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Original Paper

Insulin-like Growth Factor (IGF) and IGF Binding Proteins in Growth Hormone Dysregulation and Abnormal Glucose Tolerance in Small Cell Lung Cancer Patients

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Growth hormone (GH) regulation, glucose tolerance and serum concentrations of insulin-like growth factor (IGF) and IGF binding proteins (IGFBP) have been investigated in small cell lung cancer (SCLC) patients. Elevated serum GH was observed in the patient and smoking control groups but not in non-smoking control subjects. Glucose suppression of GH was observed in the few SCLC patients with raised basal GH but most SCLC patients exhibited a paradoxical increase in GH following oral glucose. Abnormal glucose tolerance and insulin resistance with respect to plasma glucose was observed in most patients. Patients showing GH dysregulation exhibited higher serum concentrations of IGFBP-2 than those showing no increase in GH. Abnormal glucose tolerance was associated with decreased serum concentrations of IGF-I. Given reports of elevated IGFBP secretion in SCLC and inhibition of IGF-I bioactivity by IGFBPs, these findings may indicate that increased serum IGFBPs disrupt IGF-I regulation of GH secretion and glucose homeostasis.

Key words: insulin-like growth factor, glucose, somatotropin, smoking, lung neoplasms
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INTRODUCTION

ALTHOUGH IMMUNOREACTIVE growth hormone (GH) has been detected in approximately 40% of small cell lung cancers (SCLC) [1], raised serum GH concentrations have been reported in relatively few SCLC patients. In a study of 110 non-fasted patients, only one was observed to have increased serum GH concentrations [2], and in a more recent report describing raised growth hormone-releasing hormone (GHRH) in lung cancer patients, 5 of 44 fasted SCLC patients had mildly elevated basal GH concentrations [3]. In contrast, during a study of multiple tumour markers in SCLC patients, we observed that serum GH was frequently markedly elevated compared with control subjects. However, single GH determinations may be unreliable since serum GH levels are labile and subject to rise with stress and activity. Hence, we have carried out serial GH determinations during a standard oral glucose tolerance test (oGTT) in a cohort of 20 SCLC patients to begin to elucidate the relationship between the presence of tumour and elevated GH. We now report the findings of these studies and present evidence of abnormal GH regulation rather than of tumour overproduction, and evidence for insulin resistance with respect to blood glucose in the majority of SCLC patients investigated. Since inappropriate GH hypersecretion is seen in disorders associated with low insulin-like growth factor-I (IGF-I) levels [4–7], we have examined the relationship between our observations and changes in circulating IGFs and IGF binding proteins (IGFBPs) in SCLC patients. This is particularly pertinent given previous studies demonstrating IGFBP production by lung tumour cell lines *in vitro* and *in vivo* [8] and inhibition of serum IGF-I bioactivity by IGFBPs [9].

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PATIENTS AND METHODS

Patients

In study one, sera from newly diagnosed pretreatment SCLC patients were assayed for growth hormone. The SCLC group consisted of 23 non-fasted individuals, eight females and 15 males (age range 39–75 years). 13 patients had extensive disease. The control groups comprised 28 healthy smoking individuals (nine females, 19 males; age range 23–68 years) and 17 normal non-smokers (seven females, 10 males; age range 27–57 years).

In study two, 20 consecutive SCLC patients admitted to

hospital prior to therapy were subjected to a standard oGTT. The group comprised 14 males and six females (age range 47–81 years). Diagnosis was established by sputum cytology or histology of biopsy samples. Disease extent was staged by routine chest X-ray and computed tomography (CT) scan of chest and upper abdomen. Bone scans, skeletal radiology and brain CT scans were carried out if there were relevant symptoms. Disease was staged as limited if confined to the primary site and mediastinal nodes. Extensive disease was defined as disease spread to the contralateral lymph node groups outside the chest or distant metastases. 13 patients had extensive disease. Performance status (PS) was defined according to WHO [10] criteria: 3 patients were PS 0, 3 were PS 1, 10 were PS 2 and 4 patients were PS 3. None of the patients included in the study were cachectic.

Approval for the study was granted by the local hospital ethics committee.

