An Adrenocortical Tumor Secreting Weak Mineralocorticoids

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Abstract

An adrenocortical carcinoma (15.5 g) secreting excessive amounts of steroids with weak mineralocorticoid activity in a 25-year-old woman was studied with particular reference to its in vivo and in vitro secretions of steroids. Severe hypertension, occasional low serum potassium and suppressed PRA were the major clinical findings, and were improved with removal of the tumor. In the preoperative stage, plasma levels of 11-deoxycorticosterone, 18-hydroxy-11-deoxycorticosterone, corticosterone and 18-hydroxy corticosterone were all increased. However, the plasma level of aldosterone was repeatedly normal. Although plasma levels of pregnenolone, 17-hydroxypregnenolone, progesterone and 17-hydroxyprogesterone were very high, those of other late step steroids, i.e. 11-deoxycortisol, cortisol, dehydroepiandrosterone, androstenedione and testosterone were almost normal. From these findings, a major etiological role of weak mineralocorticoids such as 11-deoxycorticosterone, 18-hydroxycorticosterone and corticosterone in her hypertension was suggested. Pregnenolone and 17-hydroxypregnenolone in tumor tissue were increased, but 11-deoxycorticosterone, corticosterone, aldosterone, cortisol and adrenal androgens such as dehydroepiandrosterone, androstenedione and testosterone were below normal or low normal. In vitro production of 11-deoxycorticosterone, aldosterone or cortisol by the tumor tissue slices was very low and scarcely responded to synthetic ACTH.

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An adrenal tumor with clinical signs of mineralocorticoid excess but no evidence of aldosterone hypersecretion is very rare. Only sporadic case reports of adrenocortical carcinoma with hypersecretion of 11-deoxycorticosterone (DOC) (Powell-Jackson et al., 1974; Devies et al., 1980) or corticosterone (B) (Fraser et al., 1968) and of adenoma excessively producing DOC (Kondo et al., 1976) or B (Mills et al., 1980) were documented. The present paper concerns in vivo and in vitro studies of steroidogenesis in a case with hypertension caused by an adrenocortical carcinoma producing exces-
sive amounts of DOC, 18-hydroxy-DOC, B and 18-hydroxy-B.

Case Report

A 25-year-old Japanese woman was admitted to Gifu University Hospital in June, 1978 because of hypertension. Five years before admission hypertension (220/150 mmHg) was first noted. On admission, her blood pressure was 190/124 mmHg, but the other physical examination results were negative. Laboratory data were as follows: ECG showed left ventricular hypertrophy and U waves in leads V₂—V₄. Serum sodium ranged 140 to 144 mEq/l (mean 142, in 12 estimations), potassium 3.4 to 4.1 mEq/l (mean 3.7, in 12 estimations) and chloride was 103 mEq/l. Potassium clearance was normal (12.0 ml/min) but increased to 33.2 ml/min after an intravenous administration of sodium thiosulfate (normal; below 30 ml/min (Abe, 1964)). Arterial pH showed mild alkalosis (pH 7.45) and base excess was +2.8 mEq/l. Basal PRA was low (0.1 ng/ml/h; normal range 0.3 to 3.2) and failed to respond to 2 h upright posture after an intravenous administration of 1 mg/kg furosemide (0.2 ng/ml/h; normal range 1.4 to 8.3). PRA responded slightly from 0.1 to 2.2 ng/ml/h to severe stimulation tests such as a combination of 2 h upright position after an intravenous administration of 1 mg/kg furosemide under sodium restriction (Na: below 30 mEq/day) and oral administration of 40 mg furosemide a day for 3 days. Plasma levels of aldosterone were always normal, but those of weak mineralocorticoids including DOC were markedly elevated. These data will be described later in detail. Adrenal scintiscanning with dexamethasone pretreatment demonstrated an enlarged left

Fig. 1. Histology of adrenocortical carcinoma: Part of the nodular proliferation of atypical small cells arranged in an alveolar or ribbon pattern. Insert is a high power view of atypical tumor cells with a mitotic figure (Hematoxylin and eosin stain, ×40 & ×160).
adrenal mass. Computed tomography revealed a round mass with a smooth margin in the left adrenal region (3.5×3.0×3.5 cm). Plasma DOC in the left renal vein was 1,900 ng/dl, while it was 260 ng/dl, 260 ng/dl and 140 ng/dl, in the right renal vein, and upper and lower parts of the inferior vena cava, respectively. Based on these findings, a left adrenal tumor secreting weak mineralocorticoids was suggested.

