Effects of MCI-727, a New Anti-Ulcer Agent, on Plasma Secretin Concentration in Rats and Dogs and Pancreatic Exocrine Secretion in Rats

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Received January 31, 1991 Accepted May 18, 1991

ABSTRACT—In the present study, we investigated the effects of MCI-727, a new anti-ulcer agent, on plasma immunoreactive secretin concentration in rats and dogs using secretin specific RIA with the ethanol extraction method. Plasma secretin levels were increased dose-dependently 10 min after oral administration of MCI-727 in rats (control: 5.5 ± 0.6; MCI-727, 10 mg/kg: 10.4 ± 2.6; 30 mg/kg: 15.3 ± 1.5; 100 mg/kg: 20.7 ± 2.6 pg/ml, n = 6). Teprenone also caused a significant increase of plasma secretin at 10 min after oral administration at the doses of 30, 100 and 300 mg/kg. Under the same conditions, MCI-727 and teprenone did not alter the plasma immunoreactive gastrin concentration in rats. From the results of the time course study, the increasing effect of MCI-727 (30 mg/kg, p.o.) on plasma secretin remained for at least 240 min after administration. On the other hand, the increasing effect of teprenone (200 mg/kg, p.o.) was only observed at 30 and 60 min after administration. Furthermore, MCI-727 had increasing effects on plasma secretin concentration in dogs, but teprenone had no effects in this species. The volume of pancreatic secretion and the pancreatic bicarbonate output increased after intra-duodenal administration of MCI-727 at 30 and 100 mg/kg in rats. Similar effects were also observed with teprenone (30–300 mg/kg, i.d.) or secretin (Secrepan®, 0.1–1.0 unit/kg, i.v.).

MCI-727 ((Z)-2-(4-methylpiperazin-1-yl)-1-[4-(2-phenyl-ethyl)phenyl]-ethanone oxime hydrochloride monohydrate) is an anti-ulcer agent with a new chemical structure, and it has more potent anti-ulcer effects than cimetidine or teprenone in various experimental ulcer models (1). The mechanism of the anti-ulcer effect of this agent has not been clearly defined. It has no effects on basal gastric acid secretion and histamine- or 2-deoxyglucose-stimulated gastric acid secretion, but suppressed the tetragastrin-stimulated one. Since a similar pattern of action has been reported with secretin (2–4), one of the gut peptides (5) which has anti-ulcer activity (6), the participation of endogeneous secretin in the anti-ulcer mechanism of this agent was suggested. Therefore, in the present study, we investigated the effects of MCI-727 on plasma secretin concentration in rats and dogs. The effects on plasma
gastrin concentration and pancreatic exocrine secretion in rats were also investigated. Teprenone, an anti-ulcer agent which has an enhancing action on the gastric mucus and mucus and mucosal barrier (7), was employed as a reference drug in these experiments. Teprenone also has been reported to increase the plasma secretin concentration (8).

**MATERIALS AND METHODS**

In experiments 1, 2 and 4, male Donryu rats (Japan SLC) weighing 250–300 g were placed in a wire cage individually and deprived of food but allowed free access to water for 24 hr before the experiments. In experiment 3, male and female mongrel dogs weighing 8–16 kg were used. Dogs were prepared with a chronic gastric fistula and were allowed to recover from the surgical operation for at least 2 weeks. Before the experiment, dogs were deprived of food but allowed free access to water for 24 hr. All animals were housed in a temperature (23 ± 1 °C) and humidity (50 ± 5%) controlled room under a 12 hr light-dark cycle (7:00 to 19:00, light on).

MCI-727 (Fig. 1) was synthesized in our laboratory. Teprenone was extracted from Selbex capsules* (Eisai). In the experiment using rats, MCI-727 was suspended with 2.5% gum Arabic solution, and teprenone was suspended with 0.5% Tween 80 solution. All test drugs or vehicle were given in a volume of 0.2 mL/100 g body weight. In the experiment using dogs, all test drugs were suspended in a solution containing 2.5% gum Arabic and 0.5% Tween 80 and administered in the volume of 10 mL/body. The pH of the drug suspensions and vehicle were adjusted to 6.0 ± 1.0 in all experiments.