Procedures

Patients undergoing oGTT were fasted overnight and breakfast was substituted by a standard oGTT with 75 g glucose at 8 a.m. To draw blood, a 18-gauge cannula was inserted in an antecubital vein of the right arm 30 min before oGTT and connected to an infusion of 0.9% saline to keep it patent. Blood was drawn into tubes containing sodium fluoride, centrifuged and analysed for glucose as described below. For both study one and study two, venous blood was also drawn into tubes for separation of serum. After clot retraction at 4°C and centrifugation at 1550*g* for 15 min at 4°C, aliquots of serum were stored at −70°C until assayed for serum levels of GH, insulin, IGF-I, IGF-II, IGFBPs and cortisol as described below.

Serum assays

In study 1 serum GH concentrations were determined using an immunoradiometric (IRMA) assay (Biogenesis Ltd, Bournemouth, U.K.), according to the manufacturer's instructions. The monoclonal antibodies used in this assay show minimal crossreactivity with human placental lactogen, human thyroid-stimulating hormone, human prolactin, human luteinising hormone or human follicle-stimulating hormone. The intra- and interassay coefficients of variation (%CV) were <8% and the assay has a sensitivity of 0.06 ng/ml at the 95% confidence limit. In study 2, serum GH levels were determined using a two-site IRMA assay calibrated against IS 80/505 in the Department of Clinical Biochemistry, Addenbrooke's Hospital (Cambridge, U.K.). The two assays gave concordant results (data not shown).

Serum glucose was measured by a standard hexokinase method on Dupont Dimension, Department of Clinical Biochemistry, Addenbrooke's Hospital, Cambridge [11]. Insulin determinations were performed using a two-site immunoenzymometric assay calibrated against IRP 66/304 with between-batch imprecision of less than 15% [12].

Serum IGF-I was determined using an IGF-I IRMA assay (DSL Europa, Frankfurt, Germany), as described previously [8]. The assay includes an extraction step to facilitate accurate determination of IGF-I. The detection limit was 0.3 ng/ml IGF-I at the 95% confidence limit and the intra- and interassay coefficients of variation were <8 and <17%, respectively. The anti-IGF-I antisera used in the assay showed no crossreactivity with IGF-II, insulin, proinsulin or GH. Serum IGF-II was determined by IRMA as previously described [13]. Prior to IGF-II determination, serum was acidified with 8 M formic acid containing Tween 20 and subsequently extracted with acetone [14]. The assay has a sensitivity (2.5 S.D. from zero concentration of IGF-II) of 30 ng/ml, and a coefficient of variation of less than 10% between 200 and 4500 ng/ml within and between assays.

The BIOTEC PP12 enzyme-linked immunoassay (Biotec Diagnostica, Hanover, Germany) was used to determine serum levels of IGFBP-1. The assay shows no crossreactivity with human chorionic gonadotrophin, alpha-fetoprotein or human prolactin. For the quantitative measurement of serum IGFBP-2, the assay was essentially that described by Schwander and Mary [15], with the exception that the anti-IGFBP-2 antiserum was supplied by Upstate Biotechnology Inc. (Lake Placid, New York, U.S.A.). This antiserum shows no significant crossreactivity with IGFBP-1, -3, -4 or -5. The assay has coefficient of variation of less than 10% within and between assays. An IGFBP-3 radioimmunoassay (Mediagnost GmbH, Tübingen, Germany) was used for the quantitative determination of serum IGFBP-3. The assay sensitivity is 0.06 ng/ml. The antiserum recognises quantitatively the complete IGFBP-3 complex and is unaffected by excess IGF-I or -II, and shows no crossreactivity with IGFBP-1 or -2.

Cortisol was measured by a solid phase radioimmunoassay (IDS) with between-batch precision (CV%) of less than 8%.

Statistical analyses

Two-tailed Mann–Whitney U-tests were used to compare serum concentrations of IGFs and IGFBPs in SCLC patients subgroups classified according to glucose tolerance status and GH response to glucose administration.