On laparotomy performed in January, 1979, a left adrenal tumor was removed. Her blood pressure and serum potassium became normal in 6 weeks after the operation. In 3 months after the operation, basal PRA recovered and responded normally to 2 h upright position after an intravenous administration of furosemide (1 mg/kg) from 1.8 to 3.9 ng/ml/h. At present, 7 years after the operation, she is well with normal blood pressure (130/86 mmHg) and normal serum potassium (4.6 mEq/l).

The tumor measured 3.5×3.0×3.5 cm and weighed 15.5 g. The cut surface, yellowish brown in color, contained several nodules and a central fibrotic lesion. Histologically (Fig. 1), the tumor was mainly composed of medium-sized cells with finely granular cytoplasm which were arranged in trabecular and alveolar structures. Sporadic clear cells were intermingled. Slight nuclear and cellular pleomorphism was observed. The nodular lesions were composed of small dark cells with hyperchromatic nuclei and occasional mitotic figures. Hemorrhage, necrosis and capsular invasion were not conspicuous, but there was a little evident vascular invasion. Histological diagnosis was differentiated adrenocortical carcinoma. Electronmicroscopically (Fig. 2), the tumor

Fig. 2. Electronmicroscopic findings of tumor: Tumor cells have an irregularly shaped nucleus and rough-surfaced endoplasmic reticulum and were predominant in elongated mitochondria. Spherical mitochondria with tubulo-vesicular cristae and smooth-surfaced endoplasmic reticula are observable (×5,600).
cells possessed large round or ovoid nuclei with well developed nucleoli. Dark cells of irregular shape with high, homogenous electron densities occasionally contained filamentous structures. Mitochondria were numerous and generally round or tubular in shape with poorly developed cristae. Large, round mitochondria with well developed lamellar cristae were rarely detected. Ribosomes were well developed and scattered smooth-surfaced endoplasmic reticula were seen. Golgi apparatus, lipid droplets and dense bodies were less.

Materials and Methods

Steroid hormone assay in plasma: Plasma levels of the following 15 steroids, i.e. pregnenolone, progesterone, DOC, B, 18-hydroxy-DOC, 18-hydroxy-B, aldosterone, 17-hydroxypregnenolone, 17-hydroxyprogesterone, 11-deoxycortisol, cortisol, dehydroepiandrosterone (DHEA), androstenedione, testosterone and estradiol were measured by previously reported specific radioimmunoassays (Ojima, 1974; Ojima et al., 1975; Ojima et al., 1980). Briefly, an aliquot of 0.5-2.0 ml of plasma was added to a tube containing tritiated steroids (about 30,000 dpm), extracted with dichloromethane, dried and applied to a Sephadex LH-20 column. The column size and solvent system (v/v) were as follows: a 0.7×6.0 cm column was used for pregnenolone, 17-hydroxypregnenolone, progesterone, 17-hydroxyprogesterone (benzene 95: methanol 5), estradiol (benzene 85: methanol 15), DHEA, androstenedione and testosterone (hexane 80: benzene 10: methanol 10), and 1×20 cm column for cortisol, B, 11-deoxycortisol (dichloromethane 99: ethanol 1), and 1×55 cm column for DOC, aldosterone, 18-hydroxy-DOC and 18-hydroxy-B (distilled water). The appropriate eluted fractions were collected and an aliquot of them was counted for the estimation of recovery, and the other aliquot was analyzed for steroid content by radioimmunoassay. The plasma concentrations of DOC, aldosterone and cortisol marked as ◆ in Tables 1 and 2 were measured by the method of Tochigi et al. (1976), and with radioimmunoassay kits using $^{125}$I-aldosterone (Commissariat a l'Energie Atomique, France) or $^{125}$I-cortisol (Daichi RI Laboratory, Tokyo, Japan), respectively.

