Nagoya J. Med. Sci. **58.** 143 ~ 147, 1995

A CASE OF PRIMARY GLUCOCORTICOID RESISTANCE

AKITOSHI KAWAKUBO[1], ATSUSHI SUZUKI[2], HISASHI YOKOI[1], SATOSHI KAKIYA[1], MITSUYA MORIKAWA[3], YUTAKA OISO[2] and MASAHIRO YAMAMOTO[1]

[1]Department of Internal Medicine and [3]Department of Dermatology, Anjo Kosei Hospital, Anjo, Aichi 446, and [2]First Department of Internal Medicine, Nagoya University School of Medicine, Nagoya 466, Japan

ABSTRACT

A 79-year-old woman developed hypokalemia and metabolic alkalosis after breast cancer surgery. She was suspected of having primary glucocorticoid resistance on the basis of high plasma ACTH and serum cortisol levels without the features of Cushing's syndrome. To clarify the end-organ resistance to cortisol, we characterized the glucocorticoid receptors (GR) in cultured skin fibroblasts from the patient. The GRs in whole cell assays decreased binding affinity (Kd=11.1 ± 0.6 nM) and the number of binding sites for [^{3}H]dexamethasone (binding capacity was 15,600 ± 1,255 sites per cell). These results strongly suggest that our patient had primary glucocorticoid resistance caused both by a decreased number of GRs and a reduction in the affinity of GRs to cortisol.

Key Words: Dexamethasone, Glucocorticoid resistance, Hypokalemia.

INTRODUCTION

Primary glucocorticoid resistance is characterized by hypercortisolism without the features of Cushing's syndrome, which was first described in 1976.[1)] Clinical manifestations are usually absent; however, when present, they are caused by overproduction of nonglucocorticoid adrenal steroids, resulting in hypertension and hypokalemic alkalosis,[1–3)] isosexual precocity in boys[4)] and hirsutism in women.[5,6)] It has been reported that this resistance is due to a decreased number of glucocorticoid receptors (GR),[5–7)] decreased binding affinity of glucocorticoid to GR,[4,6,8–11)] instability of GR,[11)] decreased binding of the GR complex to DNA,[9)] and thermolability of GR.[12)]

In this study, we report a case of a woman with primary glucocorticoid resistance, in whom end-organ insensitivity to cortisol was caused by a decrease in both the number of GRs and the binding affinity of glucocorticoid to GR.

METHODS

Dexamethasone binding to GR in cultured fibroblasts

A fibroblast strain was established from skin specimens obtained by a punch biopsy taken from the patient's right shoulder (4-mm diameter). The specimens were minced in 90-mm diameter dishes in 10 ml of Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS), and maintained at 37°C in a humidified atmosphere of 5% CO_2/95%

Correspondence: Dr. Akitoshi Kawakubo, M.D., Department of Internal Medicine, Anjo Kosei Hospital, 12–38 Miyukihon-machi, Anjo, Aichi 446, Japan

air. The cells (5×10^4) were seeded into 35-mm diameter dishes in 2 ml of DMEM containing 10% FCS. The medium was exchanged every 3 days. After confluency, the medium was exchanged for 2 ml of DMEM and the cells were used for experiments after 48 h. The cultured cells were subjected to the binding assay, essentially as described,[12] with a minor modification. In brief, the cells were incubated in 1 ml of HEPES-buffered DMEM (pH 7.4) containing various doses of [6,7-^{3}H]dexamethasone (47.9 Ci/mmol; Dupont/New England Nuclear, Boston, MA, U.S.A.) with or without a 500-fold molar excess of nonradioactive dexamethasone (Sigma Chemical Co., St. Louis, MO, U.S.A.) at 20°C for 2 h. The binding assay was done 3 times. Binding capacity, expressed as the number of binding sites per cell, and the apparent dissociation constant were calculated according to Scatchard.[13] To assess the number of cultured cells, the cells were detached by 0.5% trypsin with 0.53 mM EDTA. The cell number was then determined by direct counting with a hemocytemeter. The radioactivity of ^{3}H-samples was determined with a Beckman LS-6000IC liquid scintillation spectrometer. All data are presented as the mean ± S.D. of triplicate determinations. The patient gave informed consent to the test.

CASE REPORT

A 79-year-old woman presented with a left breast nodule and eczema in December 1992. She had a total hysterectomy for myoma of the uterus in 1956 and a ventrico-peritoneal shunt for a cerebellum tumor in 1986, both without complications. She had two children, but another 2 pregnancies had ended in spontaneous abortion.

