Mechanism of Optical Isomerization of (S)-N-[1-(2-Fluorophenyl)-3,4,6,7-tetrahydro-4-oxopyrrolo[3,2,1-jk][1,4]-benzodiazepine-3-yl]-1H-indole-2-carboxamide (FK480) in Soft Capsules Containing Polyethylene Glycol 400 and Glycerol

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FK480 is a new synthetic non-peptide antagonist of cholecystokinin (CCK)-A receptors. The dosage form of FK480 is a soft capsule containing a solution of FK480 in a mixture of polyethylene glycol 400 (PEG 400) and glycerol to improve its bioavailability. Studies on the stability of this FK480 dosage form revealed that the main degradation occurred by optical isomerization at the asymmetric C-3 position of the pyrrolobenzodiazepine ring. The degradation reaction was accelerated by formic acid formed in a mixture of PEG 400 and glycerol. Addition of amino acids to the capsule solution retarded the isomerization by reacting with formic acid. Therefore, formic acid appears to accelerate optical isomerization of FK480.

KEY WORDS: CCK-A receptor antagonist; optical isomerization; polyethylene glycol; formation of formic acid.

INTRODUCTION

Cholecystokinin (CCK), located in both the brain and intestine, is a peptide hormone of 33 amino acids which plays an important role peripherally (CCK-A) and centrally (CCK-B). Fig. 1 shows the chemical structure of the new non-peptide antagonist of CCK-A receptors, (S)-N-[1-(2-fluorophenyl)-3,4,6,7-tetrahydro-4-oxopyrrolo[3,2,1-jk][1,4]benzodiazepine-3-yl]-1H-indole-2-carboxamide (FK480, code number of Fujisawa) (1). FK480 has an asymmetric carbon at the 3-position of its pyrrolobenzodiazepine ring. The S-enantiomer was selected because of its high affinity for CCK-A receptors (1).

FK480 is only slightly soluble in water, therefore, for clinical trials, the soft capsules containing a solution of FK480 dissolved in a mixture of PEG 400 and glycerol (9:1, W/W) were selected as the dosage form. This dosage form improves the bioavailability of FK480. However, a stability study of FK480 in soft capsules revealed that the main degradation of FK480 involved optical isomerization at the 3-position of its pyrrolobenzodiazepine ring, yielding approximately 5% of the R-enantiomer after 6 month-storage at 40 °C.

In the development of optically active drugs, the production of undesirable enantiomers must be maintained at a minimum. This paper focuses on describing the isomerization mechanism of FK480 and on procedures for suppressing its isomerization.

MATERIALS AND METHODS

FK480 was synthesized at Fujisawa Pharmaceutical Co., Ltd. DL-α-alanine, L-tryptophan, and DL-phenylalanine were obtained from Wako Pure Chemical Industries (Osaka, Japan), Ishizu Seiyaku (Osaka, Japan) and Sigma Chemical (St. Louis, U. S. A.), respectively. PEG 400 and glycerol were obtained from Sanyo Chemical Industries (Kyoto, Japan) and Katayama Chemical Industries (Osaka, Japan). N-hexane, dichloromethane, absolute ethanol, and acetonitrile were of HPLC grade. All other solvents and reagents were of reagent grade.

Analysis by high-performance liquid chromatography (HPLC) was performed using a SPD-2A variable-wavelength detector (Shimadzu, Kyoto, Japan) and a solvent delivery pump model 510 (Waters, Mildford, U. S. A.). High-performance Ion chromatography (HPIC) was performed using a YOKOGAWA IC-7000 instrument (Yokogawa Electric, Tokyo, Japan). The absorbance was measured by a spectrophotometer (U-3200, Hitachi, Tokyo, Japan). Fast atom bombardment (FAB) mass spectra were obtained with a Finnigan MAT TSQ-70 (Finnigan, San Jose, U. S. A.), and ^{1}H-Nuclear Magnetic Resonance (NMR) spectra were recorded using a 200 MHz spectrometer (type AC 200P, Bruker, Karlsruhe, Germany).

