptoms show a favorable response to high doses of vitamin E, vitamin E deficiency has been established as an independent entity (Muller et al. 1983). On the other hand, it has also been shown that fat malabsorption is present along with vitamin E deficiency, in those

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with short bowel syndrome (Binder 1965) and cystic fibrosis (Farrell 1980). Concerning cystic fibrosis in particular, there are reports that red blood cell (RBC) susceptibility to hydrogen peroxide is increased and that life-span of RBCs is shortened in this pancreatic disorder. It was reported that the fasting serum vitamin E level is diminished in patients with chronic pancreatitis, vitamin E absorption is decreased in patients with chronic pancreatitis after an oral vitamin E load (Matsumoto 1983), and that enzyme activity in hydrolyzing vitamin E derivatives is suppressed in patients with chronic pancreatitis (Matsumoto et al. 1979).

In the present study, two different tocopherol nicotinate preparations, hard and soft capsules, were used in the postprandial oral vitamin E load test in patients with chronic pancreatitis and healthy volunteers, the objective being to determine the bioavailability of vitamin E after oral administration of its derivative tocopherol nicotinate. The effect of a digestive enzyme preparation on vitamin E absorption was also studied.

Materials and Methods

Subjects

Of the 13 chronic pancreatitis patients studied, 7 (6 males and 1 female averaging in age 49±12.9 years, subsequently referred to as CCP : Chronic Calcifying Pancreatitis) were positive for calcification, by abdominal radiography and 6 (males averaging in age 48.5± 4.8 years, referred to as NCP : Noncalcifying Chronic Pancreatitis) were negative for calcification. The pancreozymin-secretin test (PS test) was performed on all the patients diagnosed as having chronic pancreatitis, to evaluate exocrine pancreatic functions. Healthy age matched volunteers with normal serum amylase levels, normal fasting blood glucose levels, and normal physical findings were studied, as the controls.

Vitamin E load test

The subjects (healthy volunteers and patients with chronic pancreatitis), in principle, received hard capsules (Juvela N 100 mg capsules, Eizai Co.) or soft capsules (Juvela N soft 200 mg capsules, Eizai Co.) within 30 min of breakfast, in a dose of 400 mg of the *dl*-α-tocopheryl nicotinate base. In some patients a digestive enzyme preparation (Pancreatin) was administered concomitantly in a dose of 1 g. The healthy volunteers ate a mixed sandwich and milk for breakfast, and the patients, a hospital diet.

Test articles

Hard capsules, which contain 100 mg/capsule of dl α-tocopheryl nicotinate and obtained with silicic acid anhydrous adsorbing the active ingredient, contain official polyethylene glycol and methyl cellulose in appropriate proportions and are designed so that they are highly dispersible and soluble in artificial gastric and intestinal juice (solutions 1 and 11 described in the Japanese Pharmacopeia).

Soft capsules which contain 200 mg/capsule of *dl*-α tocopheryl nicotinate also contain appropriate amounts of a suspended official ester of fatty acids with glycerol and suspended middle chain acylglycerol.

Blood collection

Ten ml blood samples were collected before and 2, 4, 6, and 8 hr after an oral vitamin E load, and preserved as serum samples at −20°C until the analysis was done.

Analysis for vitamin E

Serum samples were assayed for vitamin E by the technique of high-pressure liquid chromatography (HPLC) described by Abe and Katsui (Abe and Katsui 1975). When assaying serum for total tocopherol, it was saponified in the presence of pyrogallol and extracted with *n*-hexane. Serum was also extracted with n-hexane without saponification. In the latter case, α-tocopherol excluding unchanged tocopherol nicotinate was determined.

High-pressure liquid chromatography was performed on all hexane extracts, using the JASCOPACK WC-03-500 (2.3 ID×500 mm) column and passing the mobile phase consisting of a 2 : 98 mixture of isopropyl ether and n-hexane through the column at a flow rate of 0.8 ml/min. The detector used was JASCO-FP-4 (EX. 298 nm, EM/325 nm). The amount of unchanged tocopherol nicotinate (tocopherol nicotinate unhydrolyzed in digestive juice) was calculated by subtracting the value obtained with an unsaponified sample from that obtained with a saponified one.