Other compounds used were as follows: Ethanol, borax (sodium tetraborate), gum Arabic (powder) (Junsei Chemical Co.); Tween 80 (polyoxyethylene sorbitan monooleate), HCl (hydrochloric acid), NaOH (sodium hydroxide), NaN₃ (sodium azide), tris (tris(hydroxymethyl)aminomethane), Na₂HPO₄ (disodium hydrogenphosphate), NaH₂PO₄ (sodium dihydrogenphosphate), SMBS (sodium metabisulfite), chloramine-T, KI (potassium iodide), sucrose (Nacalai Tesque); Sephadex G-15, Sephadex G-50, SP-Sephadex C-25 (Pharmacia); NaCl (sodium chloride) (Kishida Chemical); urethane (Sigma); BSA (bovine albumin crystallized) (Miles, Inc.); sodium pentobarbital (Sominopentyl®) (Rohm and Haas); heparin (Novo); charcoal (charcoal activated) (Merck); dextran (dextran grade C) (BDH Chemicals, Ltd.); Na[125I] (New England Nuclear Co.); secretin (synthetic porcine secretin) (Peptide Institute); cimetidine (Tagamet®) (Wellcome); Seprepan® (porcine secretin injection; extracted from porcine duodenum mucosa) (Eisai); Gastrin RIAKIT® II (Dainabo).

**Experiment 1. Effects on plasma immunoreactive secretin and gastrin concentration in rats**

Ten minutes after oral administration of test drugs or vehicle, the blood was collected from the aorta into the heparin added tube and kept on ice during the test period. Blood sampling was carried out under sodium pentobarbital (40 mg/kg, i.p.) anesthesia. Blood was centrifuged at 3000 rpm for 30 min at 4°C, and plasma was collected. For the secretin assay, a 2-ml aliquot of plasma was taken, to which 40 μl of aprotinin was added. For the gastrin assay, 0.5 ml of plasma was taken, to which 10 μl of aprotinin was added. The sample plasma was frozen at –80°C for future assay.

Plasma secretin concentration was determined by the radioimmunoassay methods (RIA) described by Chey et al. (9, 10). Prior to RIA, secretin was extracted from plasma by ethanol (11) by the following procedure: Two ml of ethanol and 2 ml of plasma sample were added into the tube and mixed. After 10 min,
the mixture was centrifuged at 3000 rpm for 30 min at 4°C, and then 6 ml ethanol was added and mixed; after 10 min, the mixture was centrifuged again under the same conditions. The supernatant was collected and evaporated to dryness in a stream of N2-gas. The dried residue was redissolved in 1 ml of standard diluents (0.05 M sodium phosphate buffer, pH 7.0, containing 0.02% NaN3 and 0.5% BSA) before the RIA. The RIA for secretin was performed as follows: secretin standard was dissolved by the standard diluent at the concentration of 1.25–160 pg/0.2 ml/tube. Anti-secretin serum R-5-6 (kindly provided by Professor W.Y. Chey, University of Rochester, NY, U.S.A.) was dissolved by antiserum diluent (0.5 M sodium phosphate buffer, pH 7.0, containing 0.02% NaN3, 0.1% BSA and 300 U/ml aprotinin) (final dilution of 1:5,000,000). 125I-Secretin was prepared to approximately 5000 cpm/0.2 ml/tube with the standard diluent. Iodination of secretin was prepared by the chloramine-T method (11): synthetic porcine secretin (5 μg/20 μl), Na[125I] (74 MBq), chloramine-T (25 μg/5 μl) and 0.5 M borax buffer, pH 8.0 (10 μl) were mixed and gently shaken at room temperature. Two minutes later, SMBS (1.25 mg/50 μl) and 80 μl of transfer solution (0.5 M sucrose, 0.05 M KI, 1.6% BSA and 0.02% NaN3) were added, and the reaction mixture was applied to a Sephadex G-15: Sephadex G-50 (7:3) column (φ0.9 × 19 cm high). The second peak fractions were collected and diluted and applied to a SP-Sephadex C-25 column at 4°C (φ0.9 × 19 cm high). The 125I-secretin was eluted from the column with 0.02 M sodium phosphate buffer, pH 5.5, containing 1% BSA with a 0.02 M to 0.2 M NaCl gradient. 125I-Secretin fractions, the major radioactive peak, were collected and rechromatographed over SP-Sephadex C-25 under the same conditions. Purified 125I-secretin was kept frozen at −20°C. All assays were set up in duplicate with 10 x 75 mm glass culture tubes at 4°C. Secretin standard (for the standard curve tube, 0.2 ml) or standard diluent (for sample tube, 0.2 ml), hormone-free plasma extract (for standard curve tube, 0.4 ml) or sample (for sample tube, 0.4 ml) and antisera (0.2 ml) were mixed and preincubated for 48 hr; then 125I-secretin (5000 cpm/0.2 ml) was added and incubated for 48 hr. The total incubation volume was 1.0 ml. Plasma coated dextran charcoal (0.4 ml of a mixture consisting of 1 part citrate-free plasma and 1 part 0.45% dextran, 4.5% charcoal, 0.02% NaN3 in 0.5 M tris-HCl buffer, pH 7.8) was added for the Bound/Free separation and incubated for 30 min, followed by centrifugation at 3000 rpm for 30 min. The supernatant fluid containing the antibody-bound secretin was decanted to another tube and counted by a gamma-ray counter. The minimum detected limit of secretin concentration was 1–2 pg/ml plasma.