Steroid concentration in tumor tissue: A portion of tumor tissue was finely sliced and homogenized in Krebs-Ringer bicarbonate buffer (pH 7.4) with a loose-fitting Teflon glass homogenizer. Steroids in homogenate were extracted with 10 vol of dichloromethane, and measured by radioimmunoassay as mentioned above. The normal adrenocortical tissues surgically resected for the treatment of three patients with breast cancer were used for the control.

In vitro steroid production by tumor tissue: Finely sliced tumor tissues, weighing about 100 to 200 mg, were suspended in a total volume of 30-50 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 200 mg/dl glucose, and incubated with or without $10^{-8}$ M synthetic $^{1-24}$ACTH at 37°C for 2 h under a 95% O₂ and 5% CO₂ gas mixture. After the incubation, aldosterone, DOC and cortisol released in the medium were measured by radioimmunoassay after extraction with dichloromethane. Similar in vitro studies were performed on the adrenal cortex adjacent to the tumor, and the control adrenal cortices obtained from six patients with breast cancer.

Results

A. In vivo steroid study

Preoperative stage (Table 1, A))

Plasma levels of pregnenolone, DOC, 18-hydroxy-DOC, B and 18-hydroxy-B were markedly higher than those of normal subjects. Plasma levels of aldosterone were repeatedly normal. Plasma concentrations of progesterone and 17-hydroxyprogesterone were clearly elevated. Plasma levels of 11-deoxycortisol and cortisol were within the normal range. Urinary excretions of 17-OHCS were also normal (4.8 mg/dl; normal range 2.9 to 9.8). An oral administration of 1 mg dexamethasone at 2100 h lowered the plasma cortisol level from 5.5 to 2.7 μg/dl at 0900 h the next day. Plasma DHEA, androstenedione, testosterone and estradiol were all within the normal range. Urinary excretions of 17-KS were also normal (3.7 mg/day; normal range 3.0 to 16.0). An
intramuscular injection of 1 mg/day $^{1-24}$ACTH-Z for 3 days induced a rise in the plasma levels of cortisol from 10.5 to 32.5 μg/dl but scarce aldosterone response from 11.0 to 15.0 ng/dl. Plasma DOC measured by the method of Tochigi et al. (1976) increased from 79 to 269 ng/dl on the morning of the 3rd day, but the result did not clearly demonstrate that the change is dependent on ACTH because of the marked daily fluctuation in basal plasma DOC levels ranged from 37 to 244 ng/dl (mean 128 ng/dl, in 6 estimations).

Postoperative stage (Table 1, B))

The basal level of plasma DOC or aldosterone 3 months after the operation was low normal (1.0 ng/dl) or low (2.3 ng/dl), and both scarcely responded to an intramuscular injection of 1 mg/day synthetic $^{1-24}$ACTH-Z for 3 days (DOC: 7.0 ng/dl, aldosterone: 4.9 ng/dl). The basal level (13.1 μg/dl) and the response to ACTH (33.0 μg/dl) of plasma cortisol was normal, as in the preoperative period.

B. Steroid concentrations in tumor tissue (Table 1)

Concentrations of pregnenolone and 17-hydroxyprogrenenolone were markedly high, but those of progesterone and 17-hydroxyprogesterone as well as DOC, B aldosterone and cortisol were markedly or moderately
low in comparison with those in control adrenal cortices. DHEA was moderately low, and androstenedione and testosterone were markedly low.

C. Steroid production by tumor tissue (Table 2)

Basal production of aldosterone, DOC and cortisol by tumor slices was markedly lower than that by control adrenal cortices and slightly responded to a pharmacological dose of synthetic $^{1-24}\text{ACTH}$ ($10^{-8}$ M). Production of these steroids before and after ACTH stimulation by adrenal cortex adjacent to the tumor was much higher than that from the tumor tissue, but lower than that by normal adrenal cortices.

Discussion

The present tumor weighing 15.5 g was histologically interpreted as a differentiated adrenocortical carcinoma based on the finding of nodular lesions compressing the surrounding tissue. The lesions were composed of small cells with increased basophilia arranged in irregular solid nests and occasional mitotic figures and vascular invasion.

