Inappropriate Secretion of Antidiuretic Hormone in Nude Mice Bearing a Human Bronchogenic Oat Cell Carcinoma

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ABSTRACT

A 58-year-old man with bronchogenic oat cell carcinoma developed a typical syndrome of inappropriate secretion of antidiuretic hormone. The tumor tissue obtained at autopsy had been serially transplanted in nude mice for more than four years with 20 passages. The levels of vasopressin were remarkably increased in the plasma of nude mice bearing this tumor [24.4 ± 18.3 (S.D.) pg/ml, n = 3] as well as in the tumor tissues [134.3 ± 72.2 ng/g, n = 3]. Furthermore, human nicotine-stimulated neurophysin was detected in both plasma and tumor tissues (7.4 ± 3.7 ng/ml, n = 3, and 2.28 ± 0.90 ng/g, n = 3, respectively). On ad libitum intake of water, nude mice bearing this tumor excreted significantly less urine with higher sodium concentration than did controls, but serum sodium concentrations did not differ from those of controls. When tumor-bearing mice were hydrated with 2 ml of water twice a day i.p., their diuretic response was found to be suppressed in parallel with the tumor size. However, these mice did not become hyponatremic because they drank less water. When a larger amount of water was loaded which could not be compensated by restriction of water drinking, serum sodium concentrations were markedly decreased. On the basis of these results, the lung cancer, when transplanted into nude mice, produced and secreted its own antidiuretic hormone, which induced inappropriate secretion of antidiuretic hormone in the mice. These mice may provide a useful experimental model for the study of excessive secretion of antidiuretic hormone and associated pathophysiological disorders.

INTRODUCTION

Since the first description of SIADH in association with malignant neoplasms (16), an increasing amount of circumstantial evidence that ADH is produced by neoplasms has been accumulated. ADH as well as neurophysins, specific proteins associated with posterior pituitary hormones, were demonstrated in the tumor tissues (1, 4, 7–9). However, it remains to be established if these malignant tumors produce and secrete ADH in vivo to induce SIADH.

Nude mice bearing transplants of malignant tumors have been well recognized as a useful system for the study of functioning tumors because functional activities of tumors are well preserved in animals. Moreover, the high concentration of released hormones in the bloodstream remarkably amplifies the host responses. Occasionally, in mice even unexpected humoral factors produced by tumors can be detected. Utilizing this technique, we have identified lung cancer producing granulopoietic factor (2) and malignant melanoma excreting cachexia-producing principles (11). The present paper gives an account of production and secretion of ADH by a lung cancer transplanted into nude mice and also of SIADH induced in the mice.

MATERIALS AND METHODS

Case Report. A 58-year-old man was admitted to Tokyo University Hospital for the examination of cough and fever. Laboratory data revealed severe hyponatremia; serum sodium was 121 mEq/liter and chloride was 87 mEq/liter. Plasma osmolality was consistently low (236 to 250 mOsmol/kg), while plasma ADH levels were elevated (19.2 to 69.8 pg/ml). A water load (20 ml/kg) resulted in a marked antidiuresis. Bronchoscopic examination showed a tumor mass on the fourth to the tenth segmental bronchi of the right lung, and the pathological diagnosis of the biopsied specimen was anaplastic small-cell carcinoma. The biopsied tumor tissue contained 88 milliunits of ADH per g of acetone, dry powder, by bioassay (19). He died of massive hemoptysis. The autopsyed tumor tissue contained 14.4 ng of arginine-vasopressin per g wet tissue and showed positive immunofluorescence with an antivasopressin serum.

Transplantation of the Tumor. Female BALB/c-<nu/><nu> mice weighing about 20 g were distributed at 4 weeks of age by Central Institute for Experimental Animals, Kawasaki, Japan, and maintained in specific-pathogen-free conditions. Each block of 5x5x5 cm of the autopsied tumor tissue was transplanted s.c. to bilateral flanks of 3 nude mice with a trocar. When the transplanted tumors grew large enough, mice were sacrificed by decapitation, and the tumors were aseptically removed for serial transplantation. The blood issuing from the vessels of the trunk was collected in chilled heparinized tubes for the subsequent determination of vasopressin and neurophysins.

Analysis of Water Balance in Nude Mice. Experiments were conducted in nude mice on ad libitum intake of food and water at constant temperature and humidity. Each nude mouse was transferred to a metabolic cage at 4 p.m. and maintained there for the succeeding 24 hr unless otherwise specified. Volumes of urine and drinking water were recorded. At the end of the experiment, nude mice were sacrificed by decapitation, and the trunk blood was collected for the determination of serum sodium concentrations. Hydration was effected by i.p. injection of 2 different doses of distilled water. In the first experiment, 2...
ml of water were injected into control and tumor-bearing mice twice a day (4 p.m. and 10 a.m.). In the second experiment, 2 ml of water were administered 3 times (10 a.m., 1 p.m., and 4 p.m.), and the animals were sacrificed at 6 p.m.