Experiment 2. Time course changes in plasma secretin concentration in rats

Test drugs were administered orally, and the blood was collected after 10, 30, 60, 120, 240 and 480 min. Control rats were administered the vehicle at 10 min before the blood sampling. Other protocols were the same as described in experiment 1.

Experiment 3. Effects on plasma secretin concentration in dogs

Each dog was fixed in the Pavlov-stand and an intracatheter for blood sampling was inserted in a peripheral vein of one of the extremities and maintained patent with a slow infusion of saline solution. The gastric fistula was opened and washed the gastric lumen with water. After the first blood sampling, cimetidine (10 mg/kg) was given intra-venously as a bolus to exclude the influence of gastric acid secretion. Test drug suspension (10 ml/body) was administered from the gastric fistula at 60 min after cimetidine treatment, and the remaining suspension at the inner space of the fistula was pushed two times by 10 ml of distilled water. Blood samples were drawn from the intra-
catheter (5 ml/sampling) at 0, 5, 10, 15, 30, 60 and 120 min after test drug administration. Other protocols were the same as described in experiment 1.

**Experiment 4. Effects on pancreatic exocrine secretion in rats**

The experiment was carried out under urethane (1.2 g/kg, i.p.) anesthesia. After tracheostomy, the abdomen was opened, and the polyethylene cannula put into the duodenum through the pylorus from the small hole of the forstomach and pylorus was ligated and the cannula was fixed. The pancreatic duct was cannulated in the duodenal wall with a polyethylene tube. The bile duct was ligated near the liver, and bile was drained out through a polyethylene tube inserted into the bile duct. The femoral vein was catheterized with a polyethylene tube. After the recovery interval (approximately 1 hr), the polyethylene cannula (internal volume of which was calibrated before the experiment) was attached to the pancreatic cannula in order to measure the pancreatic juice volume. After 60 min, the suspension of MCI-727 or teprenone was administered into the duodenal lumen through the duodenal cannula or the solution of Secrepan" was intra-venously administered from the femoral vein cannula. The volume of pancreatic exocrine secretion and bicarbonate output was measured for 120 min at 30 min intervals. Bicarbonate (HCO₃⁻) of the pancreatic juice was measured by a CO₂-analyzer (Corning). Porcine secretin (Secrepan"") was used for the positive control.

**Data analysis**

Statistical analyses were performed using the paired (experiment 3) or unpaired (other experiments) Student's t-test, and values of P < 0.05 were regarded as indicating a statistically significant difference.

**RESULTS**

**Experiment 1. Effects on plasma secretin concentration in rats**

The oral administration of MCI-727 at 10, 30 and 100 mg/kg increased the plasma secretin concentration in a dose-related manner with mean values of 10.4, 15.3 and 20.5 pg/ml, respectively (Fig. 2). The mean concentration of plasma secretin in control rats (2.5% gum Arabic solution, p.o.) was 5.5 pg/ml.

The oral administration of teprenone 10 mg/kg had no effect, but 30, 100 and 300 mg/kg increased the plasma secretin concentration with mean values of 15.4, 16.1 and 21.2 pg/ml, respectively. The mean concentration of plasma secretin in control rats (0.5% Tween 80 solution, p.o.) was 6.1 pg/ml.

The plasma gastrin concentration in 2.5% gum Arabic solution treated rats and 0.5% Tween 80 solution treated rats were 141.0 pg/ml and 130.2 pg/ml, respectively. Following administration of MCI-727 and teprenone, plasma gastrin concentration did not change significantly from the control values (Fig. 3).
Experiment 2. Time course changes in plasma secretin concentration in rats

The plasma secretin concentration was significantly increased at 10, 30, 60, 120 and 240 min after oral administration of MCI-727 (30 mg/kg) (Fig. 4, upper). The maximal increase of secretin concentration was shown at 60 min after administration with the mean value of 12.0 pg/ml.

