Primary tumorous aldosteronism due to a black adrenal adenoma: a light and electron microscopic study

IK SIENKOWSKI, RM WATKINS, VER ANDERSON

From the Departments of Morbid Anatomy and Surgery, King’s College Hospital, Denmark Hill, London SE5, and the Department of Histopathology, Dulwich Hospital, East Dulwich Grove, London SE22

SUMMARY A case of primary tumorous aldosteronism due to a black adrenal adenoma in a 37-year-old man is reported. Light microscopy showed the tumour to consist predominantly of compact cells containing intracytoplasmic lipofuscin, accompanied by zona fasciculata and intermediate type cells. Electron microscopy identified compact and interface (inner zona fasciculata) type cells, and also demonstrated spironolactone bodies in a compact cell. The morphological appearance of the tumour and adjacent adrenal gland, combined with the clinical and biochemical findings, supports both its secretory function and its classification as a rare variant of the more common non-pigmented aldosteronoma.

Case report

The patient, a 37-year-old black man, had been hypertensive for seven years and treated with β-blockers and diuretics. On further investigation he was found to be hypokalaemic. The serum aldosterone level was raised (200 nmol/l; NR < 140 nmol/l) and the supine plasma renin activity was subnormal (50 pg/ml/h; NR = 300–1300 pg/ml/h). 75Se-selenomethylcholesterol scanning showed increased uptake by the right adrenal gland (Table 1). A CT scan demonstrated a 2.5 cm x 2.0 cm lesion in the right adrenal gland. The left adrenal appeared normal. Eleven months prior to surgery the patient started treatment with spironolactone 200 mg daily and all other medication was stopped. Blood pressure was well controlled but marked bilateral gynaecomastia developed as a side effect of this therapy. At laparotomy a normal left adrenal gland was identified. The right adrenal was enlarged by the presence of an encapsulated black tumour measuring 2.5 cm x 2.5 cm x 2.0 cm. A standard right adrenalectomy was performed. The patient’s recovery was uneventful and after one year he has remained normotensive requiring no medication.

PATHOLOGY

The specimen consisted of the right adrenal gland 4.0 cm x 2.5 cm x 2.0 cm with a tan coloured...
discord from its partially invested tumour 1 month after initial sectioned routinely postfixed in natal gland and bounded was tumour. Blocks from at transversely and transferred into eosin, and stained with Luxol fast blue and the special stains listed in Table 2. Small blocks up to 0.5 cm × 0.5 cm × 0.1 cm were also taken from the tumour and transferred into Trump's fluid approximately five months after initial receipt of the specimen, postfixed in 1% osmium tetroxide in distilled water and embedded in Polarbed 812 resin. Sections (0.5 μm) were cut and stained with toluidine blue. Thin sections were cut on a Cambridge Huxley Mk II ultramicrotome and examined on a Corinth electron microscope (AEI 275).

Microscopic examination of the adrenal was confined to a distance of 1.8 cm adjacent to the tumour.

LIGHT MICROSCOPY

Sections showed a rounded cellular tumour bounded by a narrow rim of cortical tissue continuous with the adjacent adrenal gland and invested by a fibrous capsule. The rim of cortical tissue was narrower distally and absent for one quarter of the tumour circumference opposite its adrenal attachment. Tumour cells abutted directly onto the adrenal capsule in this region. A few compact cells extended beyond the capsule into the periglandular adipose tissue. The fibrous capsule was poorly developed between the tumour and the adjacent adrenal. The subcapsular rim of adrenal cortex broadly retained normal zonation but zona glomerulosa cells formed hyperplastic clusters which projected into the underlying tumour. Most of the zona glomerulosa cells contained spironolactone bodies.

