Evaluation of Hypothalamo-Pituitary-Adrenocortical Function in Children by Human Corticotropin-Releasing Hormone (MCI-028) Test

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Abstract. A dose of 1.5 µg/kg of MCI-028, human corticotropin-releasing hormone (hCRH), was administered intravenously to 38 children with non-endocrine short stature with normal function in the hypothalamo-pituitary-adrenocortical axis and to 71 children with a disorder in the same axis. Blood levels of adrenocorticotropic hormone (ACTH) and cortisol were determined to evaluate the axis. The 95% confidence limits of peak responses of ACTH and cortisol in non-endocrine short stature were between 17.2 and 135.3 pg/ml, and between 13.1 and 35.6 µg/dl, respectively, and were used as standards for children.

When compared with these standards, the hormonal responses in children with various disorders in the hypothalamo-pituitary-adrenocortical axis were as follows: in two children with Cushing’s syndrome caused by adrenal tumor, ACTH values were decreased and were not responsive to hCRH, while cortisol values, though within the normal limit, were not responsive; in children with primary adrenal insufficiency or congenital adrenal hyperplasia, cortisol values were decreased and not responsive, whereas ACTH values tended to be increased and ACTH response high except for 21α-hydroxylase deficiency of congenital adrenal hyperplasia. In two cases of pituitary dwarfism complicated with ACTH deficiency, both ACTH and cortisol values were decreased and poorly responsive; and in children who were receiving glucocorticoid, both ACTH and cortisol values tended to be decreased and to respond poorly to hCRH.

As for side effects, hot flushing was observed among 8.0% of the subjects after administration of hCRH. But this symptom was not severe and no other side effects of clinical importance were observed.

In conclusion, this study demonstrated that the hCRH test was also safe and useful in evaluating hypothalamo-pituitary-adrenocortical function in children.

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CORTICOTROPIN-releasing hormone (CRH) is one of the hypothalamic hormones that act on the anterior pituitary and stimulates synthesis and secretion of ACTH. This hormone was isolated from sheep by Vale et al. [1] and its primary structure, consisting of 41 amino acids, was determined in 1981.

Since Shibahara et al. [2] determined the amino-acid sequence of human CRH (hCRH) in 1983, it has been used in many basic and clinical studies to evaluate pituitary-adrenal function.

In Japan also, a research group from the Ministry of Health and Welfare studying hypothalamic-pituitary dysfunction conducted a study to evaluate the clinical usefulness of hCRH. Tanaka et al. completed clinical evaluations of this hCRH formulation synthesized by Mitsubishi Kasei Corporation (Code No. MCI-028) for its safety and ACTH secretion stimulating potential in healthy male adult volunteers (phase I clinical studies) [3] and, thereafter, dose-finding and reproducibility studies (phase II clinical studies) [4]. In the present study, the clinical usefulness of hCRH in evaluating pituitary-adrenal function was studied in children.

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

**Subjects**

The present study was conducted from April, 1991 to March, 1992 at six Japanese medical institutes. The study was started after being approved by the review board at the respective institutes, and “Good Clinical Practices” were followed. As shown in Table 1, subjects included children of non-endocrine short stature whose hypothalamo-pituitary-adrenocortical axis was supposed to be normal and children having or suspected of having a disorder in the same axis.

Six hCRH tests in total were conducted in two cases of Cushing’s syndrome, one adrenal tumor (Case A) and one adrenal virilization tumor (Case B).
B). Although no apparent sign of Cushing's syndrome was observed in one child with adrenal virilization tumor (Case B), analysis of the response patterns of ACTH and cortisol made it seem reasonable to diagnose this case as Cushing's syndrome. There were 2 cases with primary adrenal insufficiency: one ACTH unresponsiveness (Case C) and one adrenal hypoplasia (Case D). There were 6 children with congenital adrenal hyperplasia, 3 of whom had Prader's syndrome.

