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Duration of anti-cholecystokinin (CCK) action on the rat exocrine pancreas of new CCK receptor antagonist FK480 administered orally

YURIKO MORIYOSHI, KEIKO SHIRATORI, CHIKA IWABE, SHIN-ICHIRO WATANABE, and TADASHI TAKEUCHI

Departments of Internal Medicine and Gastroenterology, Tokyo Women's Medical College, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162, Japan

Abstract: We assessed the duration of the anti-cholecystokinin (CCK) action of FK480, a new non-peptide CCK-A receptor antagonist developed in Japan, in an in vivo study in rats, comparing it with CR 1505. Pancreatic exocrine secretion stimulated by intravenous infusion of CCK-8 (0.06 μg/kg per h) was measured at intervals of 0–24 h after the oral administration of FK480 (1.5 mg/kg) and CR 1505 (30 mg/kg). FK480 significantly inhibited both CCK-stimulated pancreatic juice volume flow and amylase output 0, 4, 8, and 12 h after oral administration, whereas the inhibitory effect of CR 1505 had completely disappeared by 8 h after oral administration. It was concluded that orally administered FK480 has a prolonged anti-CCK action.

Key words: cholecystokinin antagonist, pancreatic exocrine secretion, FK480, CR 1505

Introduction

A number of cholecystokinin (CCK)-A receptor antagonists have been developed, and the antagonistic activity of these compounds has been demonstrated in both in vitro[1–3] and in vivo systems.[4–10] One of these antagonists, the amino acid derivative CR 1505 (loxiglumide: (±)-4-(3,4-dichlorobenzamido)-N-(3-methoxypropyl)-N-pentylglutaramic acid) (Fig. 1), has been shown to be an extremely potent and highly specific antagonist of the CCK-A receptor. It has also been found to have a favorable pharmacologic and toxicologic profile, and clinical trials are now being conducted in Japan to assess its usefulness in the treatment of acute and chronic pancreatitis. When the possible therapeutic application of a CCK receptor antagonist in pancreatic disease is evaluated it is important to determine the duration of the adequate blocking of pancreatic CCK receptors that it provides.[11,12]

A new benzodiazepine derivative, FK480 ((s)-N-[1-(2-fluorophenyl)-3,4,6,7-tetrahydro-4-oxo-pyrrolo-(3,2,1-jk][1,4]benzodiazepin-3-yl]-1H-indole-2-caboxamide) (Fig. 2), has recently been developed in Japan. We have already demonstrated in vivo that the intraduodenal administration of FK480 dose-dependently inhibited increases in pancreatic exocrine secretion induced in rats by both exogenous and endogenous CCK.[13] However, since the duration of the anti-CCK effect of FK480 was unknown, we designed the present experiment to determine the duration of the anti-CCK action of FK480 on CCK-stimulated pancreatic exocrine secretion in rats, comparing it with that of CR 1505.

Materials and methods

Animal preparation for pancreatic secretion study

Male Wistar rats, weighing 250–300 g, were used in this study. The rats were first fasted for at least 18 h, with free access to water. They were then anesthetized with an intramuscular injection of urethane (50% wt/vol, 0.5 ml/100 g body weight) 1 h before surgery. The rats did not awaken from the urethane anesthesia at any time during the experiment. A midline abdominal incision was made, and the common bile duct was ligated proximal to the pancreas below the hilum of the liver. A polyethylene tube (OD 0.9 mm, ID 0.5 mm; Natsume Seisakujo, Tokyo, Japan) was inserted into the bile duct above the ligature, and bile was diverted to the exterior throughout the experiment. An identical polyethylene tube was inserted into the pancreatic duct at its entrance into the duodenum, and 1-h samples of

Offprint requests to: Y. Moriyoshi

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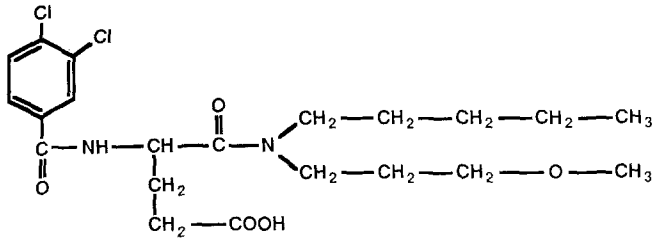
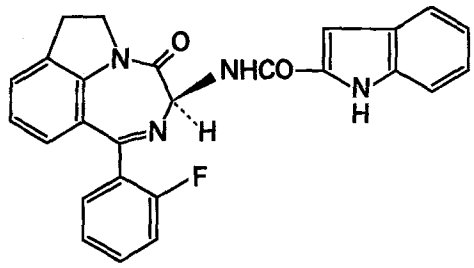


