

Brief Report

HYPOGLYCEMIA DUE TO AN INSULIN-SECRETING SMALL-CELL CARCINOMA OF THE CERVIX

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HYPOGLYCEMIA is a condition commonly seen in the emergency department and is usually caused by insulin or sulfonylurea therapy for diabetes mellitus. Tumor-induced hypoglycemia occurs more rarely and can involve several mechanisms, according to whether the tumor is of pancreatic islet-cell origin or of extrapancreatic, non-islet-cell origin.¹ The pancreatic islet beta-cell tumors (insulinomas) cause hypoglycemia by producing excessive insulin. In contrast, non-islet-cell tumors can cause hypoglycemia in any of several ways. They include release by the tumor of insulin-like growth factor II or its high-molecular-weight precursor,²⁻⁴ multiple metastases to the liver, massive tumor burden, or rarely, the production of autoantibodies to insulin or its receptor.⁵⁻⁷ However, there is considerable debate over whether non-islet-cell tumors can secrete insulin.¹

Small-cell cancers arise most commonly in the lung but can occur at many sites in the body, including the uterine cervix.^{8,9} Their origin is uncertain, but they all have a neuroendocrine phenotype characterized by the presence of neurosecretory granules

that contain several different neuropeptides. Indeed, small-cell cancers are known to secrete neuropeptides and to proliferate in response to a variety of other neuropeptides.¹⁰ Here, we describe a patient with small-cell carcinoma of the cervix who had symptomatic hypoglycemia that was relieved by intravenous administration of glucose and that was associated with inappropriately high serum immunoreactive insulin, C-peptide, and proinsulin concentrations.

CASE REPORT

In November 1997, a 29-year-old woman presented to her family doctor with intermenstrual bleeding. Physical examination revealed a tumor of the cervix, and subsequent biopsy in the gynecology department of the local hospital revealed invasive squamous-cell carcinoma. Computed tomographic (CT) scans of the chest, abdomen, and pelvis and a radionuclide bone scan showed no evidence of metastatic disease. In December 1997, a total hysterectomy and a pelvic lymphadenectomy were performed. The initial finding on histologic examination of the cervix was reported to be an invasive, poorly differentiated, nonkeratinizing, small-cell-squamous-cell carcinoma of the cervix, with 1 of 18 lymph nodes involved.

Soon after discharge, the patient became anorexic and lost weight; subsequently, she began to have increasing pain in the right buttock that radiated to the knee. She also noted intermittent symptoms of hypoglycemia, including sweating, palpitation, tremor, weakness, and confusion, which occurred predominantly in the early morning and were relieved by eating chocolate. Five weeks after surgery, she collapsed at home and was found within minutes by her husband, who reported that she was unconscious and sweating profusely. On admission to the hospital, she had a blood glucose concentration of 12 mg per deciliter (0.7 mmol per liter). She recovered consciousness rapidly in response to the intramuscular administration of 1 mg of glucagon and intravenous administration of 50 ml of 50 percent glucose. However, a continuous infusion of 10 percent glucose was required to maintain normoglycemia.

Physical examination after the patient's recovery from hypoglycemia was reported to be normal. Blood counts and serum urea, electrolyte, and cortisol concentrations were normal. Liver-function tests revealed high serum concentrations of alkaline phosphatase, alanine aminotransferase, and γ -glutamyltransferase. Magnetic resonance imaging (MRI) of the abdomen and pelvis revealed multiple metastases to the liver and a large mass extending from the right half of the sacrum into the pelvis. These results suggested that the hypoglycemia may have been due to the involvement of the liver with the tumor. However, when the patient's blood glucose concentration was less than 40 mg per deciliter (2.2 mmol per liter), the serum insulin, proinsulin, and C-peptide concentrations were inappropriately high (Table 1). The serum concentrations of insulin-like growth factors I and II were normal. A test for sulfonylurea drugs in the serum was negative. Seven days after admission, treatment with octreotide (50 μ g subcutaneously three times daily) was begun to inhibit the release of insulin. One day later, urinary retention developed, with loss of sensation in the distribution of the third and fourth right sacral nerves and weakness in the right leg. A catheter was inserted, treatment with dexamethasone (8 mg orally twice daily) was begun, and the patient was transferred to Charing Cross Hospital for further treatment of the rapidly progressing tumor.