Although hCRH was administered to 109 cases (113 tests) in total, 20 cases were excluded because they did not satisfy the subject selection criteria because they had a disease outside of the evaluation scope, they received glucocorticoid concomitantly on the day of the study or they had been diagnosed on an ill-defined basis. The remaining 89 cases (93 tests) were therefore used for the analysis. Furthermore, 4 of the 93 tests were conducted on 2 children with Cushing's syndrome after their tumors were removed. They were therefore analyzed separately as atypical cases. To analyze the change in each parameter and side effects, all the data from the 113 tests were used.

Materials and Methods

Before starting the study, informed consent was obtained from the parents or, if possible, from the subject.

The test drug, MCI-028 (Lot No. 1301), manufactured by Mitsubishi Kasei Corporation, was in a vial containing 100 µg of hCRH. In subjects who had been receiving steroid therapy, for such conditions as primary adrenal insufficiency and congenital adrenal hyperplasia, steroid administration on the test day was postponed until the hCRH test was over. After fasting overnight, the patients were kept at rest from at least 30 min before to 2 h after hCRH injection, which was administered intravenously at a dose of 1.5 µg/kg (maximum 100 µg). Thirty min before, immediately before, and 15, 30, 60, 90 and 120 min after administration, 2 ml blood samples each were drawn and the plasma was stored at −20°C. The concentrations of ACTH and cortisol in the plasma were determined at Mitsubishi Yuka B.C.L. Company by radioimmunoassay with an ACTH IRMA Kit “Mitsubishi Yuka” (Mitsubishi Yuka) and with γ-Coat Cortisol (Bacster), respectively.

Clinical signs and symptoms were observed continually and body temperature, blood pressure, and heart rate were measured 30 min and immediately before administration and 30, 60 and 120 min after administration.

Clinical laboratory examinations such as urinalysis, hematological examinations and blood chemistry were carried out 30 min before and 120 min after administration. In some patients, an insulin tolerance test was also performed to evaluate the hypothalamo-pituitary-adrenocortical axis, and the results were compared with those of the hCRH test.

Clinical data were analyzed by either paired t-test, regression analysis, using Pearson's product moment correlation coefficient, or multiple comparison (Scheffe method). P<0.05 was considered significant. Since the determination limit of ACTH and cortisol in the radioimmunoassay was 10 pg/ml and 1.0 µg/dl, respectively, values lower than the limits were all recorded as 10 pg/ml and 1.0 µg/dl. In addition, the baseline value (C₀) and peak value (Cmax) were determined as index parameters of hormonal response and were analyzed. The results were presented as the mean ± SEM, unless otherwise stated.

Results

To determine normal hormonal responses to hCRH in children, the results for 38 children of non-endocrine short stature who were supposed to have no disorder in the hypothalamo-pituitary-adrenocortical axis were analyzed. Since there was no significant difference in ACTH and cortisol responses between male and female, or between the prepubertal and pubertal states (Scheffe method) as shown in Fig. 1(a), all the data were compiled and shown in Fig. 1(b). When the patterns of hormonal responses in children and adults [5] were compared, no important differences were noted in ACTH values, but cortisol tended to be more responsive to hCRH in children than in adults.

Ninety-five % confidence limits were calculated for basal levels (C₀) and peak levels (Cmax) of ACTH and cortisol which were standardized after logarithm transformation to obtain normality. Figure 2 shows the frame-of-confidence limits for...
C₀ (ACTH: 5.3–51.1 pg/ml; cortisol: 3.9–21.3 µg/dl) and that for Cmax (ACTH: 17.2–135.3 pg/ml; cortisol: 13.1–35.6 µg/dl) together with plots of individual data; almost all the C₀ and Cmax values were found within the frame. When confidence limits for C₀ and Cmax of both hormones were compared with those in adults, the upper limits for C₀ of ACTH values and for Cmax of cortisol values were slightly higher in children than in adults. Since the frame-of-confidence limits for Cmax were revealed to be more useful than that for C₀ in evaluating the pituitary-adrenal axis, only the data for the frame of Cmax were shown in the later analysis.