She was diagnosed as having breast cancer and was admitted to our hospital in June 1993. On admission, her body weight was 48.0 kg and her height was 145 cm. She had slight facial hypertricosis, especially above her upper lip; however, she showed none of the features of Cushing's syndrome such as truncal obesity, plethoric moon face, acne, buffalo hump, purple striae or emotional disturbance. Her blood pressure was normal and routine laboratory tests during the preoperative period yielded normal results. She underwent a mastectomy in June 1993, and was well after the operation except for hypokalemia and alkalosis. Blood chemistry showed serum sodium at 145 mmol/L, potassium at 2.6 mmol/L and chloride at 98 mmol/L; blood gas analysis showed arterial blood with a pH of 7.46, PCO_2 of 6.8 kPa, HCO_3^- of 36.5 mmol/L and base excess of 11.7 mmol/L. Subsequent studies revealed elevated levels of plasma adrenocorticotropic hormone (ACTH) to 27 pmol/L (normal: <13), serum cortisol to 920 nmol/L (normal: 160—540), 11-hydroxycorticosteroid (OHCS) to 1970 nmol/L (normal: 190—630), corticosterone to 36 nmol/L (normal: 1—23), and increased urinary 17-OHCS levels to 43, 64 and 91 μmol/day (normal: 6—20). Plasma renin activity was undetectable, and serum aldosterone was within the normal range (120 pmol/L; normal: <500). Her serum testosterone level of 2.2 nmol/L was slightly high (normal: <2.0), although her 24-h urinary 17-ketosteroids level was within the normal range.

Computed tomography scans (CT) and magnetic resonance imaging (MRI) of the pituitary gland and hypothalamus were normal, and no adrenal mass was demonstrated by abdominal CT. She was normotensive throughout her clinical course.

To test the hypothesis that her hypercortisolism was not due to Cushing's syndrome, the diurnal variation in plasma ACTH and serum cortisol (and the responses of these variables to corticotropin releasing hormone (CRH) and dexamethasone) was determined. ACTH and cortisol levels were elevated and diurnal rhythms had vanished (Table 1). The administration of 100 μg CRH failed to affect ACTH and cortisol levels (basal value of ACTH was 34 pmol/L and that of cortisol was 1,980 nmol/L; peak value of ACTH was 40 pmol/L and that of cortisol was

Table 1. The Diurnal Rhythm of Circulating ACTH and Cortisol in a Patient with Glucocorticoid Resistance.

	0000h	0600h	1200h	1800h
ACTH (< 13 pmol/L[a)])	25	39	35	29
Cortisol (160–540 nmol/L[a)])	1210	1550	1560	1710

[a)] normal range

2,020 nmol/L, which were obtained at 60 min after the CRH injection). In addition, cortisol levels were not suppressed by 1 and 8mg dexamethasone suppression tests (control, 1080 nmol/L; 1 mg dexamethasone, 1030 nmol/L; 8 mg dexamethasone, 1340 nmol/L).[14,15]

Scatchard analysis of [^{3}H]dexamethasone binding to the GR of cultured fibroblasts demonstrated a single class of receptors, with a Kd of 11.1 ± 0.6 nM. The receptor-binding capacity was 15,600 ± 1,255 sites per cell (Fig. 1). The receptor binding affinity of our patient was decreased, as was binding capacity compared with the previously reported value in cultured skin