RESULTS

Study 1

Serum GH measurements in non-fasted subjects. Figure 1 shows the serum GH values obtained in non-fasted healthy non-smoking controls, smokers and SCLC patients using the Biogenesis GH immunoradiometric assay. For non-smokers, GH concentrations ranged from <0.17 to 1.44 mIU/l with 11 of 17 individuals having values below 0.17 mIU/l. For healthy smokers, GH concentrations ranged from 0.17 to 33 mIU/l. Eight of 28 smokers had levels outside the range for non-smokers and of these, four showed concentrations above 10 mIU/l, usually considered the upper normal limit. GH concentrations in SCLC patients ranged from 0.17 to 53 mIU/l. Serum GH concentrations in 11 of 23 patients were above the range for

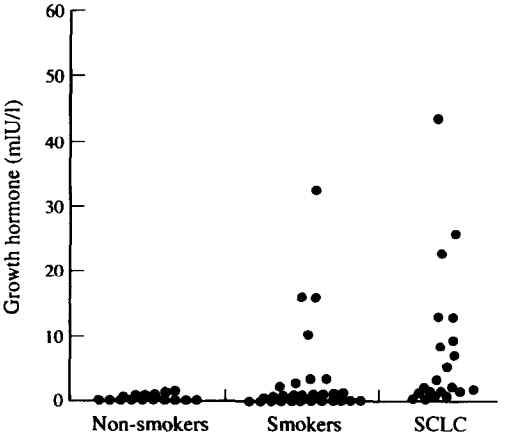

Figure 1. Serum concentrations of growth hormone in non-fasted non-smokers, smokers and small cell lung cancer patients.

normal non-smokers and of these, 6 showed concentrations above 10 mIU/l. There was no relationship between elevated GH concentrations and disease stage. Elevated serum GH concentrations were seen in patients who had ceased smoking for several years, who had recently stopped, and who were smoking at the time of the study. None of the patients with raised serum GH manifested clinical features associated with GH hypersecretion.

Study 2

Serum GH measurements during oral glucose tolerance test. Of the 20 SCLC patients included in this study, 3 had basal serum GH levels of between 9 and 10 mIU/l after an overnight fast. In all 3 patients, serum GH values fell in response to glucose administration (see Figure 2a, for example). 3 patients had basal serum GH values of <1 mIU/l which did not rise above 1 mIU/l during the oGTT. For the remaining 14 patients, basal serum GH levels were in the range of <1–6 mIU/l. Of these, 12 showed a paradoxical rise in GH levels following oral glucose administration (see Figure 2b, for example), with peak values in the range of 13–86 mIU/l. Where a paradoxical increase in serum GH was observed, this was most frequently between 30 and 60 min after glucose administration. There was no relationship between GH hypersecretion in response to glucose and disease stage or performance status. As in study one, no clinical features associated with GH hypersecretion were observed.

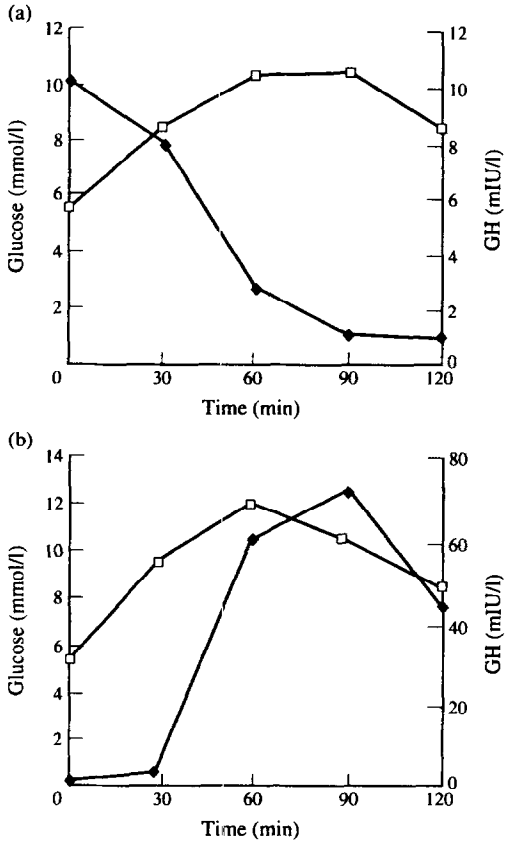

Figure 2. Serum growth hormone (GH) ◆ and glucose □ concentrations during an oral glucose tolerance test in small cell lung cancer patients. (a) shows an example of glucose suppression of GH in a patient with a raised basal GH concentration; an example of the paradoxical rise in GH concentration seen in the majority of SCLC patients following glucose intake is shown in (b).