**Light and Electron Microscopy.** The tissue specimens were processed for the routine histopathology, and sections were stained with hematoxylin and eosin. For the electron microscopic study, the tumor tissues were cut into small pieces, fixed with 2% glutaraldehyde and then 1% osmium tetroxide, dehydrated in graded ethanol, and embedded in Epon. Ultrathin sections were doubly stained with uranyl acetate followed by lead citrate.

**Tumor Tissue Extraction.** Tumors extirpated from the nude mice were homogenized in 2 volumes of 0.1 N HCl with a Potter-Elvehjem glass homogenizer. The homogenate was adjusted to pH 1.5 with 1 N HCl followed by centrifugation at 10,000 x g for 15 min. The supernatant was collected and titrated to pH 7 at 4°. White precipitate was removed by centrifugation, and the supernantant was used for the assay of vasopressin, oxytocin, and neurophysins.

**Assay of Vasopressin, Oxytocin, and Neurophysins.** Plasma concentrations as well as tissue contents of vasopressin were estimated by a radioimmunoassay previously described in detail (17). Oxytocin was measured by radioimmunoassay utilizing 125I-labeled synthetic oxytocin (Sandoz, Basel, Switzerland) and an antiserum raised against oxytocin covalently linked to bovine serum albumin. NSN and ESN were radioimmunoassayed according to the general method of Robinson (14) using the immunological materials kindly donated by the National Institute of Arthritis, Metabolism, and Digestive Diseases and the National Pituitary Agency, USPHS. Details of the assay procedure were described elsewhere (18).

**Statistical Analysis.** Comparison of 2 samples were made by an unpaired t test analysis. Linear regression analysis was performed using the least-squares method.

**RESULTS**

In all nude mice bilaterally transplanted with the autopsied tumor tissue, tumor growth was evident on at least one flank within 5 weeks. Serial transplantation was successful in more than 90% of the animals. Nude mice bearing this tumor seemed to be quite healthy, and their body weight gradually increased as tumors grew. Hematoxylin-eosin staining of the tumor tissue transplanted into nude mice showed small round and oat cell carcinoma with histology which was identical to the histology of the tumor at autopsy. The electron microscopic findings in the tumor tissue were similar to that described in previous reports (7, 13). The major portion of the tumor tissue had anaplastic features with few rough-surfaced endoplasmic reticulum and no secretary granules. Only a small number of tumor cells from the limited area possessed secretary granules with developed rough-surfaced endoplasmic reticula.

As shown in Table 1, the mean plasma vasopressin concentration in nude mice bearing the tumor [24.4 ± 18.3 (S.D.) pg/ml] was significantly higher than that in control nude mice [4.6 ± 3.1 pg/ml] (p < 0.05). Moreover, human NSN, which is considered to be synthesized and released concomitantly with vasopressin (5, 14, 15), was detected in the circulation of tumor-bearing mice with the mean plasma concentration of 7.4 ± 3.7 ng/ml (range, 4.2 to 11.4 ng/ml), while it was undetectable in the plasma from all of the 4 control nude mice.

Tumor tissues extirpated from nude mice contained large amount of vasopressin (134.3 ± 72.2 ng/g wet tissue) as well as NSN (2281 ± 898.2 ng/g wet tissue). The results are consistent with the previous reports (7–9, 12) and suggest that vasopressin biosynthesis in the tumor is closely related to that of NSN as demonstrated in the hypothalamus of the experimental animals (5, 6, 15). Tumor contained also a small amount of oxytocin (1.26 ± 0.03 ng/g wet tissue) and ESN (12.35 ± 1.91 ng/g wet tissue). ESN thus determined may be overestimated in view of the cross-reaction of a large amount of NSN in ESN radioimmunoassay (14). A control tumor tissue (a human malignant melanoma transplanted into a nude mouse contained no detectable amount of vasopressin, NSN, and ESN (Table 1). The amount of vasopressin in tumor varied considerably from tissue to tissue, but no diminution in vasopressin activity was observed during serial transplantation of the tumor.

