

Clinical case

Tumour-induced hypoglycaemia: A case report

K. Hoekman,¹ J. van Doorn,² T. Gloudemans,³ O. S. Hoekstra,⁴ J. A. Maassen,⁵
J. B. Vermorken,¹ J. Wagstaff¹ & H. M. Pinedo¹

¹Department of Oncology, Free University Hospital, Amsterdam; ²Laboratory of Endocrinology, Wilhelmina Children's Hospital, Utrecht;

³Laboratory of Physiological Chemistry, University of Utrecht; ⁴Department of Nuclear Medicine, Free University Hospital, Amsterdam;

⁵Sylvius Laboratory, Department of Medical Biochemistry, Leiden, The Netherlands

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Case report

A 50-year-old Caucasian man was admitted unconscious to the hospital in the early morning. His medical history began 10 years earlier, when he was treated for an haemangiopericytoma in the cerebellum. The tumour was resected and post-operative radiotherapy (56 Gy) was given. Two years later a thyroid metastasis was treated by a hemi-thyroidectomy followed again by radiotherapy (60 Gy). Eight years later the patient received 5 cycles of doxorubicin 90 mg/m² plus granulocyte-macrophage colony-stimulating factor (GM-CSF) because of metastases in both lungs, the left lobe of the liver and in the left adrenal gland. A minor anti-tumour response was observed. Three months after chemotherapy he was admitted to the hospital in coma.

Physical examination showed only an enlarged liver. There were no focal neurological signs. Computerised tomography (CT) of the brain showed only post-operative changes, which could not explain his reduced level of consciousness. His serum glucose level was 1.1 mmol/L and he rapidly regained consciousness after glucose infusion. Glycosylated haemoglobin (HbA_{1c}), which is an index for the integral glucose variations in the previous 2 or 3 months, was 1.3% (N: 4.5-6.5%).

His family then mentioned repeated episodes of odd behaviour over the past months, especially early in the morning, which disappeared after food intake. The patient was not taking medication, did not drink alcohol and had previously been in reasonably good physical condition.

During the admission, the hypoglycaemia could easily be re-induced by a few hours of fasting. The very low levels of serum glucose were accompanied by an undetectable serum insulin level and very low levels of c-peptide, the connecting peptide of pro-insulin. These results are pathognomonic of a completely suppressed

endogenous insulin production. Following an increase in the glucose levels to normal values, the serum insulin and c-peptide concentrations were appropriate. This result is indicative of an adequate reaction of insulin production to circulatory glucose levels, which excludes an insulinoma.

Evaluation of the tumour status showed a slow progression. The patient was then treated with 2 cycles of ifosfamide 5 g/m² with no anti-tumour response.

The hypoglycaemic episodes in this patient were initially controlled by dietary measures. Half a year later, he was readmitted because of intractable hypoglycaemias, which occurred especially in the early morning. Treatment with somatostatin 2 × 150 µg/d s.c. had no effect on insulin-like growth factor (IGF, see below) or glucose concentrations. Dexamethasone 6 mg/day taken orally prevented severe hypoglycaemias for about 3 months. When the episodes of hypoglycaemia again increased in frequency and seriousness the patient was treated with glucagon 6-12 mg/day by continuous infusion via a Port-a-Cath system in combination with dexamethasone 3 mg/day. This resulted in a more or less normal pattern of serum glucose levels throughout the entire day. After 3 months of treatment the severity of the hypoglycaemias increased and a decision was made to reduce the tumour volume by surgery. A resection of the metastasis in the left lobe of the liver and the left adrenal was performed and immediately thereafter serum glucose increased to normal levels. The liver metastasis contained a central area of fluid (Fig. 1), and 50 ml of this fluid was collected for further analysis. One day post-operatively the patient was receiving only intravenous saline solutions and had normal serum glucose concentrations. In the succeeding months only one serious hypoglycaemic episode was documented. Half a year later the patient died of sepsis. Post mortem analysis was not performed.

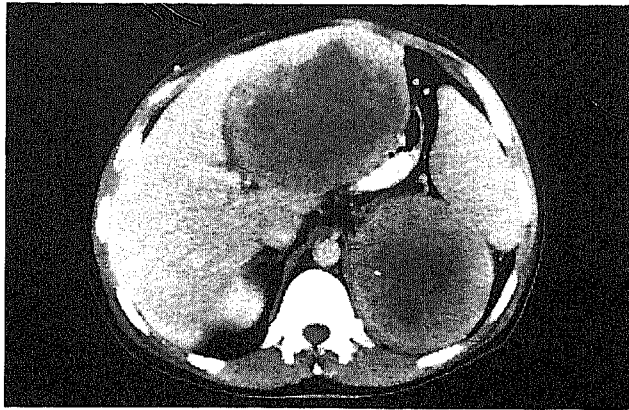


Fig. 1. Metastases from the haemangiopericytoma in the left lobe of the liver and the left adrenal gland.