(±)-4-(3,4-dichlorobenzamido)-N-(3-methoxypropyl)-N-pentylglutaramic acid

M.W. = 461.39

Fig. 1. Chemical structure of CR 1505



(S)-N-[1-(2-fluorophenyl)-3,4,6,7-tetrahydro-4-oxo-pyrrolo-[3,2,1-jk][1,4]benzodiazepin-3-yl]-1H-indole-2-carboxamide

M.W.= 438.46

Fig. 2. Chemical structure of FK480

pure pancreatic juice were collected continuously from this tube for 2 h. The jugular vein was cannulated with a polyethylene tube (OD 0.80 mm, ID 0.50 mm, Natsume Seisakujo), and a slow continuous intravenous infusion of 0.15 M NaCl solution was initiated, at the rate of 1 ml/h with an infusion pump (Harvard Apparatus, Cambridge Mass.). The rats were kept warm at a constant temperature of 38°C by the employment of heating pads under the body and a heating lamp over the abdomen.

Effect of oral administration of CCK antagonists on pancreatic secretion

A total of 96 rats was used in this study. We have shown the submaximal intraduodenal inhibitory doses of FK480 and CR 1505 on CCK-8 (0.06 μg/kg per h)-stimulated pancreatic secretion to be 1 mg/kg per h and 20 mg/kg per h, respectively.[13] Based on these observations, we administered FK480 (a gift from Fujisawa Pharmaceutical Company, Osaka, Japan), at a dose of 1.5 mg/kg, and CR 1505 (a gift from Tokyo Tanabe Pharmaceutical Company, Tokyo, Japan), at a dose of 30 mg/kg, directly into the stomach via an orogastric tube. The FK480 was emulsified with polyethylene glycol 400 (Nacalai Tesque Inc., Kyoto, Japan), and the CR 1505 was emulsified with 0.5% methylcellulose solution (Wako Pure Chemical Industries Ltd., Osaka, Japan). Pancreatic secretion in response to 1-h intravenous infusion of CCK-8 (Squibb Diagnostics, Princeton N.J.) (0.06 μg/kg per h) was measured 4, 8, 12, and 24 h after the oral administration of each of the CCK-antagonist solutions. CCK-8 was dissolved in 0.15 M NaCl solution containing 0.5% bovine serum albumin (Nacalai Tesque Inc.). To determine the effect of the CCK antagonists at 0 h, each of the agents was infused into the duodenum for 3–5 min, beginning 5 min before the CCK-8 infusion was initiated. In a control experiment, polyethylene glycol 400 alone and 0.5% methylcellulose solution alone were given instead of FK480 and CR 1505, respectively. Each of the six experimental groups, i.e., 0, 4, 8, 12 and 24 h after CCK antagonist administration and control groups consisted of eight rats. No more than one dose of CCK antagonist was administered to each rat.

Determinations

Pancreatic juice was collected continuously by connecting the pancreatic duct cannula to glass micropipettes (Drummond Scientific Company, Broomall, Pa.) with a capacity of 0.694 μl/mm tube length. Volume was determined every h by measuring the length of the columns of pancreatic juice in the micropipette. After the pancreatic juice volume had been measured, amylase concentrations were determined as changes in enzyme activity, with maltopentose being used as the substrate, and amylase output per h was calculated by multiplying by the concentration. Plasma FK480 and CR 1505 concentrations were determined by enzyme immunoassay (EIA) and high-performance liquid chromatography (HPLC),[14] respectively.

Data analysis

All values are expressed graphically as means ± SEM. The percent inhibition of amylase output was calculated according to the formula:

$$\text{Percent inhibition} = (1 - a/b) \times 100$$

where a is the increase in pancreatic secretion at an individual point in the incremental response of amylase output in pancreatic juice for 1 h, calculated by subtracting the basal 1-h secretion value from the 1-h secretion value when CCK-8 was infused intravenously in the presence of CCK antagonist, and b is the increase in pancreatic secretion calculated in the same way in the absence of CCK antagonist. Differences in re-

sponses among the six experimental groups were analyzed by ANOVA, followed by multiple comparison of the individual means, using the method of Newman and Keuls as described by Winer.[15] Student's *t*-test (two-tailed) was used to evaluate the statistical significance of differences between the control and antagonist-treated groups. A probability value of <0.05 was considered statistically significant.