Extraction Procedure of FK480 from the Capsule Solution

Preparation of FK480 capsule solution : 0.2 g of FK480 was added to 34.8 g of a mixture of PEG 400 and glycerol (9 : 1, w/w). The mixture was stirred vigorously at 50 °C for approximately 3 hr until FK480 was completely dissolved. Forty ml of water and 5 ml of chloroform were added to 0.175 g of FK480 capsule solution. The mixture was shaken for 10 min, centrifuged for 10 min at 3000 rpm, and the aqueous layer decanted. This extraction procedure was repeated once. The residual solution was then filtered using type 2S filter paper (Toyo Roshi, Tokyo, Japan). The filtrate was evaporated, and the residue was dissolved in 1 ml of acetonitrile prior to HPLC analysis (Chiral stationary-phase chromatography).

Chiral Stationary-Phase HPLC Analysis

The separation of FK480 and its enantiomer was carried out under the following condition : column, a SUMICHIRAL OA-4500 (4.6 i.d. × 250 mm, Sumitomo Chemical, Osaka, Japan); mobile phase, n-hexane:dichloromethane:absolute ethanol (18:2:1, v/v/v); flow rate, 1.0 ml/min. Retention times for FK480 and its enantiomer were 25 min and 20 min, respectively.

Assay of Formic Acid and Formaldehyde

A mixture of PEG 400 and glycerol (9:1, w/w) was stored at 60 °C and 40 °C. At appropriate time intervals, 1 g

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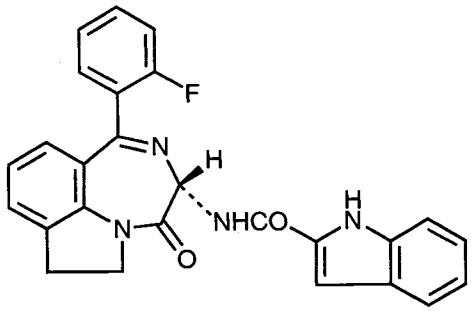

Fig. 1 The chemical structure of FK480 (S form).

of the mixture was withdrawn, and diluted to 100 ml with water before analysis. The concentration of formic acid was determined by injecting 5 μl of this solution into an HPIC system employing an Excelpak ICS-A23 column (4.6 mm i.d. × 750 mm, Yokogawa Electric) having an Excelpak ICS-A2G column (4.6 mm i.d. × 750 mm, Yokogawa Electric) as a guard column. The mobile phase was a mixture of 4 mM sodium carbonate and 1.5 mM sodium bicarbonate aqueous solution at a flow rate of 1.0 ml/min. The concentration of formaldehyde was determined by the Nash method (acetylacetone method) (2).

RESULTS AND DISCUSSION

Optical Isomerization of FK480 in the Capsule Solution

Degradation of the FK480 dosage form occurs primarily by optical isomerization. Fig. 2 shows time-course changes in the concentration of FK480 enantiomer in the capsule solution at 60 °C.

Polyethylene glycols such as PEG 400, the solvent for FK480, have been known to decompose oxidatively upon heating, liberating volatile compounds such as formaldehyde and formic acid (3) (4). The isomerization of FK480 was found to be reduced by decreasing the PEG 400 content in the dissolving solvent. For example, the amount of FK480 enantiomer formed in PEG 400 containing 20% (w/w) absolute ethanol was 2/5 when compared to the rate of isomerization in the absence of ethanol. These results indicated that a substance which has been generated in PEG 400 acted as an accelerator of the isomerization.

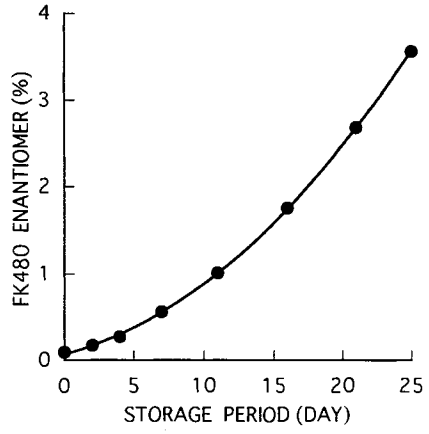

Fig. 2 The time courses for optical isomerization of FK480 in FK480 capsule solution at 60 °C.