Figure 3(a) shows the hormonal responses in 2 cases of Cushing’s syndrome obtained before surgery. In both cases, ACTH values were below the RIA sensitivity limit, while cortisol values were within normal limits but not responsive to hGRH. For these cases, the same test was also conducted 26 days and 4 months after surgery in Case A (a’ and a” in Fig. 3(b)), and 19 days and 2 months after surgery in Case B (b’ and b” in Fig. 3(b)). As shown in Fig. 3(b), in Case A there was no recovery in the pituitary-adrenocortical axis even 4 months after surgery, with Cmax plotted in the left-lower corner. In Case B, on the other hand, ACTH values were increased and highly responsive to hCRH at the 19th postoperative day, which was followed by recovery of Cmax values to the normal range 2 months postoperatively.

As shown in Fig. 4 (a)(b), cortisol values were decreased and unresponsive to hCRH in 2 cases with primary adrenal insufficiency. In contrast, ACTH values were much increased and slightly more responsive to hCRH in Case C and showed a normal basal value and an exaggerated responsiveness in Case D.

In 6 children with congenital adrenal hyperplasia, cortisol values were decreased and unresponsive to hCRH as shown in Fig. 5 (a)(b). ACTH values were increased and highly responsive in cases with Prader’s syndrome but there were
various response patterns among the remaining cases (21α-hydroxylase deficiency).

When hCRH was administered to 17 children with pituitary dwarfism, although the mean of the Cmax values was within the normal limits, some cases responded poorly to both ACTH and cortisol as shown in Fig. 6. Two children whose plots in Fig. 6 were found in the lower left corner of the confidence limits had been diagnosed as having combined ACTH deficiency.

There was one case with ACTH-ADH discharge syndrome, who had normal hormonal responses to hCRH during remission.

When hCRH was administered to 23 children with renal disease, whose adrenal function was supposed to be suppressed by glucocorticoid treatment, both ACTH and cortisol tended to respond weakly to hCRH as shown in Fig. 7.

Since the insulin tolerance test (ITT) has been traditionally used to evaluate the function of the hypothalamo-pituitary-adrenocortical axis, the results of ITT were compared with the hCRH test. The Cmax values are plotted in Fig. 8 for 20 cases whose ACTH and cortisol levels were determined by the same assay in both tests. There was no apparent correlation for ACTH values, with ITT.

Fig. 2. 95% confidence limits of C0 and Cmax values of ACTH and cortisol from 38 children of non-endocrine short stature.

Fig. 3a. ACTH (upper panel) and cortisol (lower panel) responses to hCRH in children with adrenal Cushing’s syndrome. Mean ± SEM in children of non-endocrine short stature. Shaded area shows normal range (mean ± 2SD). (See Results.)

Fig. 3b. Distributions of Cmax values of ACTH and cortisol in children with adrenal Cushing’s syndrome before and after surgery. (See Results.)
inducing higher responsiveness. For cortisol, on the other hand, a positive correlation between the two tests was noted and response in both tests was similar.

All 113 cases were observed for subjective symptoms and objective findings such as skin color change. Changes in clinical signs and symptoms were found in 10 cases (8.8%), 14 findings in total.
The most common signs were flushing including redness, sense of warmth, and burning sensation, seen in 9 cases (8.0%). The symptoms, all in the head and neck except for one case, appeared within 30 min after administration in most of the cases and disappeared within 30 min after onset in all the cases. Other side effects observed included 2 cases of chest discomfort (1.8%) and one case of feeling unwell, upper jaw discomfort, and numbness of legs (0.9%). But these were all slight and transient without any treatment needed. In conclusion, there were no severe side effects after hCRH administration and all side effects were slight and transient.

Before and after the administration of hCRH, blood pressure, heart rate and body temperature were measured and the results analyzed by paired t-test. In heart rate, no change was observed. Although systolic pressure decreased significantly 30 and 60 min after administration and body temperature increased significantly 30 min after, the changes were minimal and of no importance (data not shown).

