Brief Notes and Comments

Gastric Cancer Containing Insulin and Associated with Hypoglycemia

Susumu Miyabo, M.D., Akira Fujimura, M.D., Tamotsu Matsuda, M.D., and Mototaka Murakami, M.D., Kanazawa, Japan

SUMMARY

A patient with gastric cancer associated with hypoglycemia is described. Immunoassay of acid alcohol tissue extract revealed the presence of 7.6 mU. insulin per gram wet tissue weight in the primary tumor which weighed 10 gm. No insulin was detected in the hepatic metastasis. The concentration of pancreatic insulin was decreased and the serum insulin level was not elevated. Tissue insulinase activity was virtually absent in the primary tumor, whereas the hepatic metastasis showed activity comparable to that of normal liver. It is difficult to assume, however, that the release of insulin from the primary tumor, which contained such small quantities of the hormone, could be the sole cause of hypoglycemia. An additional mechanism(s) must have been involved in the causation of hypoglycemia in this patient. DIABETES *17*:286-89, May, 1968.

The association of severe hypoglycemia with malignant extrapancreatic neoplasms of mesothelial or epithelial origin is being reported with increasing frequency. The genesis of the hypoglycemia, however, has been the subject of controversy.[1] Little direct data have been reported to substantiate the view that excessive glucose utilization by tumors is sufficient by itself to cause hypoglycemia. Material with insulin-like activity has been found in some tumors[2-12] by bioassay technics, although, generally, immunoreactive insulin (IRI) has not been identified.[8,9,11,14]

This report describes a patient with gastric cancer hypoglycemia in whom the presence of insulin in the extract of primary tumor was confirmed immunochemically. The estimated insulin content of the tumor was too small, however, to suggest causal relationship between tumor insulin and hypoglycemia.

From the Second Department of Internal Medicine, Kanazawa University Hospital, Kanazawa, Japan.

CASE REPORT

A sixty-six-year-old man was referred to the Kanazawa University Hospital for evaluation of anorexia. His past and family history were noncontributory. The patient appeared anemic. The liver was enlarged three finger breadths below the costal margin with a hard irregular edge. The remainders of physical examination were unremarkable. The hemoglobin was 7.1 gm. per cent, white cell count 10,600 per cu. mm. Urine contained 1 plus bilirubin and 3 plus urobilinogen. The serum bilirubin was 0.8 mg. per cent of which 0.6 mg. was direct reacting. Bromsulphophthalein retention was 31.5 per cent at forty-five minutes. The alkaline phosphatase was 22.0 Bodansky units, glutamic oxaloacetic transaminase 49 units. Blood urea nitrogen and serum electrolytes were normal. Fasting blood sugar was 74 mg. per 100 ml. Plasma cortisol was normal. X-ray examination of gastrointestinal tract revealed a filling defect in the antral region. Soon after the admission, hepatomegaly increased rapidly, and the patient became progressively jaundiced. On the thirty-sixth hospital day, the patient suddenly became comatose and was treated with an infusion of glucose solution. On the thirty-eighth day, the patient had the second episode of coma. The blood glucose on that occasion was found to be 14 mg./100 ml. From that day on, he was maintained on frequent infusions of glucose and dexamethasone. He continued to suffer from recurrent attacks of hypoglycemia, however. His general condition deteriorated rapidly, and he expired on the sixtieth day. The significant autopsy findings were confined to the abdomen. Adenocarcinoma of the stomach was confirmed, which weighed 10 gm. Several lymph nodes containing metastases were noted in the periportal area. The liver was markedly enlarged and grossly nodular. It weighed 3,300 gm. and on section almost half of the normal architecture was replaced by a solid tumor mass. The pancreas weighed 85 gm. and was free from tumor invasion. The islands of Langerhans appeared slightly atrophic.

MATERIALS AND METHODS

At autopsy, the primary gastric carcinoma, two randomly selected hepatic metastases and a portion of the pancreas were stored at dry ice temperature. Crude tissue extracts were prepared by the method of Davidson et al.,[15] with acid-alcohol extraction followed by dialysis. After centrifugation, the supernatant solutions were lyophylized and stored at —20° C. One milligram of the dry powdered extracts was dissolved in

1 ml. of 1 per cent bovine serum albumin borate buffer, at *p*H 8.5. A small amount of insoluble materials was removed by centrifugation. The frozen specimen of fasting serum obtained on thirty-ninth day was thawed before use.

The tissue extracts and serum were analyzed for immunoreactive insulin by the double antibody technic but with slight modification from the original.[16] I-131-labeled porcine insulin (Abbot) was purified on a Sephadex column as described by Banerjee and Gibson.[17] All dilutions were made in 1 per cent albumin borate buffer.