Suppression Effect of Optical Isomerization by Amino Acids

The isomerization reaction can be controlled by removing the accelerator with chemical additives. Many additives were screened in a search for a compound capable of suppressing the isomerization. As shown in Table 1, the isomerization was inhibited by amino acids such as alanine, tryptophan and phenylalanine. Therefore, the mechanism of suppression was evaluated by examining the degradation of the added amino acids.

The degradation profiles of amino acids (phenylalanine and tryptophan) in a mixture of PEG 400 and glycerol at 60 °C are shown in Fig. 3, where the residual percentage was plotted against time. The concentrations of both amino acids decreased to 1/10 of their initial concentrations after 14 days. The decomposition of amino acids is due to a reaction with the accelerator formed in a mixture of PEG 400 and glycerol.

Degradation Products of Amino Acids in a Mixture of PEG 400 and Glycerol

Addition of phenylalanine to a mixture of PEG 400 and glycerol yielded 3 main degradation products. The most prominent product, P-1 was isolated, and its structure was identified by FAB-MS, ^{1}H-NMR analysis.

The mass spectrum of P-1 showed main fragments m/z 216 $(M + Na)^+$. In ^{1}H-NMR spectrum (in DMSO-d_6) signals were assigned as follows: a double of doublet at 2.9 ppm (1 H, J = 9.0, 13.8 Hz) and 3.1 ppm (1 H, J = 4.9, 13.8 Hz) from -CH_2-; a double of double of doublet at 4.5 ppm (1H, J = 4.9, 7.9, 9.0 Hz) from -CH-; a multiplet at 7.2 ppm (5H) from -C_6H_5-; a singlet at 8.0 ppm (1H) from -CHO; a doublet at 8.4 ppm (1H, J = 7.9 Hz) from -NH-. These data indicate that P-1 is a derivative of phenylalanine with the NH_2 group converted into a NHCHO moiety. Furthermore, a similar treatment of tryptophan resulted in the formation of an analogous degradation product.

These products were thought to be formed by condensation reaction between the NH_2 group of amino acids and the COOH group of formic acid. Hence, it may be inferred that formic acid generated by thermal decomposition of PEG 400 is responsible for the optical isomerization of FK480.

Table I. The Suppression Effect of Optical Isomerization by Amino Acids

Compound	Amount of FK480 enantiomer (%)		
	Initial	Day 7	Day 14
Control (no additive)	0.09	0.63	2.08
Alanine	0.09	0.30	0.80
Phenylalanine	0.15	0.33	0.89
Tryptophan	0.15	0.32	0.97

Each concentration of alanine, phenylalanine, and tryptophan in FK480 capsule solution was 0.11 mg/g, 0.41 mg/g, 0.51 mg/g, respectively. The mole ratio of these amino acids to FK480 corresponds to 0.2:1. These samples were stored at 60°C.

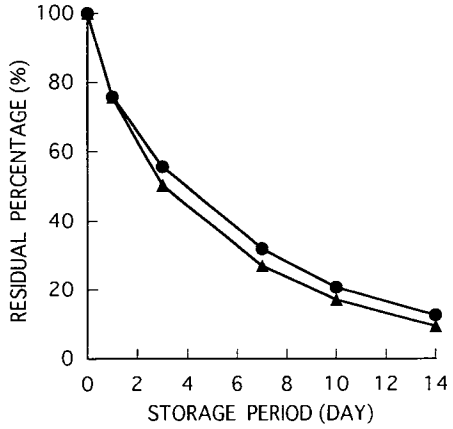

Fig. 3 The degradation profiles of phenylalanine (●) and tryptophan (▲) in a mixture of PEG 400 and glycerol at 60 °C. Each concentration of phenylalanine and tryptophan was 0.41 mg/g and 0.51 mg/g in solution, respectively.