The tumour comprised compact cells and lipid containing zona fasciculata and intermediate cells (Fig. 2). Compact cells, the commonest, were polygonal and arranged in sheets, cords and alveoli separated by fibrovascular spaces. Nuclei were vesicular, mildly pleomorphic and contained nucleoli and a few inclusions. Mitoses were infrequent (2 per 10 high power fields). Many cells were larger than typical compact cells and a few were bi-, tri- or tetra-nucleate. The cytoplasm was eosinophilic and

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Table 2 Histochemical reactions of pigment granules</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>17Se-selenomethylcholesterol scan</strong></td>
<td><strong>Stain</strong></td>
</tr>
<tr>
<td>Right adrenal</td>
<td>Left adrenal</td>
</tr>
<tr>
<td>7 days</td>
<td>0.45%</td>
</tr>
<tr>
<td>14 days</td>
<td>0.48%</td>
</tr>
<tr>
<td>Normal uptake 0-07-0-3%</td>
<td></td>
</tr>
</tbody>
</table>

- **Stain**
  - Long Ziehl-Neelsen
  - Perls
  - Schiff
  - Periodic acid Schiff
  - Masson-Fontana
  - Giemsa
  - Sudan black
  - Haematoxylin and eosin

- **Control (lipofuscin)**
  - +
  - -

- **Adenoma**
  - +
  - -
Primary aldosteronism due to a black adrenal adenoma

contained fine to coarse reddish-brown granules which stained as late reducing lipofuscin. The zona fasciculata cells possessed vesicular, mildly pleomorphic nuclei and clear cytoplasm with a low nuclear-cytoplasmic ratio. They formed small clusters and cords closely associated with intermediate cells which were smaller with higher nuclear-cytoplasmic ratios. Both types of clear cell also contained small numbers of lipofuscin granules. The tumour also contained several extracellular lipid accumulations and thin-walled blood vessels. There was no evidence of malignancy. Spironolactone bodies were not seen by light microscopy despite staining with Luxol fast blue.

The adrenal gland contained a few cortical micronodules exclusively composed of zona fasciculata cells, the largest measuring 0-14 cm (Fig. 3). Their appearance conformed to the criteria of Ferris et al14 for non-functioning nodules. Extensive prominent diffuse hyperplasia of the zona glomerulosa (Fig. 4) with focal downgrowths was present. Spironolactone bodies which were numerous in the zona glomerulosa cells were also seen in a few zona fasciculata cells. Focal lipid depletion interspersed with compact cells forming a reversion pattern was present.

ELECTRON MICROSCOPY

Tissue preservation was patchy and generally poor due partly to delay in the use of the appropriate fixative. Electron microscopy showed sheets of closely apposed cells bounded by a narrow interstitial space with an intervening basement membrane. The space contained diffuse collagen fibres. One vessel was seen which showed a uniformly thick endothelium. Two cell types, compact and interface (inner zona fasciculata), were identified at the tumour periphery. The compact cells (Fig. 5) contained round indented nuclei with occasional nucleoli. The plasma membrane was moderately folded with numerous microvilli. mitochondria were rounded with tubulovesicular or tubular cristae. Prominent in the cytoplasm were discrete rounded electron dense bodies which also formed aggregates. Slightly fewer than half contained rounded lucencies resembling lipid. Their appearances were consistent with those of lysosomes. Pigment bodies were scant. Stacked and fragmented rough endoplasmic reticulum and free ribosomes were present. Smooth endoplasmic reticulum formed densely packed small vesicles. A single compact cell contained at least three fragmented intracytoplasmic bodies character-

Fig. 2 Photomicrograph of the black adenoma showing sheets of pigmented compact cells interspersed with clusters of clear zona fasciculata and intermediate type cells mainly occupying the upper left and central portions of the picture. Haematoxylin and eosin × 280.
Fig. 3 A portion of a clear cell micronodule in the cortex of the adjacent gland. This was the largest nodule seen and consists exclusively of zona fasciculata cells. The adrenal capsule is visible at the upper margin. Note the minimal nuclear pleomorphism. Haematoxylin and eosin × 441.