Contamination of the extract of primary tumor by proteolytic enzymes was tested as follows; a trace amount of insulin-I-131 was incubated with either the tumor extract solution (0.1 mg. in 0.1 ml.) or 1 per cent albumin borate buffer (0.1 ml.) for 24 hrs. at 37° C. Then, anti-insulin serum (0.1 ml. of 1:100*) or albumin borate buffer (0.1 ml.) was added and the systems were incubated again for 24 hrs. Aliquots of the incubation mixtures were subjected to cellulose acetate electrophoresis with subsequent radioautography.

Cysteine incubation of extracts and crystalline pork insulin were performed according to the procedure of Unger et al.[18] modified by the author. One milligram of extract was dissolved in 1 ml. of 1:64 dilution of Tris-EDTA-boric acid buffer at *p*H 9.0 and further diluted with the same buffer. Cysteine was added at a concentration of 1 mg. per ml. The mixtures were incubated at room temperature for 24 hrs. and cysteine in excess was removed by dialysis against borate buffer (*p*H 8.5) for 24 hrs. at 4° C. with two changes of the external medium. Control samples were treated in an identical manner, except that cysteine was replaced by glycine.

Tissue insulinase activity was determined by the method described by Goodner and Freinkel.[19] Tissue homogenates from the primary tumor, the hepatic metastasis, as well as control normal stomach and liver were centrifuged for 30 min. at 15,000 g and the soluble supernatants were employed, i.e., 15,000 g extract. One milliliter of supernate was added to a trace amount of insulin-I-131 supplemented with crystalline insulin (Lilly) to a concentration of 100 mg. per ml. at a final volume of 2.0 ml. After 30 min. of incubation at 37° C., the radioactivity of the total incubation mixture and the precipitate obtained after addition of 10 per cent trichloroacetic acid was measured. Insulinase activity was calculated by the formula:

$$\text{I.A.} = \frac{\text{Non TCA Precipitable I-131 (\% of total I-131)}}{\text{mg. N in 1.0 ml. "15,000 g extract"}}.$$

Nitrogen was determined by the Kjeldahl method.

RESULTS

1. Serum insulin assay

The serum specimen obtained on the thirty-ninth day after overnight fast was used for assay. The level of serum IRI was 13.1 μU. per ml., which did not exceed the range of the normal fasting controls [12.2±4.5 (S.E.M.) μU. per ml.], whereas the blood sugar level in the specimen was low (48 mg. per 100 ml.).

*1:100 dilution of anti-insulin serum was potent enough to bind both labeled and unlabeled (tumor) insulin in the incubation mixture.

2. Tissue insulin assay

The results are indicated in table 1 and figure 1. The primary gastric carcinoma contained an appreciable amount of "insulin" (7.59 mU. per gm. wet tumor weight) and the total tumor content was estimated to be 76 mU., while the hepatic metastases had no assayable "insulin." Insulin concentration in the specimen of pancreas was 514 mU. per gm., or 44.7 units for the entire pancreas, assuming a uniform distribution; a value much lower than that of normal pan-

TABLE 1

Insulin content of tumor and other tissue

Tissue		Dilution of extract solution*	Insulin content of solution (μU./ml.)	Insulin content of tissue (mU./gm.)
Primary carcinoma		1:10	34.5	7.76
		1:20	16.0	7.20
		1:40	8.7	7.83
		1:80	4.2	7.56
			Mean	7.59
Hepatic metastases	I	1:1	0	0
		1:10	0	0
	II	1:1	0	0
		1:10	0	0
Pancreas		1:1,000	83.5	510.0
		1:4,000	21.2	517.9
			Mean	514.0

*Solution contained 1 mg. of dried extract/ml. prior to dilution.

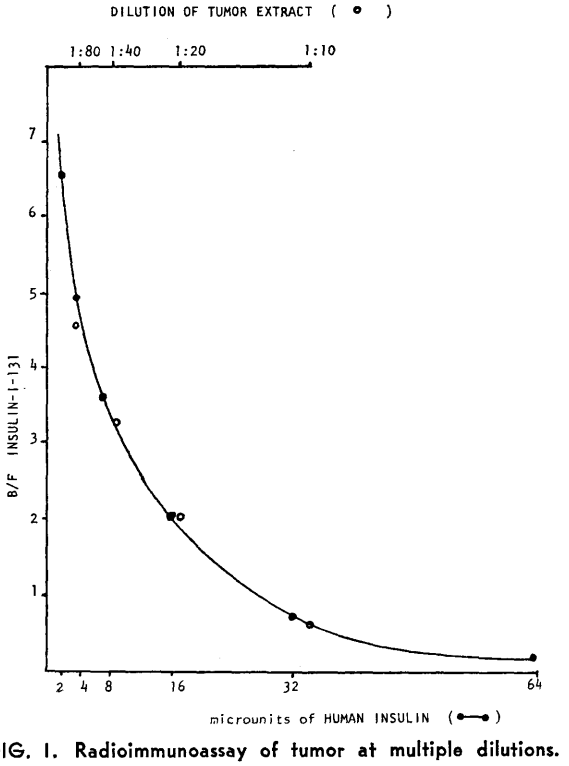

FIG. 1. Radioimmunoassay of tumor at multiple dilutions.

creas (1.31 U. per gm. or 130 U. total).[20]

3. Assay of proteolytic activity in tumor extract

On the radioelectrophoretogram (not shown), insulin-I-131 incubated with the tumor extract produced a migration pattern identical with that of insulin-I-131 in the buffer. Addition of anti-insulin serum to both systems resulted in complete shift of radioactivity towards the gamma-globulin.