On admission, an MRI of the spine revealed a pelvic-sacral mass encasing the L5 and S1 roots, an intramedullary metastasis between C4 and C7, and evidence of metastases within several vertebral bodies. A radionuclide bone scan revealed multiple bony metastases. Review of sections of the cervix obtained after the hysterectomy and lymphadenectomy revealed that the tumor was in fact a poorly differentiated small-cell carcinoma (Fig. 1A). Im-

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TABLE 1. SERUM CONCENTRATIONS OF INSULIN, PROINSULIN, AND C PEPTIDE IN THE PRESENCE OF HYPOGLYCEMIA IN A PATIENT WITH SMALL-CELL CARCINOMA OF THE CERVIX.*

VARIABLE	PATIENT	NORMAL VALUE
Blood glucose (mg/dl)	16	>40
Serum insulin (μ U/ml)	910 \pm 95	<5
Serum proinsulin (ng/ml)	1	<0.2
Serum C peptide (ng/ml)	10.1	<1

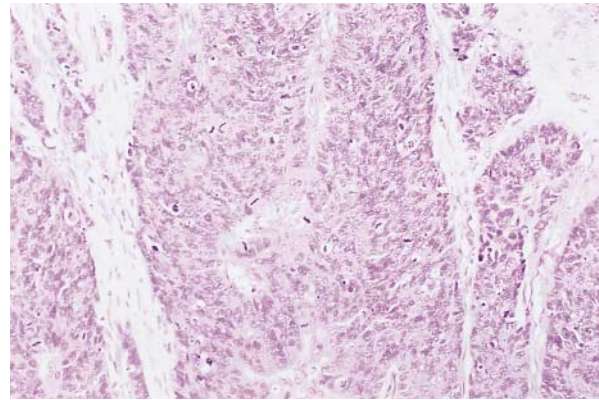
*Serum was collected when the patient's blood glucose concentration was low and was sent to three independent laboratories for measurement of insulin, which is expressed as the mean (\pm SD) concentration. The serum proinsulin and C-peptide results are from a single laboratory. To convert values for blood glucose to millimoles per liter, multiply by 0.05551. To convert values for serum insulin to picomoles per liter, multiply by 6. To convert values for serum proinsulin to picomoles per liter, multiply by 106. To convert values for serum C peptide to nanomoles per liter, multiply by 0.33.

munocytochemical studies demonstrated that the majority of the cells in the tumor contained insulin (Fig. 1B) and neuron-specific enolase. Scattered cells were positive for chromogranin, glucagon, glucagon-like peptide 2, pancreatic polypeptide, somatostatin, and gastrin but not for bombesin, amylin, or vasoactive intestinal peptide. In situ hybridization confirmed the presence of proinsulin messenger RNA (mRNA) in the tumor (Fig. 2).

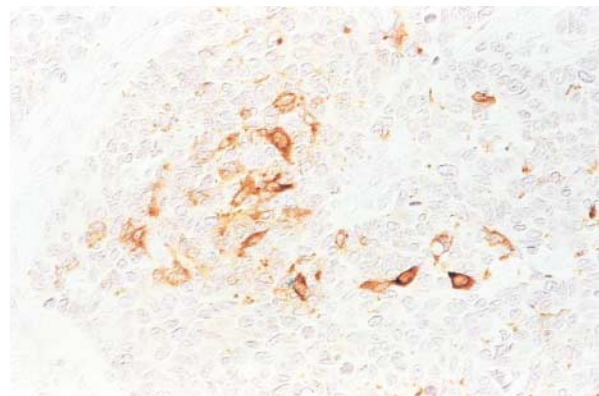
Radiotherapy of the pelvis and cervical spine and concurrent chemotherapy with cisplatin and etoposide were begun immediately.¹¹ The strength and sensory function of the patient's right leg improved, and a CT scan of the liver showed a decrease in the size of several metastases. In parallel with this clinical improvement, the serum insulin concentration dropped (Fig. 3). However, hypoglycemic control remained difficult to maintain and required the introduction of diazoxide therapy (100 mg intravenously three times daily),¹² increasing volumes and concentrations of intravenous glucose, and bendroflumethiazide (2.5 mg orally once daily). Treatment with octreotide was stopped because it did not decrease the requirement for glucose. Continuous infusions of potassium were needed to maintain normal serum potassium concentrations.