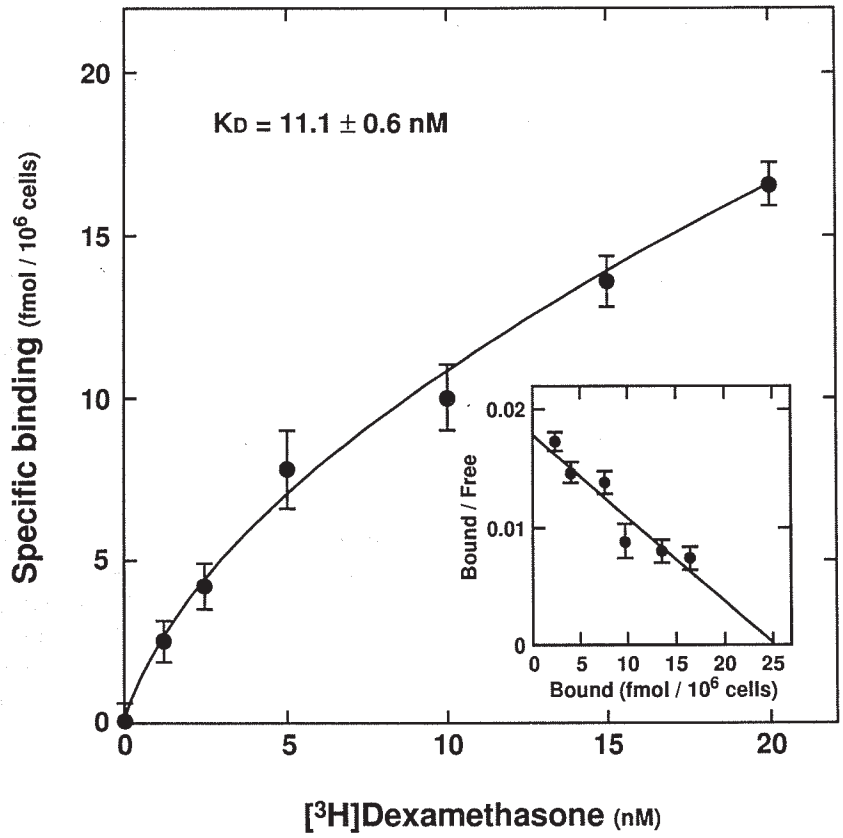

Fig. 1. Binding of [^{3}H]dexamethasone to glucocorticoid receptor in cultured fibroblasts from the patient. The cells were incubated with various concentrations of [^{3}H]dexamethasone at 20°C for 2 h, as described in *METHODS*. *Inset*, Scatchard analysis of the binding data.

fibroblasts from normal subjects (the average binding capacity of normal controls was 103,800 ± 16,144 per cell; the Kd of normal controls was 5.0 ± 1.0 nM; all data are expressed as mean ± S.D.).[9–12]

The hypokalemia and alkalosis did not worsen without treatment. However, the patient developed multiple organ failure and died in July 1993.

We also examined the family of the patient. She had 6 siblings, but 3 of them and her parents were already dead. We were able to study her 3 living siblings, all of whom had normal plasma ACTH and cortisol levels.

DISCUSSION

Primary glucocorticoid resistance is a rare disorder characterized by hypercortisolism without other clinical or biochemical features of Cushing's syndrome. As far as we know, this syndrome has only been reported in a total of 35 patients (five families and 14 individuals). In this report, we described a case of primary glucocorticoid resistance with hypokalemia and alkalosis discovered after breast cancer surgery. We demonstrated that our patient had hypercortisolemia; high plasma ACTH, 11-OHCS and corticosterone levels; and an increased urinary 17-OHCS level without any symptoms or signs of Cushing's syndrome. We also showed that ACTH and cortisol levels were elevated. The diurnal rhythms of both ACTH and cortisol were absent. ACTH and cortisol did not respond to stimulation by CRH, as the basal levels of ACTH and cortisol were high. Cortisol was not suppressed by dexamethasone. These findings strongly suggest that our patient had primary glucocorticoid resistance. Her serum potassium level and blood gas analysis were normal preoperatively; however, it has been reported that the routine laboratory data of patients with glucocorticoid resistance may fluctuate.[1] Therefore, it is likely that an increase in cortisol demand induced by the operation uncovered the glucocorticoid resistance of our patient.

To clarify the mechanism of end-organ resistance to cortisol, we characterized the GR of cultured skin fibroblasts from our patient. The Kd value was 11.1 ± 0.6 nM, and the number of binding sites was 15,600 ± 1,255 per cell. Both the receptor binding affinity and the binding capacity of our patient were decreased compared with previously reported values in cultured skin fibroblasts from normal subjects.[9–12] Therefore, our findings suggest that our patient had primary glucocorticoid resistance caused by both a decreased number of GRs and reduced affinity of GR to glucocorticoid. It has been reported that glucocorticoid resistance is due to a disorder of GR numbers or in the binding affinity of glucocorticoid to GR.[4–11] However, as far as we know, glucocorticoid resistance resulting from both lowered affinity and a decreased number of GRs has been reported in only 2 cases.[6,10] Although the relationship between the clinical presentation and the features of end-organ resistance is still unclear, it is probable that severe resistance to high-dose (8 mg) dexamethasone is due to both a decrease in the number of GRs and lowered affinity of GR to glucocorticoid. Glucocorticoid resistance occurs sporadically and in familialies.[1–12] Since our patient's siblings had normal laboratory data and did not display hypercortisolemia, it is likely that her case was sporadic.