Additional investigations

Concentrations of insulin-like growth factors (IGF) in blood and tumour fluid

The concentration of plasma IGF-I was very low (14–21 ng/ml) and IGF-II was within the normal range (515–800 ng/ml) in this patient. These values were not significantly different whether the patient was normo- or hypoglycaemic. Sampling of the hepatic, renal and femoral veins did not demonstrate increased IGF-II levels. After surgery, which was an incomplete debulking, the level of IGF-I increased and the level of IGF-II decreased, but only by a small amount. Tumour fluid contained no insulin, a very low level of IGF-I (8 ng/ml) and a strongly elevated concentration of IGF-II (2200 ng/ml).

Analysis of the IGF-I and IGF-II genes

Genomic DNA and RNA was isolated from frozen tumour tissue for Southern and Northern blot analysis. Southern blot analysis of tumour DNA revealed no amplification or rearrangements in the IGF-II gene. Messenger RNAs for IGF-I were not detected in tumour tissue but the level of expression of the IGF-II gene was about fifty times higher than in normal adult tissue. No aberrant IGF-II gene transcripts were found.

Analysis of IGF protein and interactions with IGF-binding proteins (IGFBP)

Acid gel filtration was used to investigate IGF-II size heterogeneity. In normal human serum, 90% of IGF-II was present in the mature 7.4 kD form. In contrast, preoperative patient serum had most ($\approx 80\%$) of the IGF-II as a broad peak of 10–20 kD ('big' IGF-II), decreasing to $\approx 50\%$ after surgery. In tumour fluid $>90\%$ of IGF-II appeared as big IGF-II.

The different molecular-size classes of endogenous IGFBP-IGF complexes in serum and tumour fluid were

resolved by neutral gel filtration. In normal human serum, IGF-I appeared to be exclusively present as a 150 kD complex, which also contained most of the IGF-II ($\geq 60\%$). The remaining portion of IGF-II complexes eluted slightly later than serum albumin (60 kD). In the patient's serum, the peak of IGF-II corresponded to a molecular weight of about 60 kD with a minimal contribution from the 150 kD complex. In tumour fluid, most of the IGF-II was present as a 60–65 kD complex. The conclusion is, therefore, that IGF-II was present in both the patient's serum and tumour fluid as big IGF-II, associated with a small IGFBP complex.

Counter-regulatory mechanisms

The humoral responses to hypoglycaemia, induced by fasting, were studied in this patient. In normal persons, hypoglycaemia induces a strongly increased production of glucagon, catecholamines, growth hormone (GH) and cortisol, which stimulate endogenous glucose release by the liver and/or inhibit glucose utilisation by insulin-sensitive tissues [8].

Hypoglycaemia in this patient was accompanied by a serum GH concentration which was always below the level of detection. Stimulation with 30 grams of arginine did not induce a GH response. Growth hormone-releasing hormone (100 μg i.v.) induced a weak GH response with a maximum value of 4.1 $\mu\text{g/l}$ at 30 minutes (T_{30}), whereas the glucose levels remained between 2.1 and 3.2 mmol/l during the two hours of this observation period.

During hypoglycaemia, serum glucagon increased to slightly (and inappropriately) enhanced levels (104–249 ng/l), serum cortisol remained normal (400–575 nmol/l), as did serum catecholamines [(nor)-adrenaline <3.5 nmol/L].

Glucagon 1 mg i.v. during a hypoglycaemic episode induced an increase of serum glucose from 1.8 mmol/l to a maximum of 4.0 mmol/l at T_{30} , but the glucose level at T_{90} was once again 1.9 mmol/l. The daily cortisol concentration curve was low and flat. Stimulation with synthetic ACTH demonstrated a normal cortisol response (maximum 960 nmol/L). These studies suggest that the normal counter-regulatory responses to hypoglycaemia, especially the GH response, were suppressed.

Metabolic studies

Calculated resting energy expenditure (REE), an index of the basal metabolism, was 2285 kcal/24h, which was 136% of the predicted value for the height and weight of this patient. The respiratory quotient was 0.94, indicating a preferential glucose utilisation. Basal glucose oxidation was 8.7 $\mu\text{mol/kg/min}$. Fasting glycerol concentrations were between 70–95 $\mu\text{mol/L}$, free fatty acids (FFA) <0.10 mmol/L and β -hydroxybutyrate ($\beta\text{OH-B}$) <0.01 mmol/L. The latter values are very low and correspond to an inhibited lipolysis. Both the in-

creased glucose turnover and the inhibited lipolysis suggest an enhanced insulin-like activity.

