NOTE

Human Corticotropin-Releasing Hormone Test in Patients with Hypothalamo-Pituitary-Adrenocortical Disorders

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Abstract. To assess the diagnostic usefulness of a synthetic human corticotropin-releasing hormone (CRH) formulation (Code No. MCI-028), we administered 100 µg of the peptide intravenously to 183 patients with hypothalamo-pituitary-adrenocortical (HPA) disorders, and obtained the following findings. Among the 183 patients, data from 125 patients were suitable for analyzing the effects of the test. In patients with Cushing's disease, high plasma adrenocorticotrophic hormone (ACTH) and cortisol levels increased further in response to MCI-028, while in patients with adrenal Cushing's syndrome, low ACTH and high cortisol values remained unchanged. In patients with pituitary-type hypopituitarism or isolated ACTH deficiency, low ACTH and cortisol levels responded poorly or insignificantly to MCI-028, whereas those with hypothalamic hypopituitarism showed delayed and considerable degree of responses of plasma ACTH and little increase in plasma cortisol levels. In Addison's disease, high plasma ACTH increased further in response to MCI-028, but low cortisol levels did not change. In patients with

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Cushing's syndrome soon after successful surgical treatment, plasma ACTH responsiveness was low or different depending on the clinical course of the patient. Patients treated with high doses of glucocorticoids for non-endocrine diseases tended to show impaired ACTH and cortisol responsiveness to MCI-028. Side effects, including the transient flushing which was observed most frequently in this study, did not cause any clinical problems.

Key words: Human corticotropin-releasing hormone, Hypothalamo-pituitary-adrenal disorders, Diagnostic drug, Glucocorticoid.
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CRH IS A hypothalamic hormone which directly stimulates the synthesis and secretion of ACTH in the anterior pituitary. This hormone was first isolated from sheep and its primary structure was identified by Vale *et al.* in 1981 [1]. Two years later, the structure of human CRH was disclosed by Shibahara *et al.* [2], and, since then, many basic and clinical studies have been conducted with synthetic ovine or human CRH and data obtained have shown the usefulness of CRH as a diagnostic tool, especially for differentiating disorders in the HPA axis [3–7]. However, there has been no systematic study to assess the effects and side effects of human CRH (hCRH) preparations. To introduce a hCRH preparation for diagnostic use, Tanaka *et al.* completed evaluations of a hCRH

formulation synthesized by Mitsubishi Kasei Corp. (Code No. MCI-028) for both its safety and potency in stimulating ACTH secretion in healthy male adult volunteers as a phase I clinical study [8] and, thereafter, dose finding and reproducibility confirmation studies as a phase II clinical study [9]. In the present study, we administered MCI-028 to patients with dysfunction of the HPA axis and evaluated its usefulness and safety as a diagnostic drug. The findings obtained are reported below as a phase III clinical study of MCI-028.

Table 1. Background data of 125 subjects examined

	Typical cases	Atypical cases	Total number of cases examined	Male/Female	Mean age (youngest-oldest)
Cushing's syndrome	34	10	44	6/38	37.7(17–62)
Cushing's disease	25	7	32	4/28	35.9(17–62)
Adrenal tumor	9	2	11	2/9	43.4(27–59)
Ectopic ACTH producing tumor	0	1	1	0/1	34 (34)
Adrenocortical insufficiency	40	2	42	17/25	49.5(12–75)
Primary hypothalamic dysfunction	6	2	8	2/6	37.0(12–74)
Primary pituitary dysfunction	11*	0	11	4/7	53.4(35–72)
Isolated ACTH deficiency	13	0	13	6/7	56.2(40–74)
Addison's disease	10	0	10	5/5	46.0(17–75)
Secondary adrenocortical insufficiency after excessive endogenous or exogenous glucocorticoid	39	0	39	8/31	39.6(17–65)
Cases after curative surgery for Cushing's syndrome	19	0	19	2/17	39.8(17–65)
Cases received glucocorticoid for non-endocrine disorders	20	0	20	6/14	39.5(18–64)
Total	113	12	125**	31/94	42.3(12–75)

* Total number of tests was 127, since the reproducibility test was conducted for 2 cases of Cushing's syndrome after surgical treatment. ** Pituitary adenoma, 6 cases; Sheehan syndrome, 3 cases; Rathke's cyst, 1 case; autoimmune hypophysitis, 1 case.

