Human Corticotropin-Releasing Hormone (hCRH) Test: Sex and Age Differences in Plasma ACTH and Cortisol Responses and their Reproducibility in Healthy Adults

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Abstract. The effectiveness and safety of MCI-028, a synthetic human corticotropin-releasing hormone (hCRH), as a diagnostic drug were examined in 65 healthy male and 24 healthy female adult volunteers. Mean maximum concentrations of plasma ACTH and cortisol after intravenous administration of 100 µg of MCI-028 were 3.0 and 2.0 times their basal concentrations, respectively, and there were no significant age or sex differences in the responses. Good reproducibility was observed in the responses in 59 male subjects who received a second administration after 1 to 2 weeks. Although slight adverse reactions such as mild and transient hot flushing were observed, these were not serious.

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VALE ET AL. [1] isolated and identified ovine corticotropin releasing hormone (CRH) in 1981, and Shibahara et al. [2] reported in 1983 that the structure of human CRH (hCRH) is identical to that of rat CRH [3]. Thereafter, a number of studies concerning CRH have also been reported [4–6], and the possibility of its clinical application has been suggested.

It was confirmed in a phase I clinical study [7] that the hCRH preparation (MCI-028) synthesized at Mitsubishi Kasei Corporation is highly safe, and the optimum dose was established as 100 µg in a dose determining study (phase II clinical study) [8]. In the present study, the effectiveness, including response reproducibility, age and sex differences, and the safety of hCRH as a diagnostic drug for the function of the hypothalamic-pituitary-adrenocortical system was investigated in a large number of healthy adult volunteers.

The findings obtained are reported as a phase III clinical study of MCI-028.

Subjects and Methods

Subjects

This study was performed from March, 1991 to March, 1992 at 20 Japanese institutions in compliance with the Good Clinical Practice (GCP) standard after approval of the Institutional Review Board of each institution.

Sixty-five healthy adult males aged from 20 to 64 years (mean age 35.4 years, mean weight 65.0 kg) and 24 female volunteers aged from 21 to 40 years (mean age 30.5 years, mean weight 50.3 kg) were tested. The sexual phase in the females was not recorded. Pregnant women, women who might to be pregnant and nursing women were excluded from the test. Prior to entry into the study, all subjects were informed by the physicians in charge of the purpose and method of study, the expected effect and risk, that it would not be detrimental to them even if they did not agree with the participation in the study, that they could withdraw at any time even after agreeing to participate in the study, and of other requirements concerning the protection of human rights, and gave their written consent.

Method of administration

Lyophilized MCI-028 100 µg contained in a vial was dissolved in 1 ml of physiological saline immediately prior to injection and intravenously administered.

The injection was given to each subject in the early morning after an overnight fast and after allowing the subjects to rest quietly for at least 30 min. The subjects had to rest quietly until the end of the examination at 2 h after the injection.

The second test was performed in 56 adult males aged from 20 to 59 years (mean age 34.0 years, mean weight 65.9 kg) 1 to 2 weeks after the first test as a response reproducibility study.

Determination of plasma ACTH and cortisol concentrations

The blood was withdrawn at 30 min before, immediately before, and 15, 30, 60, 90 and 120 min after the injection, and the plasma was separated, frozen and used as the sample for determination. ACTH and cortisol were determined by a radioimmunoassay (RIA) method at Mitsubishi Yuka Bio-Clinical Laboratories, Inc. using an ACTH IRMA “Mitsubishi Yuka” kit (Mitsubishi Petrochemical Co., Ltd.) and a cortisol RIA “Gamma Coat Cortisol” kit (Baxter Co., Ltd.), respectively.

The plasma concentrations at various times, peak values ($C_{max}$), maximum increase ($\Delta_{max}$) maximum rate of increase ($%_{max}$), time of peak value ($T_{max}$) and area under the concentration curve (AUC) were estimated.

Clinical signs and symptoms

Because a transient hot flushing and warmness, transient increase in the pulse rate and
hypotension were expected in the results of the phase I clinical study [7] and dose finding study (phase II study) [8] of MCI-028, the clinical signs and symptoms were regularly observed, and the body temperature, blood pressure and pulse rate were monitored with time until 2 h after administration.

General laboratory tests

Laboratory tests were performed at 30 min before and 120 min after administration to determine pH, protein, sugar, urobilinogen, occult blood, color tone, turbidity and sediment as urinalysis; erythrocyte count, leukocyte count, hematoctrit, hemoglobin, platelet count and differential leukocyte count as hematological examinations; and GOT, GPT, LDH, AL-P, γGTP, choline esterase, total cholesterol, triglyceride, total protein, albumin, A/G ratio, blood sugar, total bilirubin, BUN, uric acid, creatinine, Na, K, Cl and Ca as blood biochemical examinations.

Statistical analysis

The results are expressed as the mean ± standard error unless otherwise mentioned. For plasma ACTH and cortisol concentrations, the results were examined with a significance level of 5% by selecting an appropriate analysis technique: Pearson’s moment correlation coefficient, profile analysis or paired t-test.

Results

1) Age difference in ACTH and cortisol responses

The correlation between age and the responses of ACTH and cortisol examined in 65 adult males and 24 adult females did not seem so remarkable as to need an age-related diagnostic standard in either males or females. All subjects were therefore combined and divided by age into 3 groups, 20 to 29 (n=35), 30 to 39 (n=33) and 40 to 64 years old (n=21), and were examined by profile analysis. There were no significant age differences in either ACTH or cortisol responses, as shown in Fig. 1.

2) Sex difference in ACTH and cortisol responses

When the difference between 65 adult males after the first administration and 24 adult females was examined by profile analysis, there were no significant sex differences in either ACTH or cortisol responses (Fig. 2).