Serum glucose and insulin measurements during oGTT. Of the 20 patients studied, 5 showed normal glucose tolerance (NGT), 10 showed impaired glucose tolerance (IGT) and 5 showed diabetes mellitus (DM), as defined by WHO [16] criteria. Table 1 shows the changes in serum glucose and insulin concentrations with time after glucose administration in NGT, IGT and DM patients. Determination of insulin concentrations in the SCLC patients revealed in all cases a marked rise in serum insulin following glucose. There was no relationship between glucose tolerance status and disease stage of performance status. None of the patients exhibited symptoms associated with the development of diabetes. Abnormal glucose tolerance was observed both in patients with and without GH dysregulation.

A summary of the sex, age range and weight range of the SCLC patients subclassified according to GH response to glucose and glucose tolerance status is given in Table 2.

Serum IGFs and IGFBPs. Table 3 shows the serum levels of the IGFs and IGFBPs in fasted SCLC patients subclassified either according to GH response to glucose administration or according to glucose tolerance status. It can be seen that while there are no significant differences in the serum concentrations of IGF-I, IGF-II and IGFBP-1 between patients showing normal GH response to glucose administration (N) and those showing a paradoxical rise in GH (P) ($P > 0.05$ in all cases), marked differences in the levels of serum IGFBP-2 and IGFBP-3 exist between the two patient groups. Thus, it can be seen that serum concentrations of IGFBP-2 are significantly higher ($P < 0.025$) and serum concentrations of IGFBP-3 significantly lower ($P < 0.05$) in patients showing a rise in GH following oral glucose compared to patients showing glucose suppression of GH.

It can also be seen from Table 3 that SCLC patients with IGT or DM have lower circulating levels of IGF-I compared to serum concentrations in SCLC patients showing NGT ($P < 0.05$). In contrast, no significant difference between IGF-II concentrations in patients with NGT, IGT and DM was observed ($P > 0.05$). No significant differences in serum concentrations of IGFBP-2 and IGFBP-3 between NGT, IGT and DM patients were observed ($P > 0.05$ in all cases), although higher IGFBP-1 concentrations were observed in patients with DM.

Serum cortisol. Serum cortisol concentrations ranged from 350 to 1020 nmol/l. Of the 20 patients studied, 4 had cortisol concentrations above 650 nmol/l, the upper limit of the normal range for samples collected in the morning. No relationship was observed between cortisol concentrations and GH response to glucose or glucose tolerance status.

DISCUSSION

The finding of the oGTT study that basal GH concentrations were not raised in the majority of fasted SCLC patients, together with the observed glucose suppression of GH in those patients with higher basal concentrations, militates against the tumour as the source of elevated serum GH seen in unfasted SCLC patients. Similarly, the findings of the oGTT study make it unlikely that tumour-derived GHRH which has been detected in 25% of small cell lung tumours [3], explains the glucose-related hypersecretion of GH by SCLC patients. Rather, the observed paradoxical increases in serum GH seen after glucose administration in fasted SCLC patients suggested that elevated serum GH concentrations observed in unfasted SCLC patients reflect GH hypersecretion in response to food intake. The oGTT

Table 1. Serum glucose and insulin concentrations in small cell lung cancer patients classified according to glucose tolerance status

		Time (min) 0	30	60	90	120
NGT (n = 5)	Glucose (mmol/l)	5.3 (4.7–5.8)	9.1 (8.2–11.0)	9.0 (8.2–11.1)	6.8 (6.4–11.5)	6.4 (5.2–7.6)
	Insulin (pmol/l)	49 (32–90)	336 (212–472)	258 (222–388)	429 (116–736)	133 (68–464)
IGT (n = 10)	Glucose (mmol/l)	5.3 (3.9–5.6)	8.5 (5.2–10.1)	9.6 (6.6–14.5)	9.7 (6.7–13.6)	9.1 (6.2–10.1)
	Insulin (pmol/l)	50 (18–133)	210 (102–394)	240 (113–564)	252 (162–584)	286 (202–472)
DM (n = 5)	Glucose (mmol/l)	6.0 (5.2–7.0)	10.5 (9.3–13.5)	12.3 (9.7–14.1)	12.4 (11.4–14.4)	11.7 (11.3–13.1)
	Insulin (pmol/l)	41.5 (21–56)	220 (74–520)	308 (126–368)	400 (248–696)	408 (194–644)

Values are expressed as median (range). NGT, normal glucose tolerance; IGT, impaired glucose tolerance; DM, diabetes mellitus.