The results of the water balance study in nude mice are shown in Table 2. When water was allowed ad libitum, no significant difference was observed in the mean volumes of drinking water for control and tumor-bearing mice. However, tumor-bearing mice excreted significantly less urine with a higher sodium concentration than did controls. When 2 ml of water were administered i.p. twice a day, control nude mice excreted significantly more urine (2.4 ± 0.4 ml/day) with a

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* a p < 0.05 compared to control.
* b Mean ± S.D.
* c ND, not determined.
* d A human malignant melanoma transplanted into a nude mouse.
lower sodium concentration (87.1 ± 16.8 mEq/liter) than those in the control study (1.1 ± 0.4 ml/day, 170.6 ± 42.3 mEq/liter). In tumor-bearing mice, urine volume as well as urinary sodium concentration was not changed significantly after the water load. The volume of drinking water, on the other hand, was significantly decreased after the water load in tumor-bearing mice but not in controls. In these experiments, no significant difference was obtained in the mean serum sodium concentrations for control and tumor-bearing mice. A larger volume of drinking water (6.3 ± 1.1 ml/day, 170.6 ± 42.3 mEq/liter) was administered i.p. to both control and tumor-bearing mice twice a day. The coefficient of correlation calculated in their study. Furthermore, the host response to the ectopically produced ADH was not established. In this study, we described successful transplantation of a vasopressin-producing human bronchogenic oat cell carcinoma into nude mice. Whether ADH secreted from the tumors circulated in nude mice was not determined in their study. Furthermore, the host response to the ectopically produced ADH was not established. In this report, we described successful transplantation of a vasopressin-producing human bronchogenic oat cell carcinoma into nude mice, which has been maintained more than 4 years with 20 passages. High levels of vasopressin and also human NSN were detected in the plasma of tumor-bearing animals as well as in tumor tissues removed from mice. Moreover, it was demonstrated that the transplanted tumor could induce SIADH in mice.

Although the foregoing results indicate that the tumor transplanted into nude mice does produce and secrete ADH (Table 1), these mice did not develop hyponatremia on ad libitum water intake (Table 2). When 2 ml of water were loaded twice a day, a remarkable diuresis was observed in control mice. In tumor-bearing mice, on the other hand, the same maneuver resulted in impaired diuresis. In addition, the magnitude of suppressed diuresis was significantly correlated to the tumor size (Chart 1). However, serum sodium concentrations determined 6 hr after the second injection were not decreased. Of importance in this regard is the fact that drinking water was significantly less in tumor-bearing mice than in controls (Table 2). These results suggest that ADH produced by the tumor suppressed diuretic response but the resulting positive water balance was compensated by self-restriction of water drinking and that ingested water was mainly lost through the skin and the lung, which is not directly regulated by ADH. The thirst center may be more sensitive in mice than in humans, which results in self-restriction of water intake in response to a minute decrease in plasma osmolality. Habitual water drinking, in fact, is known to be an important trigger for the development of SIADH in nude mice bearing human lung cancer. A larger volume of drinking water (6.3 ± 1.1 ml/day, 170.6 ± 42.3 mEq/liter) was administered i.p. to both control and tumor-bearing mice twice a day. The coefficient of correlation (p < 0.01) between these 2 variables.

**DISCUSSION**

SIADH is frequently encountered in patients with malignant neoplasms and is clinically characterized by hyponatremia and antidiuresis (3). It has not been shown, however, that a neoplasm removed from a patient with SIADH was successfully transplanted to experimental animals to develop SIADH. Kamaya et al. (10) detected ADH ranging from 3.8 microunits to 5.8 milliunits per g in tumor tissues from 3 bronchogenic oat cell carcinomas transplanted into nude mice. Whether ADH secreted from the tumors circulated in nude mice was not determined in their study. Furthermore, the host response to the ectopically produced ADH was not established. In this report, we described successful transplantation of a vasopressin-producing human bronchogenic oat cell carcinoma into nude mice, which has been maintained more than 4 years with 20 passages. High levels of vasopressin and also human NSN were detected in the plasma of tumor-bearing animals as well as in tumor tissues removed from mice. Moreover, it was demonstrated that the transplanted tumor could induce SIADH in mice.

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hyponatremia in cancer patients.

If our assumption is correct, then a typical SIADH may develop in these tumor-bearing mice by forced water intake of more than 6 ml/day, because water excretion through the skin and the lung may not be able to compensate such a large water ingestion. In an attempt to test this possibility, we performed the second experiment in which 2 ml of water were administered i.p. to control and tumor-bearing mice 3 times in 8 hr. As expected, a remarkable decrease in serum sodium concentration appeared only in tumor-bearing mice. It was concluded from these results that inappropriate secretion of ADH alone is not sufficient for the appearance of hyponatremia in nude mice and that a large amount of forced water intake or disturbed thirst center, in addition, may be required for the development of hyponatremia. This lung cancer transplanted into nude mice may provide a useful experimental tool for the investigation of synthesis of ADH as well as a good experimental model for SIADH.

ACKNOWLEDGMENTS

We are grateful to Drs. N. Aoki and N. Urano (Department of Pathology, Faculty of Medicine, University of Tokyo) for their comments on pathology. We are also indebted to Dr. A. Urabe (The Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo) for his encouragement throughout the work.

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