Materials and Methods

Subjects

The present studies were conducted from March, 1991 to March, 1992, at 19 medical institutions specializing in endocrine disorders in Japan. Permission for this study was received from the respective institutional ethical committees, and "Good Clinical Practice" [10] was followed.

Of 183 patients given MCI-028, 125 patients, aged between 12 and 75 years, who were suitable for assessment of their endocrine analyses, were classified into the following three groups: Cushing's syndrome (34 cases), adrenocortical insufficiency (40 cases), secondary adrenocortical insufficiency following endogenous or exogenous glucocorticoid excess (39 cases), as indicated in Table 1.

We also separately analyzed 12 out of 125 patients who had undergone surgery, radiation, or chemical therapy which might have modified their HPA function.

Method

From each patient tested, written or oral consent was obtained after they were provided with all the necessary information. MCI-028 (Lot No. 1301), manufactured by Mitsubishi Kasei Corporation for injection, contained 100 μ g hCRH, chemically synthesized by solution synthesis per vial, and was kept in a freeze-dried state.

After the patient was fasted overnight and bed rested for at least 30 min, 100 μ g MCI-028 was dissolved in 1 ml physiological saline and administered intravenously. Blood samples (2 ml) were taken 30 min before, immediately before at time zero, and 15, 30, 60, 90 and 120 min after the MCI-028 administration, and put into cooled test tubes. Plasma was separated and frozen until measurement of hormones. The concentrations of ACTH and cortisol were determined by radioimmunoassay with an ACTH IRMA Kit "Mitsubishi Yuka" (Mitsubishi Yuka) and with the Gamma Coat Cortisol Kit (Baxter), respectively, at Mitsubishi Yuka B.C.L. The determination limits for ACTH and cortisol by radioimmunoassay was 10 pg/ml and 1.0 μ g/dl, respectively. The values obtained were next analyzed by means of several indices of the response, i.e., C_0 : each hormone

level at time zero, $C_{\max}$: peak levels after MCI-028 administration, and $\%_{\max}$: % maximum increment above C_0 .

Signs and symptoms were carefully observed during the test period, and body temperature, blood pressure, and heart rate were measured 30 min before, immediately before, 30, 60 and 120 min after MCI-028 administration.

Laboratory tests including urinalysis and blood chemistry were conducted as indicated elsewhere [11].

For the statistical analyses, data were treated by either the paired *t*-test or regression analysis to obtain Pearson's product moment correlation coefficient at the 5% level of significance (two sided).

Results

1. Cushing's syndrome

In 25 untreated cases of Cushing's disease, the C_0 values for ACTH and cortisol were 98.7 ± 23.9 pg/ml and 22.3 ± 1.7 μ g/dl, respectively, which were significantly higher than those in a group of healthy adults who underwent the trial under the same protocol [11]. After the administration of MCI-028, ACTH and cortisol levels further increased transiently, with a peak at 15–30 and 30–60 min, respectively, and returned to their initial values by 120 min (Fig. 1).

Among the seven atypical cases with Cushing's disease shown in Table 1, five patients treated with op'-DDD had lower cortisol but comparable ACTH responses to MCI-028 when compared with untreated patients. Three of the five patients who received additional pituitary irradiation therapy responded similarly to those only with op'-DDD. One patient treated with bromocriptine and another with incomplete removal of the pituitary tumor responded similarly to those in untreated patients (data not shown).

In 9 patients with untreated adrenal tumors, higher cortisol and lower ACTH levels did not change significantly after the administration of MCI-028 (Fig. 1). A patient with bilateral adrenal nodular hyperplasia and a patient with left-unilateral adrenal nodular hyperplasia responded similarly to those with adrenal adenoma (data not shown).

Fig. 1. MIC-028 tests in Cushing's syndrome. Plasma ACTH (upper panel) and cortisol (lower panel) levels in typical cases of Cushing's disease (●, $n=5$), adrenal adenoma (○, $n=11$), and a case of ectopic ACTH producing tumor after bilateral adrenalectomy (▲) are shown in comparison with those of healthy adults shown as the 95% confidence limit of the responses (area with shadow) and the mean levels (□; mean level) in healthy adults [11]. 100 μ g MCI-028 was administered at time 0. Patients' data are shown as group mean $\pm$ SEM.

