NOTE

Diagnostic Study of a Synthetic Human Corticotropin-Releasing Hormone (hCRH) in Healthy Adult Males: Its Plasma Pharmacokinetics and the Effects on the Urinary Excretion of Steroid Hormones

KOSHI TANAKA, MEGUMI NAGATA, SACHIKO ITOH, MUNEHTO KUMAGAE AND NAOKATA SHIMIZU
Third Department of Medicine, Teikyo University School of Medicine, Ichihara 299-01, Japan

Abstract. The pharmacokinetics, responses of plasma ACTH and cortisol, urinary excretion of steroid hormones, and safety of MCI-028, a synthetic human corticotropin-releasing hormone (hCRH), were examined in eight healthy adult male volunteers after intravenous administration of 33, 100 and 200 µg of the drug. The disappearance of MCI-028 from plasma could be fitted to a biexponential decay curve, the plasma half-lives (T1/2) were 0.12 to 0.15 h for α phase, and 0.57 to 0.67 h for β phase. Plasma ACTH and cortisol concentrations and the urinary excretion of steroid hormones (particularly free cortisol) increased significantly in relation to the MCI-028 dose administrated. Although hot flushing and an increase in the heart rate were observed at higher doses, they were mild and transient. It is also considered that the urinary excretion of free cortisol after the administration of MCI-028 can be an index reflecting the functioning of this system.

Key words: hCRH, Healthy adults, Hypothalamic-pituitary-adrenocortical system, Diagnostic drug, Safety.

SINCE HARRIS [1] suggested that hypothalamic hormones may regulate the secretion of anterior pituitary hormones, many investigators have directed their efforts to the isolation of corticotropin-releasing hormone (CRH), one of the hypothalamic hormones, and the elucidation of its chemical structure. Although this work was difficult, because CRH exists in the hypothalamus only in trace amounts, Vale et al. [2] succeeded in isolating and identifying of ovine CRH (oCRH) in 1981. Their report showed that CRH has the structure of a single chain polypeptide consisting of 41 amino acids, whose C terminal is amidated. Additionally, in 1983, Shibahara et al. [3] showed that the structure of human CRH (hCRH) is identical to rat CRH [4] and different from oCRH in 7 amino acids.

Many clinical studies have been conducted since the structure of CRH was elucidated; it has been confirmed that CRH causes no significant adverse reaction [5, 6] and selectively stimulates the secretion of ACTH by acting directly on the ACTH secretory cells in the anterior lobe of the pituitary [7]; and the usefulness of pituitary ACTH secretory function as a diagnostic drug has been shown [5, 6, 8]. But there are few reports concerning the pharmacokinetics of hCRH and the urinary excretion of steroid hormones in humans.

In the present study, after intravenous administration of an hCRH preparation (code number MCI-028), synthesized at Mitsubishi Kasei Corporation, to healthy adult male volunteers, changes in the plasma hCRH concentration, plasma ACTH and cortisol concentrations, urinary
excretion of steroid hormones, production of antibody and safety were examined. The findings obtained are reported as a phase I clinical study of MCI-028.

**Subjects and Methods**

**Subjects**

This study was performed from February, 1990 to September, 1990 at Teikyo University Hospital after approval of the Ethical Committee and Institutional Review Board.

Eight healthy male volunteers aged from 23 to 38 years (mean 28.8 years) and weighing 57.8 to 68.0 kg (mean 62.7 kg) were selected. They were negative in the MCI-028 intradermal reaction test (intradermal administration of 0.01 µg). Before starting the study, all subjects were informed, by the physicians in charge, of the purpose and method of the study, the expected effect and risk, that it would not be detrimental to them even if they did not agree to participate in the study, that they could withdraw at any time even after agreeing to participate in the study, and of other requirements concerning protection of human rights, and they gave their written consent.

**Dose of hCRH**

Subjects were randomly assigned to 2 groups; to one group (n=4), 33 µg, 100 µg, placebo and 200 µg were administered and placebo, 33 µg, 100 µg and 200 µg to another group (n=4) once each in order. The interval of administration of MCI-028 was 18 to 24 days, and the study was conducted by a single blind method in which subjects are not informed of the dose.

**Method of administration**

The drug used was a lyophilized injection containing 100 µg of MCI-028 in 1 vial, dissolved in 3 ml of physiological saline immediately prior to injection, and intravenously administered at the given dose.