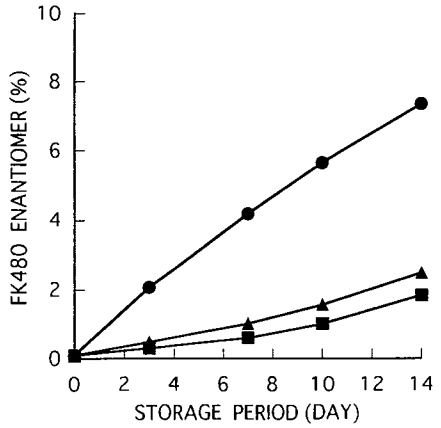

Fig. 5 The acceleration effect in the isomerization by formic acid. Each concentration of formic acid was 580 μg/g (▲), 5800 μg/g (●), and control (no addition, ■) in FK480 capsule solution, respectively. These concentrations of formic acid correspond to a mole ratio of 1 and 10 when compared to the concentration of FK480. The samples were stored at 60 °C.

Formation of Formic Acid in a Mixture of PEG 400 and Glycerol

The time-course changes of concentrations of formic acid and formaldehyde on heating a mixture of PEG 400 and glycerol (9:1, w/w) at 60 °C and 40 °C are shown in Fig. 4.

The generation of formic acid was shown to be dependent on temperature, with increases in the concentration of formic acid being 2 times and 10 times greater than the initial values after 14 days-storage at 40 °C and 60 °C, respectively. The formation curve of formic acid at 60 °C is similar to that of FK480 enantiomer (Fig. 2).

If formic acid arises from formaldehyde, a rapid increase of formaldehyde concentration must be observable at the initial stage of the storage. However, the formaldehyde concentration remained constant at 50 μg/g even after 14 days-storage at 60 °C. Therefore, formic acid may not have been formed by an oxidation of formaldehyde in PEG 400, but directly from PEG 400 as described before (3) (4). Furthermore, the amount of formic acid in glycerol was less than 100 μg/g after storage of glycerol at 60 °C for 14 days in the absence of PEG 400. Hence, formic acid did not originate from glycerol.

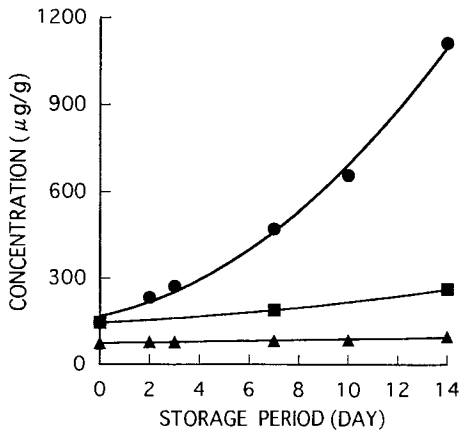

Fig. 4 The time courses for the formation of formic acid at 60 °C (●), 40 °C (■), and formaldehyde at 60 °C (▲) in a mixture of PEG 400 and glycerol.

Acceleration of Optical Isomerization by Formic Acid

The rate of optical isomerization is expected to increase upon the addition of formic acid to FK480 capsule solution. Fig. 5 shows that the rate of isomerization is dependent on the concentration of formic acid. When formic acid was added to the capsule solution at two concentrations (580 μg/g and 5800 μg/g), the enantiomer amount formed were 1.5 times and 5 times greater, respectively, than the enantiomer concentration in a capsule solution having no formic acid added.

These results demonstrated that formic acid was capable of accelerating the optical isomerization of FK480. Usage of polyethylene glycols in designing dosage forms may generate unexpected and undesirable degradation products.

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REFERENCES

1. H. Itou, H. Sogabe, T. Nakarai, Y. Sato, M. Tomoi, M. Kadowaki, M. Matsuo, K. Tokoro and K. Yoshida, Phamacological profile of FK480, a novel cholecystokinin type-A receptor antagonist: comparison to Loxiglumide. *J. Phamacol. Exp. Ther.* 268:571–576 (1994).
2. T. Nash. The colorimetric estimation of formaldehyde by means of the Hantzsch Reaction. *Biochem. J.* 55:416–421 (1953).
3. C. Weurmen and J. Dhont. Unexpected formation of volatile compounds on Gas-Liquid Chromatography columns. *Nature* 184:1480–1481 (1959).
4. M. E. Kieser and D. J. Sissons. Formation of volatile compounds on Gas-Liquid Chromatography columns. *Nature* 185:529 (1960).