Statistical analysis of data

All values were expressed in terms of ±S.E. Data were assessed by Student's *t*-test and one-way layout variance analysis, making multiple comparisons according to Tukey (Hirotsu 1984). In cases where date were not available, a two-way layout analysis was done, making multiple comparisons, according to Tukey (Hirotsu 1984).

Results

Exocrine pancreatic function in patients with chronic pancreatitis

The PS test was performed in 6 cases of NCP and 7 cases of CCP. As a result, all three parameters of exocrine pancreatic function, volume of pancreatic juice, maximum bicarbonate concentration and amylase output, were depressed to a greater extent in the CCP group than in the NCP group (Table 1).

Serum alpha-tocopherol levels before a vitamin E load

The serum α-tocopherol levels in all subjects are shown in Table 2. Briefly, the mean serum level was 7.9±0.59 for the NCP group ($p<0.05$) and 7.2±1.08

Table 1. *Pancreatic exocrine function in patients with chronic pancreatitis*

	NCP ($n=6$)	CCP ($n=7$)	Normal limit
Volume (ml/kg)	2.50±0.69	1.61±0.38	>0.9
Amylase output (SU/kg)	1568±534	693±349	>1256
Max. bicarbonate Concentration (mEq/liter)	52.58±6.19	40.59±5.46	>70.9

Table 2. *Basal serum levels of alpha-tocopherol*

		Mean±S.E.	Range
Control	($n=10$)	11.29±0.72	8.61−14.29
NCP	($n=6$)	7.92±0.59*	5.68− 9.12
CCP	($n=7$)	7.19±1.08*	3.38−11.17

* Significant difference from control ($p<0.05$).

μg/ml ($p<0.01$) for the CCP group. Both groups clearly had lower serum levels of α-tocopherol than did the control group. However, the CCP group showed a tendency toward greater depression than did the NCP group, although the difference was of no statistical significance.

Vitamin E load test with soft capsules

Serum levels of total tocopherol were determined in healthy subjects and in patients with chronic pancreatitis, after ingesting two soft capsules, each containing 200 mg of *dl* α-tocopheryl nicotinate (i.e., 400 mg in each load test), after breakfast. The data obtained were compared between healthy subjects and patients with chronic pancreatitis (Fig. 1). The absorption of α-tocopherol, that is, the increase in serum α-tocopherol level expressed as AUC (area under the curve), was 12.7 ± 2.0 μg/ml/hr for healthy subjects, 13.0 ± 3.2 μg/ml/hr for the NCP group and 9.1 ± 3.1 μg/ml/hr for the CCP group : the last group showed the greatest depression in serum level of α-tocopherol. The maximum concentration of α-tocopherol in serum (C_{max}) was about the same for the NCP and control groups, but the CCP group had the largest decline, though the change was not statistically significant. The time to reach C_{max} (T_{max}) showed no statistical significance between the healthy subjects and the patients with chronic pancreatitis (Fig. 2).

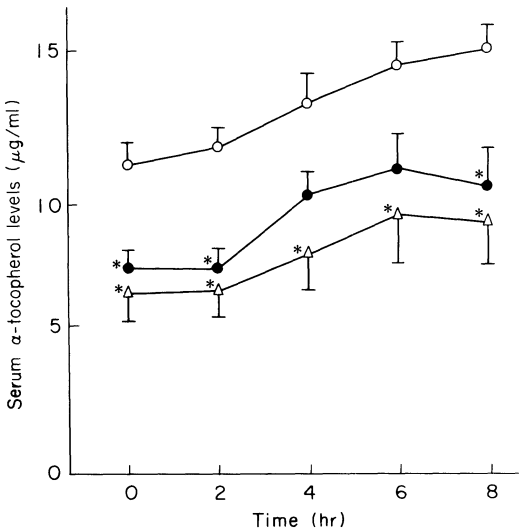

Fig. 1. Serum levels of total alpha-tocopherol after oral administration of tocopherol nicotinate 400 mg (soft capsules).
*Significant difference from controls ($p<0.05$)
○——○, control ($n=10$); ●——●, NCP ($n=6$); △——△, CCP ($n=7$).