The study was started after an overnight fast in the early morning after allowing the subjects to rest quietly for more than 30 min. The subjects had to remain quiet in bed until the end of the examination 4 h after the drug administration and, if possible, until the end of the examination 24 h after administration.

**Determination of the plasma concentrations of hCRH, ACTH and cortisol**

The blood was withdrawn at 30 and 15 min before, immediately before, 5, 10, 15, 30, 45, 60, 90, 120, 240, 480 min and 24 h after administration, and the EDTA plasma was obtained, frozen and stored until assayed. The hCRH concentration was determined by radioimmunoassay (RIA) according to the method of Toyama et al. [9] at Mitsubishi Yuka Bio-Clinical Laboratories, Inc.

Plasma ACTH and cortisol concentrations were determined by RIA at the Central Laboratory Department, Teikyo University Hospital with a Mitsubishi Yuka ACTH IRMA kit (Mitsubishi Petrochemical Co., Ltd.) and an Eiken cortisol RIA kit (Eiken Kagaku Co., Ltd.), respectively.

**Determination of urinary hormone concentration**

Complete urination was conducted immediately before administration, and the urine was collected at 4 h after administration and used as the sample for determination. 17-Hydroxycorticosteroid (17-OHCS) and 17-ketosteroid (17-KS) with colorimetry and free cortisol with RIA were determined at the Central Laboratory Department, Teikyo University Hospital. For calculation, the values found were corrected from the urinary excretion of creatinine.

**Production of antibody**

The hCRH antibody in the plasma of each subject before and 24 h after the last administration (40 to 67 days after the first injection) of MCI-028 (200 µg) was examined by the polyethylene glycol sedimentation method at Mitsubishi Yuka Bio-Clinical Laboratories, Inc.

**Clinical signs and symptoms**

Clinical signs and symptoms were regularly observed; blood pressure, heart rate, body temperature and respiration rate were determined and electrocardiograms (ECG) were taken with time until 8 h after drug administration.
Results

1) Plasma hCRH concentration

The plasma concentration of hCRH reached 11, 26 and 60 ng/ml at 5 min after the administration of 33, 100 and 200 µg of MCI-028, respectively, and quickly decreased to the basal level by 24 h (Fig. 1). Since basal plasma hCRH did not fluctuate with time after administration of the placebo, the hCRH value before administration was subtracted from the measurement at each time point. The pharmacokinetic parameters such as plasma half-life for the α phase (T1/2 α) and β phase (T1/2 β), the area under the concentration curve (AUC₀₋∞), the distribution volume (Vd) and the total clearance (CL) were calculated (Table 1). T1/2 α and T1/2 β were 0.12 to 0.15 h and 0.57 to 0.67 h, respectively. And there was good linearity between the dose and AUC₀₋∞.

2) ACTH and cortisol concentrations

After a single intravenous administration of each dose of MCI-028, plasma ACTH and cortisol concentrations increased significantly and dose-dependently (Fig. 2). Plasma ACTH and cortisol concentrations reached their maxima at 15 or 30 min and 30 or 60 min after administration, respectively, and thereafter quickly decreased. When 33, 100 and 200 µg of MCI-028 were administered, the rates of mean maximum increase in the plasma ACTH concentration were 94.3, 187.7 and 165.9%, and those in the plasma cortisol concentration were 55.8, 80.4 and 57.9%, respectively.

Table 1. Pharmacokinetic parameters after administration of various doses of MCI-028

<table>
<thead>
<tr>
<th>Parameters</th>
<th>[unit]</th>
<th>33 µg (8)</th>
<th>100 µg (8)</th>
<th>200 µg (8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1/2 α</td>
<td>[h]</td>
<td>0.15±0.04</td>
<td>0.15±0.09</td>
<td>0.12±0.07</td>
</tr>
<tr>
<td>T1/2 β</td>
<td>[h]</td>
<td>0.66±0.25</td>
<td>0.67±0.29</td>
<td>0.57±0.09</td>
</tr>
<tr>
<td>AUC₀₋∞</td>
<td>[ng·h/ml]</td>
<td>3.67±0.56</td>
<td>8.80±1.92</td>
<td>24.74±2.73</td>
</tr>
<tr>
<td>Vd</td>
<td>[l]</td>
<td>2.48±0.49</td>
<td>3.98±1.68</td>
<td>2.58±0.73</td>
</tr>
<tr>
<td>CL</td>
<td>[l/h]</td>
<td>9.16±1.36</td>
<td>11.84±2.53</td>
<td>8.18±0.96</td>
</tr>
</tbody>
</table>