Results

There was no significant change in either volume flow or amylase output during the 1st and 2nd h after the diversion of pancreatic juice in the control groups to which polyethylene glycol 400 alone or 0.5% methylcellulose solution alone had been administered orally.

Effect of oral administration of FK480 on CCK-8-stimulated pancreatic secretion

The intravenous infusion of CCK-8, 0.06 μg/kg per h, significantly increased pancreatic juice volume flow to 67.4 ± 2.6 μl/h and amylase output to 3511 ± 254 IU/h ($P < 0.001$). FK480 significantly inhibited both CCK-8-stimulated pancreatic juice volume flow and amylase output 0, 4, 8, and 12 h after oral administration ($P < 0.01$–0.05), whereas the inhibitory effect of CR 1505 on CCK-8-stimulated pancreatic juice volume flow and amylase output was significant only at 0 and 4 h ($P < 0.01$) after oral administration and had disappeared by 8 h, based on comparisons with the values in the control rats (Figs. 3 and 4). There were no statistical differences in basal pancreatic secretion between any of the groups.

Percent inhibition of amylase output, and plasma concentrations of CCK antagonists

Inhibition of amylase output by FK480 0, 4, 8, 12, and 24 h after administration was 100 ± 2.6%, 93.6 ± 1.0%, 93.3 ± 3.0%, 82.0 ± 2.9%, and 10.4 ± 5.0%, respectively (Fig. 5). A significant inhibitory effect of

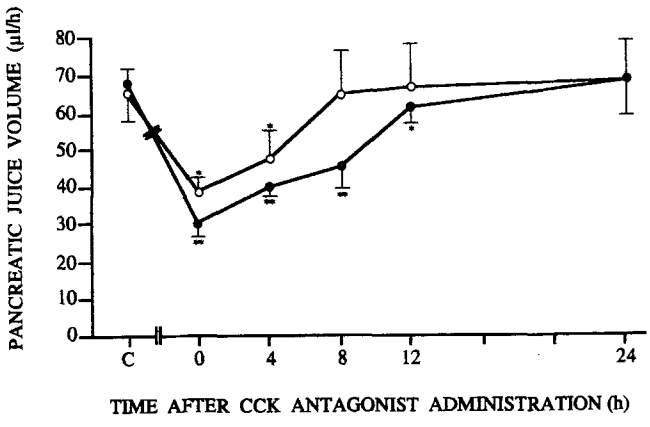


Fig. 3. Time course of changes in pancreatic juice volume, stimulated by cholecystokinin (*CCK*)-8 (0.06 μg/kg per h), after administration of CCK antagonists. *Filled circles* indicate FK480 (1.5 mg/kg), and *open circles* indicate CR 1505 (30 mg/kg). Each point represents the mean ± SEM of the results obtained in eight rats. $^*P < 0.05$; $^{**}P < 0.01$, significant difference between the treated and control (*C*) groups (two-tailed Student's *t*-test following one-way ANOVA). *C*, CCK-8-stimulated pancreatic secretion without CCK antagonists

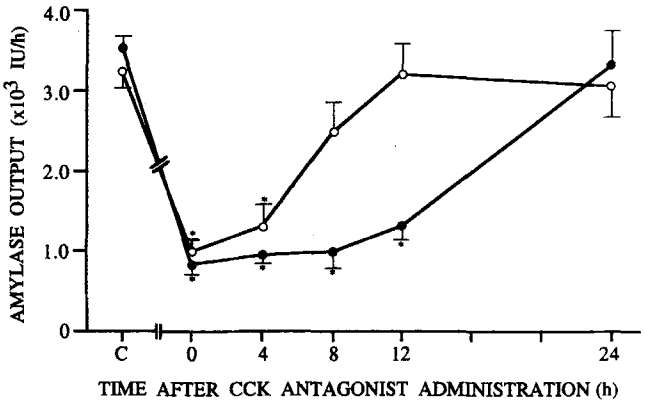


Fig. 4. Time course of pancreatic amylase output, stimulated by CCK-8 (0.06 μg/kg per h), after administration of CCK antagonists. *Filled circles* indicate FK480 (1.5 mg/kg), and *open circles* indicate CR 1505 (30 mg/kg). Each point represents the mean ± SEM of the results obtained in eight rats. $^*P < 0.01$, significant difference between the treated and control (*C*) groups (two-tailed Student's *t*-test following one-way ANOVA). *C*, CCK-8-stimulated pancreatic secretion without CCK antagonists