The oral treatment of teprenone (200 mg/kg) significantly increased plasma secretin concentration at 30 and 60 min after administration, and the maximal increase was observed at 30 min with the mean value of 13.7 pg/ml (Fig. 4, lower).

Experiment 3. Effects on plasma secretin concentration in dogs

The basal plasma secretin concentration in dogs was 5–10 pg/ml, and it was not altered by cimetidine (10 mg/kg, i.v.) pretreatment. The intra-gastric administration of MCI-727 (30 mg/kg) significantly increased plasma secretin concentration after 5 to 30 min. The maximal response was shown at 5 min with the mean value of 30.0 pg/ml (Fig. 5, upper).

The intra-gastric administration of teprenone (200 mg/kg) caused no significant changes in the plasma secretin concentration (Fig. 5, lower).

The plasma secretin concentration remained unaltered after intra-gastric administration of vehicle.

Experiment 4. Effects on pancreatic exocrine secretion in rats

The volume of basal pancreatic exocrine secretion was approximately 10 μl/30 min, and HCO₃⁻ secretion was approximately 0.35 μEq/30 min. The intra-duodenal treatment of...
MCI-727 (30 and 100 mg/kg) significantly increased the volume (21.5 and 19.0 μl/30 min, respectively) and the HCO₃⁻ (0.84 and 0.97 μEq/30 min, respectively) at 30 min after administration (Fig. 6).

The intra-duodenal treatment of teprenone (30, 100 and 300 mg/kg) significantly increased the volume (16.1, 30.5 and 34.5 μl/30 min, respectively) and the HCO₃⁻ (0.67, 1.56 and 2.15 μEq/30 min, respectively) at 60 min after administration (Fig. 7).

The intra-venous treatment of Secrepan" (0.1 and 1.0 unit/kg) increased the volume (14.7 and 29.0 μl/30 min, respectively) and the HCO₃⁻ (0.47 and 1.66 μEq/30 min, respectively) at 30 min after administration (Fig. 8).

No significant changes in the volume and HCO₃⁻ secretion were observed in the control rats.

Fig. 5. Effect of MCI-727 (upper) and teprenone (lower) on plasma secretin concentration in dogs. All dogs were treated with cimetidine (10 mg/kg, i.v.) before intra-gastric administration (i.g.) of MCI-727 or teprenone. Control dogs were administered the combination of 2.5% gum Arabic and 0.5% Tween-80 solution. Results are expressed as the mean ± S.E. (n = 3–4). *P < 0.05: Significant difference from 0 min in each group. □: control, ● (upper): MCI-727, 10 mg/kg, i.g., ▲: MCI-727, 30 mg/kg, i.g., ● (lower): teprenone, 200 mg/kg, i.g.
Fig. 6. Effects of MCI-727 on pancreatic juice and bicarbonate secretion in rats. Results are expressed as the mean ± S.E. (n = 6–10). *P < 0.05, **P < 0.01: Significant difference from the control rats. ○: control, ●: MCI-727, 10 mg/kg, i.d., ▲: MCI-727, 30 mg/kg, i.d., ■: MCI-727, 100 mg/kg, i.d.

Fig. 7. Effects of teprenone on pancreatic juice and bicarbonate secretion in rats. Results are expressed as the mean ± S.E. (n = 6–10). *P < 0.05, **P < 0.01: Significant difference from the control rats. ○: control, ●: teprenone, 30 mg/kg, i.d., ▲: teprenone, 100 mg/kg, i.d., ■: teprenone, 300 mg/kg, i.d.

DISCUSSION

Based upon the results of the previous study on the effect of MCI-727 on gastric acid secretion (1), we assumed that the endogenous secretin may participate in part in the mechanism of the anti-ulcer effect of this agent. In the first experiment, we determined the plasma secretin concentration after oral administration of MCI-727 in rats. MCI-727 (10, 30 and 100 mg/kg) increased the plasma secretin concentration dose-dependently (Fig. 2). This finding indicates that MCI-727 has endogenous secretin releasing action. According to Shiratori et al. (12), the infusion of secretin (0.05 CU/kg/hr) increased the plasma secretin concentration with the value of 21.6 pg/ml (basal value was 4.1 pg/ml) and inhibited the
tetragastrin-stimulated gastric acid secretion in man. Chey and Konturek (13) also reported that the exogenous secretin (0.03 CU/kg/hr) treatment increased the plasma secretin concentration with the value of approximately 15 pg/ml (basal value was 6 pg/ml) and stimulated the pancreatic exocrine secretion in dogs. These results suggest that the low level of plasma secretin can produce the physiological action (12, 13).