T1/2, half-life; AUC₀₋∞, area under the concentration curve; Vd, distribution volume; CL, total clearance. Each measurement is the mean ± SD for 8 subjects.
3) Urinary hormone concentration

Significant and dose dependent increases in the urinary excretion of free cortisol (with 33, 100 and 200 µg dosages of MCI-028), 17-OHCS (with 100 and 200 µg) and 17-KS (with 200 µg) were observed (Fig. 3). The rates of the mean increase in urinary free cortisol following the administration of 33, 100 and 200 µg were 78.4, 106.0 and 117.7%, respectively.

4) Production of antibodies

Both before and after the administration of MCI-028 in all subjects, there was no sign of hCRH antibodies in the plasma.

5) Clinical signs and symptoms

Facial warmness was observed in 1 case (12.5%) and 6 cases (75.0%) after the administration of 100 and 200 µg of MCI-028, respectively. It was evident in all within 5 min after drug administration and disappeared within 30 sec to 30 min, was mild and did not require treatment. No other abnormality was observed.

6) Effects on blood pressure, heart rate, ECG, body temperature and respiration rate

There was no significant change in blood pressure after the administration of each dose of MCI-028. After the administration of 100 and 200 µg of MCI-028, the heart rate increased (mean maximum of 10 and 17 beats/min, respectively) and returned to the basal rate within 20 and 120 min (Fig. 4). Throughout the study, no abnormality or significant difference was observed in the ECG, body temperature or respiration rate after the administrations of each dose of MCI-028 or the placebo.

7) Laboratory test values

No notable abnormality was observed in any subject.

Discussion

Pharmacokinetic analysis of hCRH has been previously examined by Schurmeyer et al. [11]. They reported monoexponential plasma elimination of $T_{1/2} = 0.07$ h after the intravenous administration of 1 µg/kg and biexponential plas-
ma elimination of $T_{1/2}^\alpha = 0.09$ h and $T_{1/2}^\beta = 0.42$ h after 5 $\mu$g/kg. In the present experiments with 33 to 200 $\mu$g of MCI-028 (0.5 to 3.2 $\mu$g/kg), the $T_{1/2}^\alpha$ value (0.12 to 0.15 h) and the $T_{1/2}^\beta$ value (0.57 to 0.67 h) appeared to be greater than those in the previous report. The difference may be due to the assay method for hCRH and/or time points used for pharmacokinetic analysis, although the exact reason remains to be clarified.

Since the pharmacokinetic parameters $T_{1/2}$, $V_d$ and $CL$ were almost constant independent of the dose, and $AUC_0^\infty$ increased with good correlation to the dose, it is presumed that the metabolic fate of MCI-028 may not change so much at doses ranging from 33 $\mu$g to 200 $\mu$g.

Changes in plasma ACTH and cortisol concentrations after the administration of MCI-028 were similar to those in previous studies with synthetic hCRH [6, 11, 12]. And urinary excretion of steroid hormones increased dose-dependently, corresponding to the increase in plasma ACTH and cortisol concentrations. Since significant increases were observed in urinary excretion of free cortisol and 17-OHCS particularly following the administration of MCI-028 at 100 $\mu$g and more, it is suggested that these parameters can be used as a diagnosis index for the hCRH loading test.

Since there was no adverse reaction other than mild and transient facial warmness and an increase in the heart rate, it is considered that MCI-028 should be quite safe and appeared to be useful when used as a diagnostic drug.

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**Fig. 4.** Systolic blood pressure (upper panel), diastolic blood pressure (middle panel), and heart rate (lower panel) after administration of MCI-028. O, 0 $\mu$g (placebo); ●, 33 $\mu$g; □, 100 $\mu$g; ■, 200 $\mu$g of MCI-028. Each value is the mean ± SEM for 8 subjects. Paired t-test was performed for the value following administration of 0 $\mu$g. **: $P<0.01$, ***: $P<0.001$. 
References