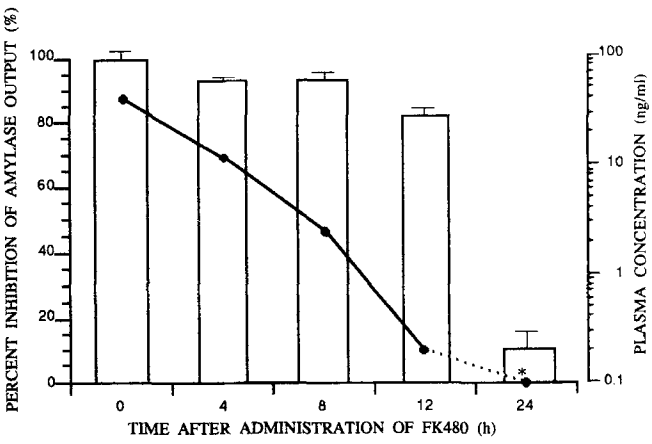


Fig. 5. Percent inhibition (*columns*) by FK480 of pancreatic amylase output induced by CCK-8 (0.06 μg/kg per h), and plasma concentrations of FK480 (*filled circles*). Values are means ± SEM of the results obtained in eight rats. *ND, Not detectable

FK480 on amylase output was demonstrated for 12 h after oral administration, despite the time-dependent decreases in plasma FK480 concentration from 40.5 ± 2.0 to 0.2 ± 0.06 ng/ml (Table 1). In contrast, as shown in Fig. 6, the inhibition of amylase output by CR 1505 had diminished to 35.8 ± 8.4% by 8 h after the beginning of administration, and the time-dependent decreases paralleled the reduction in plasma CR 1505 concentration (9.82 ± 1.95 → 0.20 ± 0.05 μg/ml (Table 1).

Discussion

FK480, a new CCK receptor antagonist containing a benzodiazepine ring, was developed in Japan. FK480 has been shown to selectively antagonize peripheral CCK receptors, as opposed to brain CCK receptors, in the light of findings that it was 180 times more potent in inhibiting CCK binding to the pancreas than in inhibiting CCK binding to brain tissue.[16] Akiyama and Otsuki[17] have reported that FK480 is a potent, competitive, and specific antagonist of CCK's stimulatory action on the exocrine pancreas. FK480 has been reported to be 1000 times more potent than loxiglumide in inhibiting CCK-8-stimulated amylase release from rat pancreatic acini and to be comparable in potency and selectivity to MK-329, the most potent CCK receptor antagonist known. We have previously demonstrated that the intraduodenal administration of FK480, in graded doses of 0.0016–1.0 mg/kg per h, produced dose-dependent inhibition of both pancreatic juice volume flow and amylase output stimulated by the intravenous infusion of CCK-8 at a dose of 0.06 μg/kg per h in vivo in rats, indicating that FK480 is 208 times more potent than CR 1505.[13] Thus, FK480 has been shown to be a potent antagonist of CCK in both in vitro and in vivo systems. However, the duration of the anti-CCK effect of FK480 has not been reported previously.

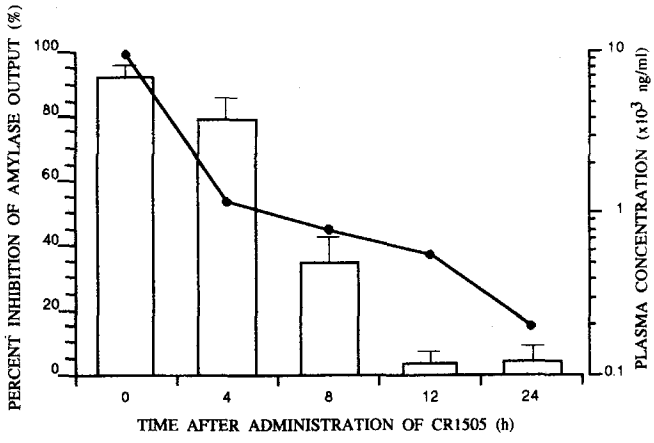


Fig. 6. Percent inhibition (*columns*) by CR 1505 of pancreatic amylase output induced by CCK-8 (0.06 μg/kg per h), and plasma concentrations of CR 1505 (*filled circles*). Values are the means ± SEM of the results obtained in eight rats