3) Mean values and confidence interval of the responses of ACTH and cortisol

Because there was no significant difference in response due to age or sex, all 89 subjects were combined. Since sufficient logarithmic normality was observed in skewness and kurtosis, the mean value for each parameter was logarithmically converted, the 95% confidence interval was calculated, and mean value and the upper and lower limits of the 95% confidence interval were established by actual number conversion. The results are shown in Fig. 3 and Table 1.
A significant negative correlation was observed between the Co of cortisol and the %maxs of ACTH or cortisol in 89 adult males and females (ACTH, r=0.43 P<0.001; cortisol, r=0.74 P<0.001; Fig. 5).

**5) Response reproducibility**

No significant difference was observed between the ACTH and cortisol responses at the first and the second administrations of MCI-028, when analyzed by paired t-test in 56 adult males (Fig. 6). The Cmax for each subject exhibited good correlation between 2 administrations (ACTH, r=0.42 P<0.01; cortisol, r=0.45 P<0.001; Fig. 7).

**6) Clinical signs and symptoms (Tables 2 and 3)**

The most common signs and symptoms were hot flushing such as facial suffusion or warmness

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**Table 1.** The mean and 95% confidence limits (shown in parenthesis) of the response parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>ACTH</th>
<th>Cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax</td>
<td>43.9 (18.1 to 134.3) pg/ml</td>
<td>18.6 (12.2 to 28.3) µg/dl</td>
</tr>
<tr>
<td>Δmax</td>
<td>30.6 (6.6 to 141.7) pg/ml</td>
<td>8.8 (3.1 to 24.5) µg/dl</td>
</tr>
<tr>
<td>%max</td>
<td>200.7 (39.7 to 1013.9) %</td>
<td>103.1 (21.8 to 487.7) %</td>
</tr>
<tr>
<td>Tmax</td>
<td>33.9 (13.6 to 84.6) min</td>
<td>53.3 (31.7 to 110.4) min</td>
</tr>
<tr>
<td>AUC</td>
<td>27.7 (4.3 to 178.9) pg/h/ml</td>
<td>9.8 (2.7 to 38.1) µg-h/ml</td>
</tr>
</tbody>
</table>

Cmax, peak value; Δmax, maximum increase; %max, maximum rate of increase; Tmax, time of peak value; AUC, area under the concentration curve.
which were observed within 15 min after administration, but disappeared within 30 min in most cases.

7) Effects on body temperature, blood pressure and pulse rate

No marked change was observed in body temperature, blood pressure or pulse rate during the test.

8) General laboratory test

Changes in laboratory tests after MCI-028 administration were negligible except for one case. In this case, GPT increased from 29 to 39 IU (the normal value is 7 to 30 IU) at the first administration, but did not change at the second administration (from 25 to 23 IU). No notable changes in GOT, LDH or γGTP or other abnormalities were observed in this case.

Discussion

When the basal levels of plasma ACTH and cortisol are high due to stress, poor responses to exogenous CRH are observed [5, 6]. We therefore used data obtained only from subjects who could be kept at rest during the test.

It has been reported that there were no signi-
ficant sex or age differences in the ACTH and cortisol responses to hCRH [6, 9, 10]. The present results appeared to be in good agreement with these previous reports. Thus, age and sex are not important factors in evaluating the results of the CRH test in adults. There was a difference between males and females in mean body weight, but no significant correlation was observed between body weight and the ACTH or cortisol response in males and females.

The mean $T_{\text{max}}$ and the $C_{\text{max}}$ of ACTH and cortisol in the present study corresponded well to previous reports on hCRH [6, 11, 12]. Also in the present study, a negative correlation was confirmed, in which the higher the basal plasma cortisol concentration, the smaller the response of ACTH and cortisol.

There was almost no significant difference between the first and second administrations of MCI-028 in the response of ACTH and cortisol, suggesting high reproducibility when used as a diagnostic drug.

It is reported that synthetic growth hormone releasing factor (GRF) produced hot flushing in 97 (75.8%) of 128 administrations when a clinical dose of 100 µg was intravenously injected into healthy adults [13]. So the frequency of hot flushing produced by 100 µg of MCI-028 is not thought to be so high as that produced by other synthetic hypothalamic hormones. The adverse reaction in a laboratory test, which was judged to be caused by MCI-028, was only one case of GTP increase. Even in this case, it was considered that there is little fear that this drug may adversely affect liver function, in view of other indices such as GOT and the results of the second administra-
In conclusion, it is considered that MCI-028 is effective and highly safe as a diagnostic drug, so it is expected to be useful for functional tests for disorders of the hypothalamic-pituitary-adrenocortical system.

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Table 3. Time of occurrence and duration of hot flushing after MCI-028 administration

<table>
<thead>
<tr>
<th>Time after MCI-028 administration (min)</th>
<th>Number of administrations</th>
<th>%</th>
<th>Cumulative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occurrence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–1</td>
<td>42</td>
<td>71.2</td>
<td>71.2</td>
</tr>
<tr>
<td>2–5</td>
<td>14</td>
<td>23.7</td>
<td>94.9</td>
</tr>
<tr>
<td>6–15</td>
<td>3</td>
<td>5.1</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–5</td>
<td>16</td>
<td>27.1</td>
<td>27.1</td>
</tr>
<tr>
<td>6–15</td>
<td>27</td>
<td>45.8</td>
<td>72.9</td>
</tr>
<tr>
<td>16–30</td>
<td>11</td>
<td>18.6</td>
<td>91.5</td>
</tr>
<tr>
<td>31–60</td>
<td>4</td>
<td>6.8</td>
<td>98.3</td>
</tr>
<tr>
<td>over 61</td>
<td>1</td>
<td>1.7</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>59</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

References