A second course of chemotherapy identical to the first was given, after which pancytopenia developed. Radiotherapy was discontinued, and supportive therapy, including administration of granulocyte colony-stimulating factor, blood products, and antibiotics, was begun. Twelve days after the second course of chemotherapy was begun, small-bowel obstruction developed. Septic shock and disseminated intravascular coagulation followed. Despite continued supportive measures, multiorgan failure ensued, and the serum insulin concentrations increased (Fig. 3). The patient died 28 days after her admission to Charing Cross Hospital.

Postmortem examination confirmed that death was due to disseminated intravascular coagulation caused by *Escherichia coli* septicemia, with associated extensive bowel infarction. In addition to the tumor within the pelvis, tumor tissue identical to that of the original primary tumor of the cervix was found to have largely replaced the liver. Sections of the adrenal glands and the pancreas obtained at 5-mm intervals were macroscopically and histologically normal.



A



B

Figure 1. Sections of Small-Cell Carcinoma of the Cervix in a Patient with Hypoglycemia.

The tumor was composed of cells with round-to-oval vesicular nuclei and scant cytoplasm, arranged in cords and nests that infiltrated the wall of the cervix (Panel A). Numerous mitotic figures are visible (hematoxylin and eosin, $\times 80$). In Panel B, the majority of cells contain insulin (stained brown) (anti-insulin serum, $\times 100$).

METHODS

Hormone Assays

Serum insulin was measured by three methods, each at an independent laboratory: a one-step, chemiluminescent, immunoenzymatic assay (Sanofi-Pasteur Diagnostics, Marnes-La-Coquette, France) in which cross-reactivity with proinsulin is less than 0.2 percent; an automated enzyme-linked immunosorbent assay (Roche Diagnostics, Lewes, United Kingdom) in which cross-reactivity with proinsulin is 40 percent; and a manual enzyme-linked immunosorbent assay (Mercodia Diagnostics, Newark, United Kingdom) in which cross-reactivity with proinsulin is 54 percent. Time-resolved fluorometric assays were used to measure serum C peptide (Wallac Oy, Turku, Finland, manufactured by Dako, High Wycombe, United Kingdom) and serum proinsulin.¹³ Serum insulin-like growth factor I was measured by enzyme-linked immunosorbent assay (Immunodiagnostic Systems, Boldon, United Kingdom), and serum insulin-like growth factor II and its precursor were measured by competitive radioimmunoassay.¹⁴