In patient with Cushing's syndrome who had ectopic ACTH production from a lung carcinoid tumor and bilateral adrenalectomy, the higher ACTH level closely responded, whereas undetectable plasma cortisol levels remained unchanged after MCI-028 injection (Fig. 1).

2. Adrenocortical insufficiency

In all 10 patients with Addison's disease, high basal ACTH levels further increased in response to MCI-028, and low and almost undetectable plasma cortisol values remained completely unresponsive to the MCI-028 administration.

In patients with hypopituitarism attributable to the pituitary lesion detailed in Table 1, their low C_0 ACTH and cortisol levels showed no or little

Fig. 2. MCI-028 tests in Addison's disease, isolated ACTH deficiency and pituitary adrenocortical insufficiency. Hormonal responses in typical cases of Addison's disease (●, $n=10$), isolated ACTH deficiency (▲, $n=13$) and pituitary adrenocortical insufficiency (○, $n=11$) are shown. Data are shown as group means $\pm$ SEM. Shaded areas are the same as in Fig. 1.

response to MCI-028 (Fig. 2). Low ACTH and cortisol levels in isolated ACTH deficiency also responded similarly (Fig. 2).

The results for eight patients with hypopituitarism attributable to hypothalamic lesions varied (Fig. 3). In one patient with untreated suprasellar germinoma (#1), the response of low C_0 ACTH to MCI-028 was a considerable but delayed. Patients with suprasellar germinoma after irradiation had a delayed response (#3) or none at all (#2). Both surgically not-treated (#4 and #5) and treated (#6–8) patients with craniopharyngioma had no response or a delayed or intermediate response to MCI-028. Plasma cortisol responded poorly in all these patients.

3. Secondary adrenocortical insufficiency after excessive endogenous or exogenous glucocorticoids

In 14 patients who needed glucocorticoid supplements after curative surgery for Cushing's

Fig. 3. MCI-028 tests in hypothalamic adrenocortical insufficiency. 1: suprasellar germinoma, 2, 3: suprasellar germinoma irradiated, 4, 5: craniopharyngioma, 6–8: craniopharyngioma surgically treated. Shaded areas are the same as in Fig. 1.

syndrome, the C_0 values for ACTH were comparable to those in healthy adults, whereas the $C_{\max}$ values were decreased, and both C_0 and $C_{\max}$ values for cortisol were obviously lower than in healthy adults (Fig. 4). On the other hand, in 5 patients who needed no glucocorticoid supplementation, high normal C_0 ACTH and low normal C_0 cortisol values responded almost normally to MCI-028.

Fig. 5 shows the correlation between several indices of ACTH and cortisol responses in the MCI-028 test in patients after curative surgery for Cushing's syndrome with or without glucocorticoid supplements shown in Fig. 4. A positive correlation existed between the C_0 values and $C_{\max}$ values for cortisol in these patients (Fig. 5A). Moreover, a positive correlation which was not evident in normal controls [11] existed between the C_0 values for cortisol and $C_{\max}$ values for ACTH in these patients (Fig. 5B). A comparison of the C_0 and $\%_{\max}$ values for cortisol (Fig. 5C), revealed no relationship, in contrast to the obvious negative relationship in normal volunteers [11].

In 20 patients after treatment with glucocorti-

Fig. 4. MCI-028 tests in patients recovering from curative surgery for Cushing's syndrome. Fourteen patients requiring supplemental glucocorticoid (○), including 7 patients with Cushing's disease, 5 with adrenal adenoma, 1 with ectopic ACTH producing tumor due to thymic carcinoid, and 1 with unilateral nodular hyperplasia, and 5 patients not requiring it (△), including 4 with Cushing's disease and 1 with adrenal tumor, are shown. Data are presented as group means $\pm$ SEM. Shaded areas are the same as in Fig. 1.

coids with diverse regimens concerning the kinds of steroids used and periods of their administration for non-endocrine diseases including 6 patients with systemic lupus erythematosus and 4 with nephrotic syndrome, plasma hormone responses varied, but their response patterns were generally similar to those for Cushing's syndrome recovering after curative surgery, as shown in Fig. 4. In these patients the correlation between the C_0 and $C_{\max}$ values for cortisol (Fig. 6A) and that between the C_0 and $\%_{\max}$ values for cortisol (Fig. 6C) were comparable to those obtained for the treated cases of Cushing's syndrome shown in Fig. 5. In contrast, there was no apparent correlation between C_0 values for cortisol and $C_{\max}$ values for ACTH in these cases (Fig. 6B).