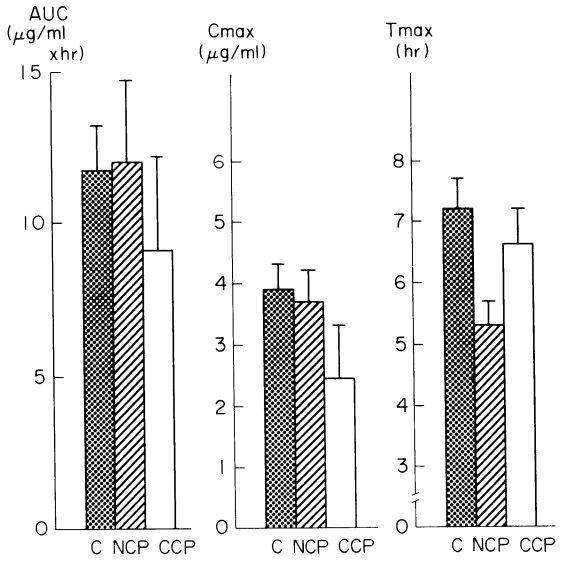

Fig. 2. Parameters of bioavailability of Vitamin E (AUC, area under the curve; Cmax, maximum concentration of α-tocopherol; Tmax, time to reach Cmax) after oral administration of tocopherol nicotinate 400 mg (soft capsules).

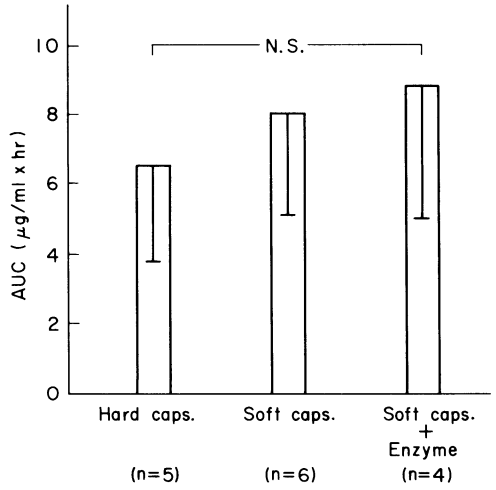

Fig. 3. Comparison of bioavailability of vitamin E between dosage forms in patients with chronic pancreatitis (AUC, area under the curve).

Vitamin E load test with different forms of vitamin E and effect of a co-administered digestive enzyme preparation

Four days after the ingestion of soft capsules in 6 CCP inpatients, 4 hard capsules, each containing 100 mg of tocopherol nicotinate, were administered to 5 of the 6 patients, after breakfast. The results of this test are represented in Fig. 3. There was no statistically significant difference in either AUC, C_{max} or T_{max}

TABLE 3. *Rates of hydrolysis of tocopherol nicotinate*

		AUC (μg/ml × hr)		Rate of hydrolysis (%)
		Total vitamin E	Free vitamin E	
Control	($n=10$)	12.69±2.01	9.52±1.46	75.77±4.37
NCP	($n=6$)	12.96±2.74	9.54±1.33	79.15±6.82
CCP	($n=7$)	9.11±3.12	5.93±2.35	57.32±7.59

among those three forms of drug administration, but the mean AUC was greater with soft capsules than with hard ones and similarly larger with the co-administration of capsules and the digestive enzyme preparation than with soft capsules alone. This tendency was of no statistical significance, but was also noted with the C_{max}. On the other hand, there was no particular tendency with the T_{max}.

Hydrolysis of tocopherol nicotinate in healthy subjects and patients with chronic pancreatitis

To assess the in vivo hydrolysis of a vitamin E ester in healthy subjects and in patients with chronic pancreatitis, the content of unchanged tocopherol nicotinate in the serum was determined by HPLC and the level of free tocopherol calculated by subtracting the value obtained with unsaponified serum from that obtained with saponified serum. Assuming that the tissue distribution volume was the same for tocopherol nicotinate and hydrolyzed tocopherol, the rate of hydrolysis of tocopherol nicotinate was determined, as shown in Table 3. The mean rate of hydrolysis of tocopherol nicotinate was 75.8±4.37% for the control group, 79.2±6.82% for the NCP group, and 57.3±7.59% for the CCP group. Although there was no statistically significant difference, the CCP group showed the lowest rate of hydrolysis.

DISCUSSION

The few reports concerning the absorption of vitamin E in man, are broadly divisible into ones that deal with the relationship between dosage and absorption in healthy subjects (Gross and Melhorn 1972; Baker et al. 1980) and ones that discuss the results of absorption tests, according to dosage given (Bateman and Uccellint 1984). On the other hand, the vitamin E loading test seems to have been performed only on premature infants (Miyao et al. 1972) considered to have malabsorption and chronic pancreatitis patients (Matsumoto 1983).