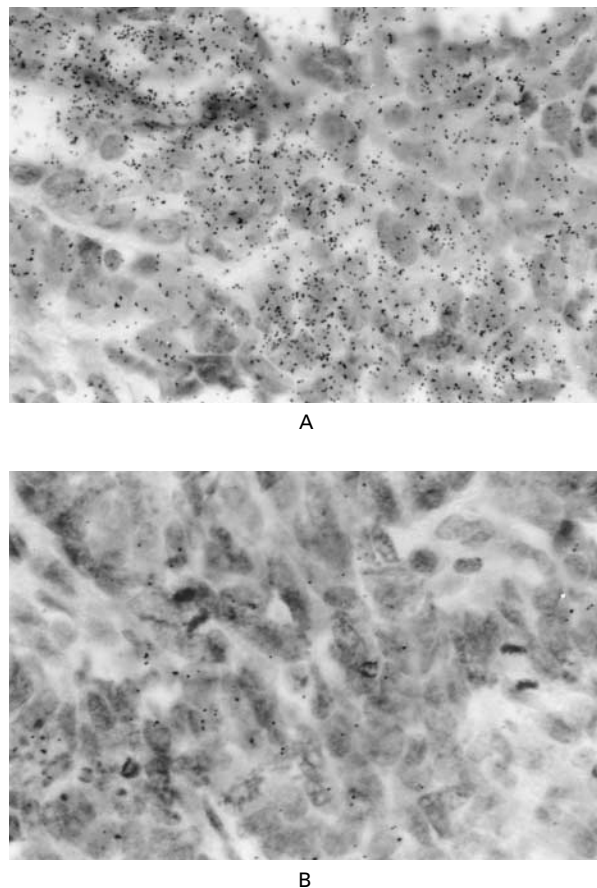


Figure 2. Detection of Proinsulin Messenger RNA in the Tumor by in Situ Hybridization.

In situ hybridization of a section with radiolabeled antisense complementary RNA shows a clear signal (silver grains in Panel A) over tumor cells ($\times 360$). The sense (control) probe produced no signal (Panel B, $\times 360$).

Immunohistochemical Studies

Sections 5 μm thick were immunostained by incubation with avidin–biotin–peroxidase complex (Vector Laboratories, Burlingame, Calif.)¹⁵ and exposure to diaminobenzidine. The primary antibodies were directed against neuron-specific enolase (Dako), chromogranin (Boehringer Mannheim, Lewes, United Kingdom), insulin (Miles Laboratories, Slough, United Kingdom), pancreatic polypeptide (Eli Lilly, Indianapolis), and somatostatin (Immuno Nuclear, Stillwater, Minn.). The rabbit polyclonal antibodies to glucagon, glucagon-like peptide 2, gastrin, vasoactive intestinal polypeptide, and bombesin were produced at Hammersmith Hospital as previously described.^{16–19}

In Situ Hybridization

During the patient's second admission, 10- μm -thick sections of the primary cervical tumor and of a normal pancreas (as a positive control) were prepared for reexamination by removal of wax, rehydration, and permeabilization with proteinase K (1 μg per milliliter). In situ hybridization was performed with use of antisense and sense (control) ³⁵S-labeled uridine triphosphate riboprobes transcribed from a human proinsulin complementary DNA (the gift of K. Docherty, Aberdeen University, Aberdeen, United

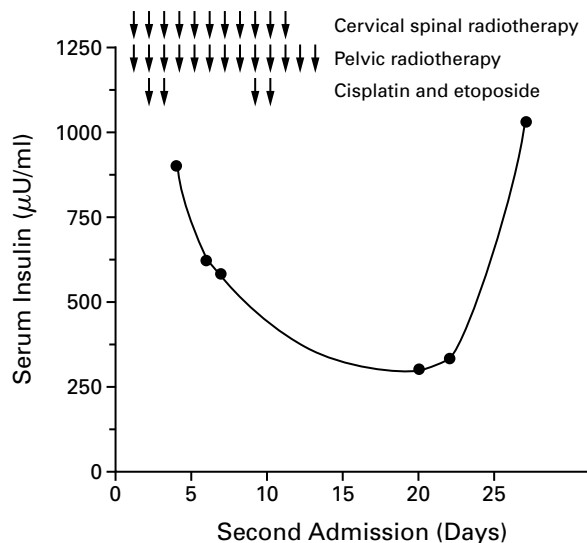


Figure 3. Serum Insulin Concentrations before, during, and after Chemotherapy and Radiotherapy.

The serum insulin concentrations appeared to change according to the course of the disease. Although the patient required a continuous intravenous infusion of glucose, the infusion was reduced or stopped for the purpose of sampling blood for insulin measurements (denoted by points on the curve), which were performed when the blood glucose concentration was less than 40 mg per deciliter. The patient died on day 28. Arrows indicate times at which treatment was administered. To convert values for insulin to picomoles per liter, multiply by 6.

Kingdom), as previously described.²⁰ After hybridization and high-stringency washes, the sections were exposed to autoradiographic film for 48 hours and then dipped in photographic emulsion, exposed for 17 days, developed, and counterstained with hematoxylin and eosin. Sections of the normal pancreas contained high-intensity signals for proinsulin mRNA, confined to specific cells of the islet (not shown). No signal was seen with sense probes on any section of either the normal pancreas or the patient's tumor.