Fig. 7 summarizes the findings described above. The normal range for C_0 and $C_{\max}$ shown was

Fig. 5. Comparison of several indices obtained in MCI-028 test in patients recovering from curative surgery for Cushing's syndrome and in normal controls. Data are modified from those shown in Fig. 4. ● represents an individual case of the disease, and r and P values seen at the upper-right corner in each panel represent the degree of correlation between indices for the patients. ○ indicates an individual subject in a control group ($n=89$) detailed elsewhere [11]. Correlation coefficients in these control groups are as follows: in panel A: $r=0.367$, $P=0.0004$; in panel B: $r=0.112$, $P=0.2953$; and in panel C: $r=-0.743$, $P=0.0001$.

determined with data from normal adult volunteers [11] as a rectangle surrounded by 95% confidence limits. The characteristic distribution for each disease group was evident in these figures.

4. Clinical signs, symptoms and laboratory examinations

Among 127 tests of MCI-028, 45 (35.4%) showed some of the clinical symptoms or signs indicated in Table 2. As anticipated in the results of the phase I [8] and phase II [9] clinical studies of MCI-028, hot flushing, including redness and a sense of warmth, were seen most frequently in 37 tests (29.1%). The most frequently affected site was the face and neck (86.5%) and the symptom appeared within 5 min after administration in 83.8% of the tests and disappeared within 15 min after their onset in 81.1%. Other side effects were all transient and disappeared by the end of the sampling period.

Blood pressure and heart rate were not significantly changed, although a slight but significant increase in body temperature was noted after administration.

Laboratory examinations revealed some changes in the hematological data: significant decreases in the red blood cell count, hematocrit, and lymphocyte fraction, although the differences were all very small. Slight changes were noted in blood chemistry in 15 of 20 parameters, but could not be attributed to the MCI-028 test. No abnormal changes in urinalysis were observed (data not shown).

Discussion

The present study has shown that intravenous injection of hCRH evokes characteristic responses of plasma ACTH and cortisol in different types of disorders of the HPA axis.

Fig. 6. MCI-028 tests in patients dosed with pharmaceutical glucocorticoid for non-endocrine disorders. As in Fig. 5, ● represents a patient who had been receiving glucocorticoid before an MCI-028 test. ○ indicates a control subject as in Fig. 5.

Following the administration of MCI-028, both increased basal ACTH and cortisol values further increased in Cushing's disease but neither high ACTH nor low cortisol levels responded in Cushing's syndrome due to glucocorticoid-producing adrenal adenoma. These patterns are consistent with those reported previously in patients given ovine CRH [3–5, 12–14] or human CRH [6]. MCI-028 is therefore useful in the differential diagnosis of Cushing's syndrome.

One of the difficulties in the differential diagnosis of Cushing's syndrome is to distinguish ACTH-producing tumors in the pituitary from ectopic ACTH-producing tumors. Although CRH may be useful in discriminating these types of Cushing's syndrome [12–17], this point could not be sufficiently evaluated in this study because no typical case of ectopic ACTH producing tumor was studied. In the patient whose data are shown in Fig. 1, the ACTH response after MCI-028 could not be attributed exclusively to ectopic ACTH production, since ACTH secretion from the pituitary might respond well to CRH without endogenous glucocorticoids.

Concerning patients with adrenocortical insufficiency, the present study demonstrates that patterns of response of ACTH and cortisol to MCI-028 differed according to the site of the lesion (Figs. 2, 3). These findings, which are in agreement with previous reports [18–20, 23], indicate that, if ACTH values are responsive to MCI-028 in the presence of low cortisol, the hypothalamus is the probable site of the lesion; and if ACTH response is poor, a pituitary lesion with or without hypothalamic involvement is strongly suggested.

In cases of successfully treated Cushing's syndrome, low basal cortisol level did not respond well to MCI-028 as in healthy subjects with similar low basal cortisol levels [6, 7, 11, 21]. Therefore, the cortisol response after MCI-028 administration could be a more useful index than its C₀ value in assessing the restoration of the HPA function in patients with Cushing's syndrome after curative surgery. A positive correlation between the C_{max} values for ACTH and C₀ values for cortisol, which was not observed in normal subjects, is compatible with the notion that functional recovery of the adrenal cortex after glucocorticoid excess depends on the functional restoration of the pituitary gland [22].