Glucose-scan

18F-fluoro-deoxyglucose (18FDG) scintigraphy was performed with a conventional gamma-camera, equipped with a collimator specially designed for 511 keV photons (Nuclear Fields, Boxmeer, The Netherlands). The 18FDG scan showed a normal 18FDG distribution as well as an abnormal accumulation in the upper abdomen, reflecting the hepatic and adrenal metastases. The 18FDG tumour uptake was, however, less than we commonly observe in other tumours such as lymphomas, which are not associated with hypoglycaemias. This suggested that the hypoglycaemias were not primarily induced by an increased glucose consumption by the tumour.

Stimulation of insulin and IGF-I receptors by serum and tumour fluid

The metabolic effects of IGF-II are mediated through both the insulin and IGF-I receptor [9]. The in vitro ability of serum and tumour fluid to stimulate partially purified insulin and IGF-I receptors was, therefore, examined [10]. Control and patient serum had no effect, but tumour fluid stimulated the insulin receptor as strongly as 75 nM insulin (normal serum values <0.5 nM) whilst the IGF-I receptor was stimulated to the same degree as by 60 nM (\approx 450 ng/ml) free IGF-II. These findings show an enormous insulin-like activity in tumour fluid but not in the patient's serum.

Discussion

Pathophysiology of tumour-induced hypoglycaemia

Hypoglycaemia induced by a tumour-related mechanism is a rare phenomenon [4]. It has been associated with tumours producing insulin (insulinomas), antibodies against insulin or insulin receptors [5, 6] and tumours producing an insulin-like growth factor. These IGFs are small peptides of 70 (IGF-I) and 67 (IGF-II) aminoacids, respectively, with homology to proinsulin. Their properties include both mitogenic and metabolic activity. IGF-I is the mediator of many of the effects of growth hormone whereas IGF-II is involved in embryogenesis but has no well defined function in the adult life. A great variety of tumours have been shown to be able to induce an IGF-mediated hypoglycaemia. Among them are primary liver cell carcinomas (the most frequent reason for tumour-induced hypoglycaemia in the eastern part of the world), adrenal and neuroendocrine tumours, Wilms' tumour and sarcomas. In nearly all cases, the sarcomas which show this syndrome are large and slow-growing, and the hypoglycaemia is a late event in the disease history.

Table 1. The causes of tumour-related hypoglycaemia.

Enhanced transcription of the IGF-II gene in tumour tissue
Secretion of big (immature) IGF-II by the tumour
Suppression of the production of mature IGF-II and GH-IGF-I-IGFBP-3 in normal tissues
Sequestration of big IGF-II in small IGFBP-complexes
Facilitated transcapillary transport resulting in enhanced bioavailability of tumour derived IGF-II
<i>Tumour-induced insulin-like activity</i>
increased glucose turnover
instability of glucose balance due to insufficient counter regulation
inhibited lipolysis

In the group of sarcomas, haemangiopericytomas are frequently associated with hypoglycaemias. The causes of tumour-induced hypoglycaemia are shown in Table 1.

Tumour-derived IGF-II is thought to be largely responsible for the hypoglycaemia induced by sarcomas, although increased circulatory concentrations of IGF-II are exceptional in these patients. The evidence for an IGF-II-mediated mechanism is the finding that these sarcomas invariably show an enhanced expression of the IGF-II gene [7]. This results in a tumour product which is present in the circulation as a high-molecular-weight (10–20 kDa) protein ('big' IGF-II) [8]. This protein represents abnormally or incompletely processed IGF-II, resulting in the appearance of a mixture of precursors of IGF-II. The production of mature 7.5 kDa IGF-II by normal tissues is suppressed.

The association of big IGF-II with the IGF-binding proteins (IGFBPs) is abnormal. In normal human serum both IGFs are complexed with IGFBPs which regulate their access to target tissues as well as their clearance rates [9]. Less than 5% of the IGFs appears in the free form. Six IGFBPs have been characterised so far. IGFBP-3 is the most abundant IGFBP, existing in a ternary complex of 150 kDa, which, besides an IGFBP-3 (β -subunit), also contains an acid-labile 85 kDa glycoprotein (α -subunit) and either IGF-I or IGF-II (γ -subunit). Both IGFs are associated with IGFBP-3 in normal adult serum and these 150 kDa IGF-BP complexes are too big to cross the capillary barrier.