The present study has demonstrated that orally administered FK480 significantly inhibited both the pancreatic juice volume flow and amylase output stimulated by the intravenous infusion of CCK-8 at a dose of 0.06 μg/kg per h. The inhibitory effect of FK480 was still present 12 h after oral administration, whereas that of CR 1505 had completely disappeared by 8 h. Although a number of classes of CCK-A receptor antagonists, including the substituted glutaramic acid analogues, such as CR 1409 (lorglumide)[2,6–8] and CR 1505,[4,7–9] and the benzodiazepine derivative MK329,[3,5,7] have been described and well characterized, there have been only a few reports of studies on the duration of the anti-CCK effect of these antagonists. Lotti et al.[18] evaluated the duration of the inhibitory effect of oral MK329, 0.04 mg/kg, on gastric emptying induced by CCK-8 (80 μg/kg, s.c.) in mice, and reported that the maximum duration of effective action appeared to be 5 h. Corazziari et al.[19] evaluated the effect of a single oral dose of loxiglumide on postprandial gallbladder contraction in humans and found that a dose of 800 mg was followed by significant inhibition throughout the entire 300-min observation period. Further, Watanabe and Otsuki[20] reported significant inhibition of cerulein-stimulated pancreatic secretion by loxiglumide for 8 h after subcutaneous administration and for 12 h after oral administration. Nevertheless, little detailed information has been available on the duration of the anti-CCK effect of CCK receptor an-

Table 1. Plasma concentration of CCK antagonists (ng/ml)

	Time after CCK antagonist administration (h)				
Antagonist	0	4	8	12	24
FK480	40.5 ± 2.0	11.9 ± 2.4	2.5 ± 0.7	0.2 ± 0.06	ND
CR 1505 (×10³)	9.82 ± 3.2	1.17 ± 0.17	0.8 ± 0.08	0.56 ± 0.11	0.24 ± 0.05

Values are means ± SEM of the results obtained in eight rats in each group
ND, Not detectable

tagonists on pancreatic secretion when administered orally. In the present study, we have clearly shown that FK480 administered orally is highly potent and antagonizes responses to exogenously administered CCK-8 for much longer than CR 1505. The duration of action of CR 1505 we observed differs from the results reported by Watanabe et al.[20] in the rat. They reported that the oral administration of loxiglumide (10 mg/kg and 50 mg/kg) caused prolonged inhibition of both pancreatic protein secretion (6 h and 8 h, respectively) and pancreatic juice secretion (8 h and 12 h, respectively). The difference in results regarding duration of action in the two studies is probably attributable to the dosage used and the difference in stimulus to the exocrine pancreas, i.e., they used a 100 ng/kg bolus injection of cerulein, whereas we used continuous infusion of CCK-8 at a physiological dose of 0.06 μg/kg per h.

A significant inhibitory effect of FK480 on CCK-stimulated amylase output was shown for 12 h after oral administration, despite the time-dependent decreases in FK480 plasma concentrations. The inhibitory action of CR 1505 on CCK-stimulated amylase output, on the other hand, exhibited time-dependent decreases that paralleled the reduction in plasma CR 1505 concentration. These findings suggest that FK480 is bound to receptors on acinar cells in a slowly dissociating state and remains active for a much longer period than CR 1505. Differences in biochemical properties may explain this phenomenon. The benzodiazepine derivative FK480 is hydrophobic and only slightly soluble in water, whereas the glutaramic acid analogue CR 1505 is water soluble. Our findings seem to be corroborated by the study of Akiyama and Otsuki,[17] who reported that FK480 caused residual inhibition of the action of CCK-8. They found that acini incubated with FK480 for 30 min showed one-tenth the sensitivity to CCK-8 of acini preincubated without FK480. Thus, this residual inhibition may explain the long duration of the inhibitory action of FK480.

In conclusion, the present study in rats has shown that FK480 administered via the oral route has a much longer duration of anti-CCK action than CR 1505. The potency, oral bioavailability, and long duration of action of FK480 as a CCK antagonist indicates that this agent has potential value for clinical application in the treatment of pancreatitis.

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