The effects of hCRH on clinical laboratory data were evaluated by paired t-test. Significant variations were noted for 6 of 10 hematological parameters and for 10 of 20 blood biochemistry parameters. The changes were all small, however. Similarly, for urinalysis data, there was no abnormal variation attributable to hCRH. When considered individually, there was no case showing change or severe side effects which could be causally related to hCRH (data not shown). In conclusion, although a number of clinical laboratory parameters showed variations after administration, they were all slight and no side effects of
Clinical importance were observed.

**Discussion**

The usefulness of sheep and human CRH as a diagnostic agent of hypothalamo-pituitary-adrenocortical axis function has been reported in children as well as in adults [6–14]. Although many studies recommend a dosage of children of 1 µg/kg, there have also been reports that dosages from 1 to 2 µg/kg produce no difference in responsiveness [15]. In the present study, therefore, 1.5 µg/kg was adopted to make the dosage coincide with that for adults established in the phase II clinical study [4].

Among children of non-endocrine short stature whose hypothalamo-pituitary-adrenocortical axis was supposed to be normal, the patterns of response of ACTH and cortisol to hCRH were not influenced by pubertal maturation or sex. Moreover, when compared with adults, there was no difference in hormonal response patterns except that cortisol values were slightly more responsive in children than in adults. These results were consistent with those reported by Ross et al. [7] and Attanasio et al. [9], and the 95% confidence limits of baseline and peak values were also comparable to those of adults.

In cases with Cushing’s syndrome due to adrenal tumor, ACTH values were decreased and not responsive, while cortisol values were within normal limits but not responsive to hCRH. The hCRH loading test was useful for diagnosis in this case because, as reported by Muguruza et al. [12], the response pattern indicated that the hypothalamic-pituitary axis was suppressed by cortisol which was being secreted autonomously, even if Co values for both ACTH and cortisol were within normal limits and cortisol values were not necessarily increased.

The change in responsiveness after surgery was also interesting. In Case B, ACTH was increased and cortisol had begun to recover its responsiveness 19 days after surgery; almost normal responses were observed 4 months after. This case required supplemental hydrocortisone but the dosage was low; it was considered that this case represented the process of recovery in the pituitary-adrenal gland axis. In contrast to this, in Case A, which received a high dose of a supplemental therapy, hormonal responses were suppressed by the glucocorticoid administered.

In cases with primary adrenal insufficiency and Prader’s syndrome, cortisol values were decreased and not responsive, while ACTH values were increased and not responsive or excessively responsive to hCRH. In cases with 21a-hydroxylase deficiency, although cortisol values were decreased and not responsive, some ACTH Cmax values were within normal limits; this pattern might suggest the presence of feedback effects by a small amount of glucocorticoid produced endogenously or glucocorticoid administered in steroid therapy. This result did not conflict with the report by Moreira et al. [14] stating that hormonal responses differed according to the degree of steroid synthesis impairment.

Since most of the children with renal disease had impaired function of the pituitary-adrenal axis caused by glucocorticoid therapy, their ACTH and cortisol responses were suppressed. In addition, ACTH and cortisol Cmax values tended to decrease with the increased cumulative dose of prednisolone, suggesting that possibly the degree of suppression in adrenal function might be assessable to some extent (data not shown). It was considered that repeated hCRH tests would be useful in evaluating the recovery of adrenal function in such cases.

In the hCRH test, when compared with the response to the traditionally used insulin tolerance test, ACTH was less responsive but cortisol was comparably responsive. This agreed with the finding reported by Goji [11] and suggested that even an ACTH concentration of 20–40 pg/ml might exert maximum stimulation of the adrenal gland and, therefore, the insulin loading might be too stimulative to serve as a function test.

After the administration of hCRH, facial flushing was observed as a side effect in 8% of the subjects. The incidence was lower than in adults, however, and the sign disappeared completely within 30 min after onset. There were no other side effects of clinical importance.

The present study demonstrated that the function of the hypothalamo-pituitary-adrenocortical axis can be assessed on the basis of the distribution of Cmax values, in particular, of ACTH and cortisol, in comparison with the frame of 95% confidence limits, and that the hCRH test was useful in differential diagnosis of various children’s disorders of the axis.
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References