The absorption of vitamin E is as materially affected as that of lipids by the motility of the gastrointestinal tract and the volume and composition of bile, pancreatic juice and duodenal juice (Gallo-Torres 1970). It has been reported that the shorter the period of gestation, the lower the absorption of vitamin E in premature infants (Miyao et al. 1972). It is believed that diminished absorption of vitamin E has similar implications to malabsorption of lipids. On the other

hand, meals have a pronounced effect on the motility of the gastrointestinal tract and the secretion of digestive juice. In the study with radiolabelled vitamin E in rats, there was a 45% absorption rate using a vitamin E preparation added with skim milk (Nakamura et al. 1975). Further, Hasegawa et al. found that the absorption of tocopherol nicotinate was 2-fold in rats, 5-fold in dogs and 29-fold in man, as compared to the absorption after fasting (Hasegawa et al. 1981). They therefore suggested that vitamin E be administered after meals, to enhance the absorption. It has been reported that the absorption of vitamin E is diminished in patients with chronic pancreatitis (Matsumoto 1983). In the present study two different forms of vitamin E were tested after meal loading, the objective being to try to improve the absorption of vitamin E in patients with chronic pancreatitis. The absorption of vitamin E was obviously diminished in the CCP group of patients, but when the absorption and maximum concentration of vitamin E in the blood were compared in patients with chronic pancreatitis, both absorption and concentration were clearly lower in patients with chronic pancreatitis than in healthy subjects, in our previous study (Matsumoto 1983), whereas there was no difference between these two groups of subjects in the present study. Various factors, such as whether vitamin E is esterified and difference in dose, conditions of administration, analysis and number of subjects should be taken into account. Nevertheless it appears that the stimulation of pancreatic enzyme secretion by ingestion of food and vitamin E accounts for the better absorption of vitamin E after meals than after fasting. The absence of difference in T_{max} between healthy subjects and chronic pancreatitis suggests that the time of lipid absorption does not differ. This is a subject of inquiry which should be further pursued along with the question of gastric evacuation in patients with chronic pancreatitis.

We noted a tendency toward a better absorption of vitamin E when soft capsules of tocopherol nicotinate were given. Such was also the case when capsules of tocopherol nicotinate plus a digestive enzyme preparation were given. It therefore seems desirable that when vitamin E is prescribed for patients with chronic pancreatitis, soft capsules be administered concomitantly with a digestive enzyme preparation.

To elucidate in vivo hydrolysis of esterified vitamin E in healthy subjects and patients with chronic pancreatitis, serum was assayed by HPLC for unchanged tocopherol nicotinate and the level was calculated as the difference between values of saponified and unsaponified serum samples. The rate of hydrolysis of tocopherol nicotinate was 75.77% for healthy subjects and 79.15% for NCP patients, there being no difference between these two groups, but was as low as 57.32% for CCP patients. However, the difference was of no statistical significance. Asano et al. investigated, in vivo, the hydrolysis of tocopherol nicotinate in healthy subjects, determining the serum levels of total cholesterol and unchanged tocopherol nicotinate, using gas chromatography. They found that 90% of the dose of tocopherol nicotinate is hydrolyzed in vivo (Asano et al.

1982). On the other hand, Nakamura et al. (1975) reported that whereas the rate of hydrolysis in vivo was about 90% for tocopherol nicotinate, that for tocopherol acetate was nearly 100%. Matsumoto compared, in vitro, the hydrolysis of tocopherol acetate and noted that the hydrolysis was affected by increases in bile and suppressed in chronic pancreatitis patients (Matsumoto et al. 1979). The in vivo hydrolysis of vitamin E has apparently not been reported. The present study revealed that the severer the pancreatitis, the lower the rate of hydrolysis of tocopherol nicotinate. This may be attributed to a depressed esterase activity in duodenal juice following a diminution in exocrine pancreatic function.

Conclusions

1. The fasting blood levels of α-tocopherol in patients with chronic pancreatitis, notably in the presence of calcification, were significantly depressed.

2. Vitamin E was well absorbed in healthy subjects and patients with chronic pancreatitis, when an oily suspension of vitamin E (soft capsules) was administered after meals and the absorption of vitamin E further improved after the co-administration of soft capsules of tocopherol nicotinate and a digestive enzyme preparation.

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