DISCUSSION

Small-cell carcinomas of the cervix are rare. Like their bronchial counterparts, they are highly metastatic and are associated with poor long-term survival.^{8,9} These tumors may secrete several neuropeptides of both pancreatic and nonpancreatic origin, but whether small-cell carcinomas or other non-islet-cell tumors can induce hypoglycemia as a result of insulin secretion has been debated.¹

The few case reports that have suggested that non-islet-cell tumors may produce insulin^{21–24} include one report of a woman with squamous-cell carcinoma of the cervix who had hypoglycemia and a slightly increased serum insulin concentration.²⁵ However, in these cases, there were alternative mechanisms for hypoglycemia or the possibility of pancreatic insulinoma was not excluded. For example, some early studies used biologic-receptor and radio-

receptor assays that could not distinguish among insulin-like growth factor I, insulin-like growth factor II, and proinsulin, which are structurally similar to one another.¹ In addition, although the serum insulin concentrations in some cases were inappropriately normal or slightly elevated, in none was the concentration markedly elevated.²⁵

The results of our studies of this patient with metastatic small-cell carcinoma of the cervix clearly show that a non-islet-cell tumor can be associated with massively increased serum insulin concentrations (about 200 times the normal concentration) and profound hypoglycemia. The hyperinsulinemia was confirmed by measuring serum insulin with three different assays. The endogenous nature of the hyperinsulinemia was verified by the finding of high serum C-peptide and proinsulin concentrations (Table 1), and the small-cell tumor was identified as the most likely cause of the hyperinsulinemia on the basis of the detection of proinsulin mRNA and insulin protein within the tumor cells. Although we did not test the patient's serum for insulin antibodies, the results of these studies strongly suggested the presence of endogenous hyperinsulinemia. Interestingly, the processing of insulin by the tumor appeared to be similar to that in normal pancreatic tissue, in that the serum concentration of 32-33 split proinsulin was higher than that of 65-66 split proinsulin (data not shown).¹³ Furthermore, the tumor contained several other pancreatic peptides, including glucagon, glucagon-like peptide 2, and pancreatic polypeptide. It could therefore be argued that the tumor originated from the pancreas. However, at postmortem examination, the appearance of the pancreas was both macroscopically and microscopically normal. Although the patient had liver metastases, her liver function was not markedly abnormal. It is very unlikely that other causes contributed to the hypoglycemia in our patient. For example, there was no evidence of adrenal insufficiency or of aberrant production of insulin-like growth factor II. We therefore believe that this case of hypoglycemia was induced by insulin secretion from a non-islet-cell tumor.