In contrast to normal 7.5 kDa IGF-II, tumour-derived big IGF-II circulates in a 60 kDa complex [10]. This is caused by a decreased concentration of circulatory IGFBP-3 and the acid-labile component together with a reduced ability of the α -subunit to combine with the $\beta\gamma$ -complex [11]. This impaired formation of normal IGFBP complexes may be indirectly affected by the excess of IGF-II, which inhibits the production of GH, IGF-I and the IGF-I-dependent IGFBP-3 [12] and by an abnormal structural appearance of the $\beta\gamma$ -complex when it includes big IGF-II. It has been postulated that these small tumour-derived IGF-II-IGFBP complexes have an easier transcapillary transport and result in an enhanced bioavailability of tumour derived IGF-

II. There has as yet, however, been no direct demonstration of the insulin-like activity of a tumour product.

In this patient an enhanced expression of the IGF-II gene in tumour tissue, an increase of IGF-II in tumour fluid, the appearance of big IGF-II in patient serum and tumour fluid and the association of IGF-II with small IGFBP complexes were all observed. In addition, it has been demonstrated for the first time that tumour fluid possessed enormous insulin-like activity *in vitro*. The finding that tumour fluid, but not patient serum, had this capacity confirms the above hypothesis of increased IGF-II bioavailability. In addition, it was demonstrated that glucose consumption by the tumour itself was not of great importance for the hypoglycaemias in this patient. Finally, the normal counter-regulatory responses against hypoglycaemia, namely, increased glucagon, GH, cortisol and catecholamine levels were shown to be insufficient to prevent hypoglycaemic episodes in this patient. These perturbations in counter-regulatory responses are the consequence of abnormal IGF-II activity on the one hand and the prolonged periods of hypoglycaemia on the other [14]. These facts are important in explaining the pathophysiology of tumour-induced hypoglycaemia [13] and are illustrated by the preferential occurrence of hypoglycaemias in the early morning. The increased insulin-like activity due to an IGF-II excess and the insufficient counter-regulatory responses easily explain the severe hypoglycaemias observed in this patient (see Table 1).

Insulin-like growth factors and sarcomas

The involvement of IGFs in tumour biology has been demonstrated for many tumour types [15–17]. Observations which indicate this include enhanced concentrations of IGFs and IGF receptors in tumours, the effect of IGFs on the proliferation of tumour cells and the anti-proliferative effects of IGF receptor inhibition *in vitro*. The data do, however, show considerable variability. Recently, IGF-II expression in leiomyosarcoma [18] and rhabdomyosarcoma [19] cells and the stimulatory effect of IGF-I on the growth of sarcoma cells [20] has been reported. The role of IGFbps in tumour biology has not been investigated thoroughly thus far. This work should be undertaken because of the dominant effect of IGFbps on the biological activity of IGFs. In addition, it is important to mention that a standard RIA cannot discriminate between normal and tumour-derived IGF-II. Column chromatography can demonstrate the presence of big IGF-II. Recently, a RIA has been developed [21] which detects the E-region of the molecule, this being the carboxy-terminal extension of the IGF-II precursor. This RIA can directly show the presence of tumour-derived IGF-II precursors in biological fluids.

Soft tissue sarcomas are frequently associated with areas of 'necrosis', which can be easily detected by standard radiological techniques. These areas may be of great interest because of the fact that they contain

enhanced concentrations of tumour products, as has been demonstrated in the present case. Aspiration of tumour-derived fluid is an easy procedure which may be of great help in the investigation of tumour biology.

Management of tumour induced hypoglycaemia

Any excessive insulin-like activity can be detected by measuring the glucose concentration in a blood sample taken after fasting when hypoglycaemia will be present. In such a situation measurable levels of both insulin and c-peptide are indicative of an insulinoma. Unmeasurable levels of these proteins suggest that other insulin-like mechanisms are operating. This is decisive for the discrimination between insulin and insulin-like mechanisms of tumour-induced hypoglycaemia. A further indication for an IGF-II-induced mechanism is the ratio of IGF-I/IGF-II which is low in these patients [22] due to normal or slightly elevated IGF-II levels and low IGF-I concentrations.

The best therapy for tumour-induced hypoglycaemia is reduction of the tumour load. In the case of bulky mesenchymal tumours a partial removal of tumour may be worthwhile, as demonstrated here. Symptomatic treatment consists of dietary guidelines, including strategies to prevent long periods to fasting and advice for the patient and his family of what to do in case of an imminent hypoglycaemia. In addition, drugs can be used which stimulate endogenous glucose production. These medications include glucagon [23], growth hormone [24] and corticosteroids. Somatostatin was not successful in reducing IGF-II production or the frequency of hypoglycaemic episodes in this patient. Treatment with IGFBP-3 is a theoretical possibility, which has not previously been attempted.

The study of the role of IGFs, their binding proteins and IGF-receptors in the biology of sarcomas may offer a new understanding of and new treatment modalities for this type of tumour.

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Correspondence to:

Dr. J. Wagstaff, MB ChB, MD, MRCP
 Department of Oncology
 Free University Hospital
 de Boelelaan 1117
 1007 MB Amsterdam,
 The Netherlands