The removal of the tumor with adjacent tissue resulted in the restoration of normal blood pressure and serum potassium. Sustained low or low normal levels of aldosterone or DOC and their poor response to ACTH even 3 months after the operation suggested suppressed preoperative secretion of these steroids by the contralateral adrenal.

Normal suppressibility of plasma cortisol by dexamethasone suggested that cortisol was secreted normally even in the preoperative period mainly from the contralateral adrenal gland and/or from the adjacent tissue surrounding the tumor.

The normal plasma aldosterone level in the preoperative period in this case is in line with the findings of almost normal preoperative plasma aldosterone levels observed in DOC (Powell-Jackson et al., 1974; Kondo et al., 1976; Davies et al., 1980) or B (Fraser et al., 1968; Mills et al., 1980) producing adrenal tumor reported previously. We could not examine the aldosterone content and/or histological view of the adjacent adrenal tissue, but the basal aldosterone release from it in this case was much less than that in control adrenal tissue, although it was far more than that in tumor tissue. This is comparable with the low postoperative basal plasma aldosterone level, and suggests that the preoperative normal aldosterone level would be mainly due to the aldosterone production from the tumor.

Concerning the responsiveness of aldosterone release to ACTH in the slice experiment on adjacent tissue, a slight response was observed, although it was much less than
that in control adrenal tissue. Generally zona glomerulosa in tissue adjacent to an aldosterone producing adenoma, even when it shows morphologically hyperplasia, is not active in respect to aldosterone secretion (Neville et al., 1982). In fact, aldosterone response to angiotensin II in tissue adjacent to an aldosterone producing adenoma was negligible or absent in the face of considerable response of aldosterone release to ACTH (Brown et al., 1980). Thus, the patient's hypertension and occasional mild hypokalemia with suppressed PRA were found to be induced by the excessive amounts of mineralocorticoids other than aldosterone secreted by the adrenal carcinoma, and as judged from the known relative potencies among these weak mineralocorticoids, DOC may play a primary role in symptoms. However, it is still possible that aldosterone, even though in the normal range, might participate in part in these symptoms.

Cases of adrenal carcinoma with hypermineralocorticoidism are usually associated with concomitant hypersecretion of the other adrenocortical hormones such as glucocorticoids and androgens, and they should be considered to be mixed hypercorticoidism rather than pure hypermineralocorticoidism. Adrenocortical tumors producing DOC (Powell-Jackson et al., 1974; Davies et al., 1980; Kondo et al., 1976) or B (Fraser et al., 1968; Mills et al., 1980), either malignant (Powell-Jackson et al., 1974; Davies et al., 1980; Fraser et al., 1968) or benign (Kondo et al., 1976; Mills et al., 1980), have been documented as hypermineralocorticoidism other than hyperaldosteronism. All these patients had severe hypertension and hypokalemia. A patient with an adrenal carcinoma with a high plasma level of DOC alone among mineralocorticoids reported by Powell-Jackson et al. (1974) excreted large amounts of urinary 17-KGS. In a B producing adrenal carcinoma reported by Fraser et al. (1968), preoperative studies on adrenal androgens and DOC were not performed. In a benign DOC producing adrenal tumor reported by Kondo et al. (1976), plasma B and 18-hydroxy-B were not measured. In the present case, hypersecretion of DOC, 18-hydroxy-DOC, B and 18-hydroxy-B and normal levels of plasma cortisol, urinary 17-OHCS, plasma adrenal androgens and urinary 17-KS confirmed the diagnosis of a rare form of pure hyperweak mineralocorticoidism.

In our case, the results of tumor slice experiments did not always support the in vivo data. We cannot adequately explain this discrepancy: i.e. why were progesterone, 17-hydroxyprogesterone and DOC levels in the tumor low, and why was DOC production by the tumor slice decreased in the condition of high levels of these steroids in plasma. Low efficacy of steroid production which is a well known characteristics of adrenocortical carcinoma, and relatively large tumor size, may have in part contributed to low in vitro steroid concentrations and high plasma levels, respectively. In addition, because adrenal cortical carcinomas were rarely uniform in their structure in general, this would be related to the fact that only a portion of the tumor tissue was used for slice experiments. Further study of similar cases is required.

References