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REFERENCES

- Marks V, Teale JD. Tumours producing hypoglycaemia. *Endocr Relat Cancer* 1998;5:111-29.
- Gorden P, Hendricks CM, Kahn CR, Megyesi K, Roth J. Hypoglycemia associated with non-islet-cell tumor and insulin-like growth factors: a study of the tumor types. *N Engl J Med* 1981;305:1452-5.
- Daughaday WH, Kapadia M. Significance of abnormal serum binding of insulin-like growth factor II in the development of hypoglycemia in patients with non-islet-cell tumors. *Proc Natl Acad Sci U S A* 1989;86:6778-82.
- Shapiro ET, Bell GI, Polonsky KS, Rubenstein AH, Kew MC, Tager HS. Tumor hypoglycemia: relationship to high molecular weight insulin-like growth factor-II. *J Clin Invest* 1990;85:1672-9.
- McFadzean AJS, Yeung RT. Further observations on hypoglycaemia in hepatocellular carcinoma. *Am J Med* 1969;47:220-35.
- Redmon B, Pyzdrowski KL, Elson MK, Kay NE, Dalmasso AP, Nuttall FQ. Hypoglycemia due to a monoclonal insulin-binding antibody in multiple myeloma. *N Engl J Med* 1992;326:994-8.
- Walters EG, Tavaré JM, Denton RM, Walters G. Hypoglycaemia due to an insulin-receptor antibody in Hodgkin's disease. *Lancet* 1987;1:241-3.
- Remick SC, Hafez GR, Carbone PP. Extrapulmonary small-cell carcinoma: a review of the literature with emphasis on therapy and outcome. *Medicine (Baltimore)* 1987;66:457-71.
- O'Hanlan KA, Goldberg GL, Jones JG, Runowicz CD, Ehrlich L, Rodriguez-Rodriguez L. Adjuvant therapy for neuroendocrine small cell carcinoma of the cervix: review of the literature. *Gynecol Oncol* 1991;43:167-72.
- Seckl MJ, Rozengurt E. Neuropeptides, signal transduction and small cell lung cancer. In: Martinet Y, Hirsch FR, Martinet N, Vignaud J-M, Mulshine JL, eds. *Clinical and biological basis of lung cancer prevention*. Basel, Switzerland: Birkhäuser Verlag, 1998:129-42.
- Hoskins PJ, Wong F, Swenerton KD, et al. Small cell carcinoma of the cervix treated with concurrent radiotherapy, cisplatin, and etoposide. *Gynecol Oncol* 1995;56:218-25.
- Ernesti M, Mitchell ML, Raben MS, Gilboa Y. Control of hypoglycaemia with diazoxide and human growth hormone. *Lancet* 1965;1:628-30.
- Sobey WJ, Beer SF, Carrington CA, et al. Sensitive and specific two-site immunoradiometric assays for human insulin, proinsulin, 65-66 split and 32-33 split proinsulins. *Biochem J* 1989;260:535-41.
- Teale JD, Blum WF, Marks V. Alleviation of non-islet cell tumour hypoglycaemia by growth hormone therapy is associated with changes in IGF binding protein-3. *Ann Clin Biochem* 1992;29:314-23.
- Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *J Histochem Cytochem* 1981;29:577-80.
- Hamid QA, Bishop AE, Sikri KL, Varndell IM, Bloom SR, Polak JM. Immunocytochemical characterization of 10 pancreatic tumours, associated with the glucagonoma syndrome, using antibodies to separate regions of the pro-glucagon molecule and other neuroendocrine markers. *Histopathology* 1986;10:119-33.
- Facer P, Bishop AE, Lloyd RV, Wilson BS, Hennessy RJ, Polak JM. Chromogranin: a newly recognized marker for endocrine cells of the human gastrointestinal tract. *Gastroenterology* 1985;89:1366-73.
- Facer P, Bishop AE, Moscoso G, et al. Vasoactive intestinal polypeptide gene expression in the developing human gastrointestinal tract. *Gastroenterology* 1992;102:47-55.
- Hamid QA, Addis BJ, Springall DR, et al. Expression of the C-terminal peptide of human pro-bombesin in 361 lung endocrine tumours, a reliable marker and possible prognostic indicator for small cell carcinoma. *Virchows Arch* 1987;411:185-92.
- Yau JL, Noble J, Seckl JR. Site-specific regulation of corticosteroid and serotonin receptor subtype gene expression in the rat hippocampus following 3,4-methylenedioxymethamphetamine: role of corticosterone and serotonin. *Neuroscience* 1997;78:111-21.
- Hayes DM, Spurr CL, Felts JH, Miller EC Jr. Von Recklinghausen's disease with massive intra-abdominal tumor and spontaneous hypoglycemia: metabolic studies before and after perfusion of abdominal cavity with nitrogen mustard. *Metabolism* 1961;10:183-99.
- Olesky S, Bailey I, Samols E, Bilkus D. A fibrosarcoma with hypoglycaemia and a high serum-insulin level. *Lancet* 1962;2:378-80.
- Shames JM, Dhurandhar NR, Blackard WG. Insulin-secreting bronchial carcinoid tumor with widespread metastases. *Am J Med* 1968;44:632-7.
- Lyall SS, Marieb NJ, Wise JK, Cornog JL, Neville EC, Felig P. Hyperinsulinemic hypoglycemia associated with a neurofibrosarcoma. *Arch Intern Med* 1975;135:865-7.
- Kiang DT, Bauer GE, Kennedy BJ. Immunoassayable insulin in carcinoma of the cervix associated with hypoglycemia. *Cancer* 1973;31:801-5.