In patients who were clinically suspected of

Fig. 7. Plasma ACTH and cortisol levels in an MCI-028 test for various disorders in the HPA axis. C_0 and C_{max} represent hormone levels before the test and the highest levels after MCI-028 administration. Squares in figures represent 95% confidence limits of normal responses in a group of healthy adults detailed separately [11], and other marks are as follows; ■, Cushing's disease; □, Cushing's syndrome due to adrenal adenoma; ○, hypothalamic hypocortisolemia; ▲, pituitary-lesioned hypocortisolemia; △, isolated ACTH deficiency; ●, Addison's disease.

Table 2. Side effects seen after administration of MCI-028 (185 tests in total)

a) Clinical signs and symptoms	Number of findings (incidence %)	b) Signs and symptoms of hot flushing	Number of tests (incidence %)
Hot flushing symptoms	55(29.7)	Facial redness	18(9.7)
Facial redness	22(11.9)	Sense of warmth, hot flushes, sense of flush	25(13.5)
Sense of warmth, hot flushes, sense of rush, sense of flush	33(17.8)	Sense of rush	4(2.2)
Others	26(14.1)	Facial redness, sense of warmth, hot flushes	3(1.6)
Palpitation	6(3.2)	Facial redness, sense of rush	1(0.5)
Headache, heavy feeling of head	5(2.7)	Number of tests showing hot flushing	51(27.6)
Strange feeling/malaise at the pharynx	5(2.7)	c) Site of hot flushing symptoms	Number of tests (%)
Pressure/strange feeling in the chest	4(2.2)	Face and neck	45(88.2)
Chilly feeling at the dorsum and pedis	1(0.5)	Whole body	2(3.9)
Pain in the neck	1(0.5)	Finger tip (injected side)	1(2.0)
Abdominal pain	1(0.5)	Chest	1(2.0)
Feeling high	1(0.5)	Lower extremities	1(2.0)
Feeling unsteady	1(0.5)	Unknown	1(2.0)
Whistling sensation in the nose	1(0.5)		
Total number of findings	81	d) Time of onset of hot flushing symptoms	Number of tests (%)
Total number of tests	61(33.0)		Cumulative number of tests (%)
		Immediately-1 min.	26(51.0)
		2-5 min.	16(31.4)
		6-15 min.	4(7.8)
		16-30 min.	4(7.8)
		31-30 min.	1(2.0)
		e) Duration of hot flushing symptoms	Number of tests (%)
			Cumulative number of tests (%)
		Within 5 min.	30(58.9)
		6-15 min.	10(19.6)
		16-30 min.	6(11.8)
		31-60 min.	3(5.9)
		61-120 min.	2(3.9)

having HPA hypofunction due to long-term and/or large-dose glucocorticoid therapy for non-endocrine disease, no indices have been accepted so far as suitable in assessing adrenocortical functions accurately, safely, and easily. For this purpose, cortisol responses expressed as %_{max} which were apparently different from those of healthy adults, may be a better index than simply measuring the C₀ cortisol value which is controversial as an index of HPA dysfunction [23, 24].

As shown in Fig. 7, the C₀ and C_{max} for ACTH and cortisol levels collected in this study were distributed characteristically on these kinds of charts. This type of analysis may therefore also be useful in the diagnosis of various diseases in the HPA axis.

Hot flushing was reported to be one of the most common side effects induced by peptide preparations such as CRH (28.3%) [25], GRF (17.1%) [26] and TRH (50.0%) [27]. In this study, the flushing, including redness and a sense of warmth, induced by MCI-028 was transient and the incidence in this study (29.1%) was comparable with that reported previously [25], indicating no limitation for its clinical use.

To our knowledge, there has been no detailed description of changes in blood biochemistry or

urinalysis data after the administration of CRH to patients with HPA dysfunction [25]. In the present study, slight but significant variations were detected for 16 of 31 clinical laboratory parameters examined before and after the MCI-028 test, but the changes were all very small, being of no clinical significance.

We therefore conclude that MCI-028 is a safe and unique drug to use in diagnosing patients with the HPA dysfunction.

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