4. Effect of cysteine on tumor "insulin"

As seen in table 2, cysteine almost completely destroyed tumor "insulin" as well as pancreatic insulin.

5. Assay of tissue insulinase activity

TABLE 2

Effect of cysteine on insulin

	Control (glycine incubation)		Cysteine incubation	
Dilution of extract solution*	1:10	1:20	1:10	1:20
Tumor "insulin" (μU./ml.)	33.5	17.0	2.1	0
Dilution of extract solution*	1:1,000	1:4,000	1:1,000	1:4,000
Pancreatic insulin (μU./ml.)	80.2	21.0	1.2	0
Pork insulin, 10 × cryst. (Lilly) (μU./ml.)	200.0 50.0		1.5 0	

*Extract solution contained 1 mg. of dried extract/ml. prior to dilution.

The results are illustrated in table 3. The primary tumor contained only a trace of insulinase activity, whereas the hepatic metastases exhibited activity almost equal to that of the control liver.

DISCUSSION

The significance of tumor "insulin" must be interpreted with caution. It seems unlikely that the apparent presence of tumor "hormone" is a consequence of nonspecific inhibition of the antigen-antibody reaction by a substance other than insulin. The extract showed specific hormone-like reactivity over a wide range of dilutions. Incubation of insulin-I-131 with tumor extract did not cause any change in its electrophoretic mobility. The possibility of contamination of the tumor extract by proteolytic enzymes cleaving insulin-I-131 can thus be ruled out. Cysteine almost completely abolished the immunological activity of the tumor extract. The interchain disulfide bridges in insulin molecule are known to be susceptible to reductive cleavage by thiol or sulfite reagents.[21] Since functional islet neoplasms arising from ectopic pancreas usually retain their histological characteristics,[22] it is less likely that the apparent gastric carcinoma had originated from aberrant islet cells, although this possibility cannot be excluded.

TABLE 3

Insulinase activity of tumor and other tissue

		Insulinase activity per cent non-TCA-precipitable I-131/mg. N in ml. extract	
Primary carcinoma		0.02	
Hepatic metastases	I	33.6	
	II	30.2	
Normal stomach		0.007	(mean of 6 cases)
Normal liver		31.2	(mean of 6 cases)

So far, there has been no convincing evidence for the production of true insulin by nonpancreatic neoplasia. In tumors associated with hypoglycemia, attempts to demonstrate tumor insulin by immunoassay have been unsuccessful in the majority of cases.[8,9,11,14] Only Floyd et al.[7] and Carey et al.[10] have shown assayable IRI in their tumors. Floyd did not state that the amount was significant, however, and in the studies of Carey the "insulin" content of control tissues was not determined in the same laboratory or by the same method as the content of the tumor in question. Plasma insulin has been consistently low,[7-10,13] with one exceptional high value reported by Oleesky et al.[23] On the other hand, Unger et al.[18] identified large quantities of insulin in the bronchogenic metastasis of a patient who did not show clinical hypoglycemia. They suggested that the tumor might merely entrap rather than synthesize insulin.

In the present case, "insulin" in the primary tumor was 7.6 mU. per gm., an amount exceeding insulin accounted for by extracellular fluid. It is impossible, however, to state whether the tumor "insulin" was actively synthesized or passively accumulated by the tumor. Furthermore, the "insulin" content of the entire tumor seems to have been too small to effect a lowering of blood glucose, even if it could have been released from the tumor by any mechanism (i.e., secretion of synthesized hormone or leak of entrapped hormone). Thus, we suspect that hypoglycemia and insulin in the tumor were probably, or causally at least, not related to each other in the present case. Absence of "insulin" in the hepatic metastasis could be explained in terms of its high insulinase activity. Serum insulin was not elevated, despite the presence of hypoglycemia. This might be related to the subnormal concentration of insulin in the pancreas.

The mechanism of the patient's hypoglycemia re-

mains unsettled. The most likely and currently favored idea is that the tumor might elaborate some factor which is chemically distinct from insulin and yet shares some of the biological properties with insulin or mediates (or potentiates) the action of insulin. The nature of this substance is, of course, unclear. It might be a peptide or protein. But other classes of substances such as nucleic acid components,[1,24] or tryptophan metabolites[25] have also been proposed as candidates. The search for the existence of an yet unidentified substance which may be responsible for hypoglycemia in some cases of nonpancreatic tumor associated with hypoglycemia does deserve renewed attention.

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