In order to demonstrate the physiological action of plasma secretin induced by MCI-727, we examined the effect of MCI-727 on pancreatic exocrine secretion in rats. The volume of pancreatic exocrine secretion and pancreatic HCO₃⁻ outputs were significantly increased after MCI-727 treatment (Fig. 6). A similar effect was also observed with teprenone (Fig. 7) and Secrepan™ (Fig. 8). Thus, the study clearly indicates that MCI-727 has endogenous secretin releasing activity, and the rise in secretin is sufficient to induce the physiological action. In addition to this effect, MCI-727 inhibited the gastrin-induced gastric acid secretion and depressed the gastric motility (1). Corresponding effects were also observed for secretin (2–4, 13–15).

In the present study, we also investigated the effects of MCI-727 and teprenone on plasma gastrin concentration in rats. The plasma gastrin level was not altered significantly by the oral administration of MCI-727 and teprenone (Fig. 3), so it was suggested that both drugs stimulate the secretin release without influencing the gastrin release.

In the second experiment, the time course changes in the effects of MCI-727 (30 mg/kg) and teprenone (200 mg/kg) on plasma secretin concentration in rats were investigated. The increasing effect of MCI-727 (30 mg/kg) on plasma secretin concentration was already apparent after 10 min of oral administration and this remained for 240 min (Fig. 4, upper). On the other hand, oral administration of teprenone (200 mg/kg) also increased the plasma secretin concentration, but the significant increases were only observed at 30 and 60 min after administration (Fig. 4, lower). Up to now, there have been no time course studies on plasma secretin concentration after the treatment of secretin releasing agents. However, with regards to plasma secretin concentration induced by ingestion of a meal, it was reported that the increasing effect was observed until approximately 3–4 hr after the meal in man (14). From these data, it may be suggested that the duration of action on plas-
ma secretin concentration of MCI-727 was long enough. Furthermore, we previously demonstrated that MCI-727 (30 mg/kg, p.o.) and teprenone (200 mg/kg, p.o.) prevented HCl-ethanol induced gastric lesion (1) with the same inhibitory potency at 30 min after administration, but the duration of the anti-ulcer action of MCI-727 was more than 4 times longer than those of teprenone. We also observed that the administration of synthesized secretin prevented the formation of HCl-ethanol induced gastric lesion (M. Kawamura et al., unpublished data). In the present study, the duration of the effect of MCI-727 on plasma secretin concentration was shown to be about 4 times longer than the case of teprenone. The potency ratio of MCI-727 to teprenone (MCI-727 : teprenone = 4 : 1) in the duration of the increasing effect on plasma secretin agreed well with that in the duration of the anti-ulcer effect (HCl-ethanol ulcer model).

We have further shown in the present study that the plasma secretin increasing effect of MCI-727 was also observed in dogs. The maximal response of MCI-727 (30 mg/kg) on plasma secretin concentration was higher than that after teprenone (200 mg/kg), which showed no significant increases in contrast with rats (Fig. 5). The reason for the difference is not clear at present.

The regulating factors of secretin-release are poorly understood. Release of secretin is not under parasympathetic nerve control (16, 17), and other nerves controls are not known. The mechanisms of secretin release of the compounds which have increasing action on plasma secretin such as fatty acids (18, 19), bile (20), licorice extract (Fm 100) (21), teprenone (8) and plaunotol (22) are yet unclear. As for HCl which also increases the plasma secretin in vivo (23), it may be suggested that secretin release was induced by the direct stimulating action on the duodenal mucosa from an in vitro study (24). In our preliminary study, MCI-727 also stimulated secretin secretion from isolated rat duodenal mucosa in vitro, so we assumed that the increasing action of MCI-727 on plasma secretin was caused by a direct action to the duodenum.

In conclusion, we have clearly shown that MCI-727 increased the plasma secretin concentration and pancreatic secretion without influencing the plasma gastrin concentration. The increasing effect on plasma secretin may play a role, in part, in the mechanisms of the anti-ulcer effects of this agent.

Acknowledgment
The authors thank Professor William Y. Chey, University of Rochester School of Medicine and Dentistry, Rochester, NY, U.S.A., for his kind gift of anti-secretin serum R-5-6 and his helpful suggestions.

REFERENCES
10 Chey, W.Y., Kim, M.S., Lee, K.Y. and Chang, T.M.: Effect of rabbit antisecretin serum on post-
prandial pancreatic secretion in dogs. Gastroenterology 77, 1268–1275 (1979)