Fig. 4 Hyperplasia of the zona glomerulosa of the adjacent adrenal gland. Zona glomerulosa cells adjacent to the capsule have a higher nuclear cytoplasmic ratio than the underlying zona fasciculata cells and contain numerous spironolactone bodies. Haematoxylin and eosin × 700.
Primary aldosteronism due to a black adrenal adenoma

Fig. 5 Electronmicrograph of a compact type cell. Numerous lysosomes, some containing lipid, are present. Microvilli arising from the cell membrane at the upper left can be seen. Mitochondria (see also insert) are tubulo-vesicular.

Discussion

There are several morphological features in the tumour described which are consistent with a secretory function. Some differences from pigmented nodules are shown in Table 3. Most significantly the groups of clear cells interspersed among the compact cells, also observed by Caplan and Virata,11 constitute a heterogeneity of type in keeping with that of non-pigmented adenomas causing aldosteronism.15 These usually consist of zona glomerulosa, zona fasciculata intermediate and compact type cells in varying proportions. Zona glomerulosa cells may be absent, as in this tumour, or sparsely arranged on the periphery.

Spironolactone bodies have been described in zona glomerulosa16,17 and compact cells18 of aldosteronomas following the preoperative administration of spironolactone. They are a specific marker of aldosterone secreting cells occurring when aldosterone biosynthesis is blocked19 and their presence in this tumour therefore supports its secretory function. The observed micronodularity of the adjacent adrenal and the hyperplastic zona glomerulosa have also been described as concomitants of tumorous aldosteronism. An alternative adenomatous source of aldosterone was not found in the portion of adja-
The identity of the compact cells was supported by the tubulovesicular mitochondrial morphology which is also consistent with non-specific secretory function. Tubular cristae were often prominent but a definite tendency to a lamellar arrangement as described by Tsuchiyama et al. and regarded by them as indicating a functional relationship between compact cells and zona glomerulosa cells was not seen in this case. These authors concluded that compact cells were responsible for aldosterone secretion in aldosteronomas on the basis of enzyme histochemistry, the presence of spironolactone bodies and electron microscopic features.

The numerous lysosomes with the staining properties of lipofuscin form a complex with the lipid inclusions which is probably a precursor of pigment bodies. The large number of lysosomes in this particular tumour are probably the result of a defect within them which does not impair secretory function but results in the characteristic dark colour of this and other black adenomas. The incidental nature of this defect is supported by the rarity of the black adenoma compared with the non-pigmented adenoma.

An alternative explanation to the one previously described for the relationship between morphology and function of aldosteronomas is provided by Neville and O'Hare. This is based on the measurement of steroid products of the tumours and of their constituent cells following in vitro culture by high performance liquid chromatography. It proposes that an aldosterone secreting zona glomerulosa cell microadenoma by its expansion alters the adjacent microcirculation and hence hormonal climate of its microenvironment. The result is conversion of some neoplastic zona glomerulosa cells to zona fasciculata cells by way of intermediate cells and finally to compact cells. The steroid products of each cell type tend to promote the sequence and the proportion of zona glomerulosa cells remaining is reduced. This hypothesis would explain the absence or scarcity of the latter cell type in this and other aldosteronomas. There is a concomitant reduction in aldosterone secretion by the tumour which continues to secrete more aldosterone than the hyperplastic zona glomerulosa of the adjacent adrenal. There is no satisfactory explanation for this hyperplasia which is accompanied by a functional atrophy.

Although the predominance of compact cells can be encompassed by the above hypothesis it does not readily explain the presence of spironolactone bodies in them seen in this case and reported by Tsuchiyama et al. Despite the difficulties of interpretation, the morphology of the tumour and the adjacent adrenal as reported in this case confirms the association of
Primary aldosteronism due to a black adrenal adenoma

black adenomas with aldosteronism and supports the current concept that they are a rare variant of the commoner non-pigmented aldosteronoma.

We would like to thank Mr HE Berry and Dr DEH Llewelyn for permission to report this case under their care. We are grateful to Mr G Anderson, Mr K Davies and Mr R Senkus for their assistance with the light and electron photomicrography. Dr M Driver is thanked for her help and encouragement in the preparation of this paper.

References