In conclusion, our patient had primary glucocorticoid resistance, in which end-organ insensitivity to cortisol was due to decreases in both the number of GRs and the binding affinity of glucocorticoid to GR.

REFERENCES

1) Vingerhoeds, A.C.M., Thijissen, J.H.H. and Schwarz, F.: Spontaneous hypercortisolism without Cushing's syndrome. *J. Clin. Endocrinol. Metab.*, 43, 1128–1133 (1976).
2) Chrousos, G.P., Vingerhoeds, A.C.M., Loriaux, D.L. and Lipsett, M.B.: Primary cortisol resistance: a family study. *J. Clin. Endocrinol. Metab.*, 56, 1243–1245 (1983).
3) Lamberts, S.W.J., Poldermans, D., Zweens, M. and de Jong, F.H.: Familial cortisol resistance: differential diagnostic and therapeutic aspects. *J. Clin. Endocrinol. Metab.*, 63, 1328–1333 (1986).
4) Malchoff, C.D., Javier, E.C., Malchoff, D.M., Martin, T., Rogol, A., Brandon, D., Loriaux, D.L. and Reardon, G.E.: Primary cortisol resistance presenting as isosexual precocity. *J. Clin. Endocrinol. Metab.*, 70, 503–507 (1990).
5) Vecsei, P., Frank, K., Haack, D., Heinze, V., Ho, A.D., Honour, J.W., Lewicka, S., Schoosch, M. and Ziegler, R.: Primary glucocorticoid receptor defect with likely familial involvement. *Cancer Res.*, 49 (suppl.), 2220s–2221s (1989).
6) Lamberts, S.W.J., Koper, J.W., Biemond, P., den Holder, F.H. and de Jong, F.H.: Cortisol receptor resistance: The variability of its clinical presentation and response to treatment. *J. Clin. Endocrinol. Metab.*, 74, 313–321 (1992).
7) Kontula, K., Pelkonen, R., Andersson, L. and Sivula, A.: Glucocorticoid receptors in adrenocorticoid disorders. *J. Clin. Endocrinol. Metab.*, 51, 654–657 (1980).
8) Iida, S., Gomi, M., Moriwaki, K., Itoh, Y., Hirobe, K., Matsuzawa, Y., Katagiri, S., Yonezawa, T. and Tarui, S.: Primary cortisol resistance accompanied by a reduction in glucocorticoid receptors in two members of the same family. *J. Clin. Endocrinol. Metab.*, 60, 967–971 (1985).
9) Nawata, H., Sekiya, K., Higuchi, K., Kato, K.I. and Ibayashi, H.: Decreased deoxyribonucleic acid binding of glucocorticoid-receptor complex in cultured skin fibroblasts from a patient with the glucocorticoid resistance syndrome. *J. Clin. Endocrinol. Metab.*, 65, 219–226 (1987).
10) Werner, S., Thoren, M., Gustafsson, J.Å. and Brönnegård, M.: Glucocorticoid receptor abnormalities in fibroblasts from patients with idiopathic resistance to dexamethasone diagnosed when evaluated for adrenocortical disorders. *J. Clin. Endocrinol. Metab.*, 75, 1005–1009 (1992).
11) Chrousos, G.P., Vingerhoeds, A., Brandon, D., Eil, C., Pugeat, M., DeVroede, M., Loriaux, D.L. and Lipsett, M.B.: Primary cortisol resistance in man: A glucocorticoid receptor-mediated disease. *J. Clin. Invest.*, 69, 1261–1269 (1982).
12) Brönnegård, M., Werner, S. and Gustafsson, J.Å.: Primary cortisol resistance associated with a thermolabile glucocorticoid receptor in a patient with fatigue as the only symptom. *J. Clin. Invest.*, 78, 1270–1278 (1986).
13) Scatchard, G.: The attractions of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.*, 51, 660–672 (1949).
14) Nugent, C.A., Nichols, T. and Tyler, F.H.: Diagnosis of Cushing's syndrome. *Arch. Intern. Med.*, 116, 172–176 (1965).
15) Liddle, G.W.: Tests of pituitary-adrenal suppressibility in the diagnosis of Cushing's syndrome. *J. Clin. Endocrinol. Metab.*, 20, 1539–1560 (1960).