Spironolactone Inclusions in an Aldosteronoma

DELVER R. CAIN, M.D., ROBERT L. VAN DE VELDE, PH.D., AND STEPHEN J. SHAPIRO, M.D.

Cedars-Sinai Medical Center, Cedars of Lebanon Hospital Division, 4833 Fountain Avenue, Los Angeles, California 90029

ABSTRACT

Cain, Delver R., Van De Velde, Robert, and Shapiro, Stephen J.: Spironolactone inclusions in an aldosteronoma. Am. J. Clin. Pathol. 61: 412-416, 1974. Spironolactone cytoplasmic inclusions were found in an aldosteronoma but not in the adjacent zona glomerulosa of an adrenal gland that was removed because of aldosteronism. Their light and electron microscopic appearance was similar to that of previous reports. No other reports of these inclusions in an aldosteronoma were found in the literature. (Key words: Adrenal, adenoma; Aldosteronoma; Inclusions; Spironolactone; Zona glomerulosa.)

CYTOPLASMIC INCLUSIONS occurring in the zona glomerulosa of the adrenal cortex in patients who have been on spironolactone (Aldactone) medication have been reported.1-3,4 The bodies are found as early as ten days after treatment with spironolactone and persist for as long as four months after the last dose of the drug is given.4 No report of the finding of these inclusions in an aldosteronoma has been found.

Clinical History

A 45-year-old Negro man was admitted with a 10-year history of hypertension that had been controlled with Hydromox, 50 mg. daily, so that his blood pressure remained in the range of 160/100 mm. Hg. In addition, he had had bouts of palpitation, decreased exercise tolerance, tiredness, weakness, and a recent episode of chest pain. His condition was very resistant to potassium replacement; serum potas-

Received May 8, 1973; received revised manuscript August 15, 1973; accepted for publication September 13, 1973.

FIG. 1. Gross specimen with central adenoma protruding superiorly. The cut is off-center from the greatest diameter of the adenoma.
FIG. 2 (upper). Overall view of microscopic section of adenoma and part of adjacent adrenal gland. Lighter-staining areas within tumor contained no inclusions. Darker-staining areas of tumor showed many inclusions. Smaller dark nodule to left of adenoma contained no inclusions. Bouin's fixed. Hematoxylin and eosin. × 8.

FIG. 3 (lower). Photomicrograph of cells from center of adenoma. The inclusions are intracytoplasmic and eosinophilic. Many show a surrounding clear halo. Four of the inclusions are marked by arrows, but there are many in the field. Bouin's fixed. Hematoxylin and eosin. × 800.
renin levels were reported as low. Aldosterone levels in the adrenal venous blood were 135 ng per 100 ml on the left and more than 3,000 ng per 100 ml on the right. The normal range is 2 to 9 ng per 100 ml supine and as much as five times that in the upright position. After injection of angiotensin, blood pressure rose from 160/110 to 200/130 mm Hg during a 40-minute period. Other electrolyte values were: sodium, 139 mEq per 1.; carbon dioxide, 25 mEq per 1.; chloride, 103 mEq per 1. Hemoglobin was 12.0 Gm per 100 ml and leukocyte count was 5,200 per cu. mm., with a normal differential. After removal of the tumor there were minor postoperative complications, but blood pressure and electrolyte values eventually stabilized in the normal ranges. Prior to surgery, for a period of 28 days, the patient had received 400 mg of spironolactone daily, giving a total preoperative dose of 11,700 mg. He had also been taking Aldomet at various times, with dosages of 250 mg four times daily.

Materials and Methods

The specimen removed consisted of a 9.5-Gm. right adrenal gland with 7 Gm. of periadrenal fat. Within the gland was a 1.6 x 1.0 x 0.7 cm. nodule which protruded from the superior aspect. The cut surface of the nodule bulged slightly and was variegated dark yellow to dark tan. There was no hemorrhage or necrosis. Adjacent adrenal had a yellow cortex with a thickness that varied from 0.1 to 0.2 cm. The thicker portion of the cortex showed a nodular pattern.

Fresh tissue was taken within minutes after surgical resection and fixed in Sym-Collidine-buffered paraformaldehyde (pH 7.4). This was later cut into 1-mm. cubes, postfixed in 1% Sym-Collidine-buffered osmium tetroxide, and dehydrated in a graduated series of acetone. Embedding was done in Epon 812. One-half-micron sections were stained with azure II and methylene blue. Sections for electron microscopy were stained with uranyl acetate followed by lead citrate.

For histologic sections, 10% formalin and Bouin's were used as fixatives. In those sections stained with hematoxylin and eosin, the Bouin's-fixed tissue was superior to the formalin-fixed tissue for identifying and counting the inclusions. The only special stains done on the tissue were periodic acid-Schiff and hematoxylin-phloxine-safranin with a luxol-fast blue counterstain.

Microscopic Findings

The interesting aspect of the histologic examination of the tissue was the occurrence of the spironolactone inclusions only within the aldosteronoma and not in the zona glomerulosa of the adjacent cortex. In addition, the zona glomerulosa was not thickened, as has been reported in adrenal glands that contain spironolactone bodies. Most of the cells of the tumor were granular, but scattered throughout were aggregates of foamy lipid-laden cells that were similar to the cells of the zona fasciculata. There was a thin, poorly-defined capsule, and adjacent adrenal tissue was not compressed.
The inclusions were similar to those in other reports in that they were intracytoplasmic, laminated, and often surrounded by a clear halo. With hematoxylin and eosin, the inclusions were cosinophilic. With hematoxylin-phloxine-safranin and a luxol-fast blue counterstain, the inclusions were medium blue. Generally, there was one inclusion per cell, occasionally two. The sizes ranged from 4 to 18 microns, but most were nearer the larger size. Generally, the inclusions were as large as, or slightly larger than, the nuclei.

We were impressed with the large number of inclusions that were present in the aldosteronoma. Although it is difficult to compare accurately with data from other studies, it appears that the concentration (number of inclusions per 10 high-power fields) in this aldosteronoma is at least three times greater than the concentration of the inclusions in the adrenal glands of any of the patients, except one, in two previous reports. The dose of the spironolactone given in our patient was five times greater (400 mg. daily for 28 days) than the largest dose given to any of those patients, however.

Electron Microscopy

The electron microscopic structure of the inclusions was similar to that of previous reports. They generally had a central lipid core that was surrounded by concentric rings of double-layered membranes. The numbers of rings ranged from four or five in inclusions with large lipid cores to about 30 in those with smaller or absent cores. At the periphery, there was a merging of the outer ring with, and/or a concentration of, the rough endoplasmic reticulum. The mitochondria were generally vesicular, but some showed plate-like cristae, as in the zona glomerulosa.

Comment

It has been suggested that spironolactone inclusions are morphologic manifestations of aldosterone production and also that they may form as a result of blocking action. In either case, the finding of the inclusions only in the aldosteronoma of the tissue removed and not in the adjacent zona glomerulosa would suggest that the adenoma was (1) the sole source of the aldosterone in the patient's blood and/or (2) the only tissue that showed the effects of the spironolactone treatment. The very large number of inclusions found may reflect (1) the intense production of aldosterone by the tumor and/or (2) the high dose of spironolactone (400 mg./day) that this patient received.

References