Table 2. Details of small cell lung cancer patients showing abnormal glucose tolerance and growth hormone dysregulation

	NGT	IGT	DM	N	P
Male:female	3:2	8:2	3:2	5:3	9:3
Median age, years (range)	69 (66–72)	65 (47–81)	68 (64–76)	65 (47–81)	69 (58–77)
Median weight, kg (range)	54 (52–102)	71 (55–88)	70 (51–81)	74 (51–102)	66 (53–78)
Steroids (n)*	2	0	0	1	1

NGT, normal glucose tolerance; IGT, impaired glucose tolerance; DM, diatetes mellitus; P, paradoxical rise in growth hormone; N, no rise in growth hormone following oral glucose. *Where n is the number of patients receiving steroids at the time of blood sample collection. One patient had stopped steroid treatment 2 weeks prior to the study. All other patients had received no steroid treatment.

Table 3. Serum IGFs and IGFBPs in SCLC patients showing growth hormone dysregulation and abnormal glucose tolerance

	Median (range) IGF-I (ng/ml)	IGF-II (ng/ml)	IGFBP-1 (ng/ml)	IGFBP-2 (ng/ml)	IGFBP-3 (ng/ml)
SCLC*					
N (n = 8)	168 (100–340)	330 (238–773)	16 (2–60)	780 (365–1425)	6610 (3610–16 830)
P (n = 12)	157 (95–315)	336 (122–650)	25 (2–85)	1300 (725–3370)	3650 (1530–12 020)
SCLC†					
NGT (n = 5)	285 (205–340)	379 (216–650)	23 (2–73)	1350 (365–3370)	5530 (1980–7510)
IGT (n = 10)	110 (95–290)	343 (122–560)	23 (2–85)	1160 (550–1710)	4090 (1530–16 830)
DM (n = 5)	130 (100–271)	333 (307–773)	44 (10–85)	1000 (600–1800)	3660 (3000–14 100)

*SCLC patients subclassified according to growth hormone response to glucose: N, growth hormone—no paradoxical rise; P, paradoxical rise. † SCLC patients subclassified according to glucose tolerance status: NGT, normal glucose tolerance; IGT, impaired glucose tolerance; DM, diabetes mellitus; IGF, insulin-like growth factor; IGFBP, IGF binding protein; SCLC, small cell lung cancer.

study also revealed that the majority of SCLC patients, most of whom were of good performance status, showed abnormal glucose tolerance and insulin resistance with respect to blood glucose. Although it is well recognised that GH hypersecretion and impaired glucose tolerance are often associated, dysregulation of GH secretion and abnormal glucose tolerance did not co-exist in all SCLC patients. In the present study, 75% of SCLC patients showing a paradoxical rise in GH following glucose exhibited impaired glucose tolerance or DM and 60% SCLC patients with impaired glucose tolerance showed GH hypersecretion. Thus, inappropriate GH hypersecretion following glucose administration, and insulin resistance with respect to blood glucose occur frequently in patients with SCLC. Indeed, of the 20 patients studied for glucose tolerance, only 2 showed neither GH dysregulation nor impaired glucose tolerance.

Aberrant regulation of both GH secretion and glucose homeostasis can occur when insulin-like growth factor is deficient [4–7, 17] or its bioactivity diminished by IGFBPs [9]. Importantly, serum concentrations of IGFBP-2 have been found to be elevated in SCLC patients taken as a whole (1259 ± 162 ng/ml) compared to healthy age and sex-matched adults (362 ± 39 ng/ml), indicating hypersecretion of IGFBP-2 in many SCLC patients. *In vitro* and *in vivo* studies have demonstrated the frequent expression of IGFBP genes, particularly of IGFBP-2 [18, 19], and binding protein secretion by SCLC and non-SCLC tumours [8] suggesting that the observed increase in

serum concentrations of IGFBP in SCLC patients probably results from tumour overproduction. One possible explanation for the findings of the present study is that in SCLC reduced IGF bioavailability and/or bioactivity consequent to IGFBP excess, contributed at least in part to GH dysregulation and to altered insulin sensitivity and impaired glucose homeostasis. Several lines of evidence support this contention. Firstly, patients with inappropriate GH hpersecretion showed almost a 2-fold increase in serum concentrations of IGFBP-2 compared with SCLC patients showing glucose suppression of GH, consistent with the contention that GH dysregulation may result from IGFBP-mediated disruption of IGF-I feedback effects. Secondly, impaired glucose tolerance and DM in SCLC patients was associated with reduced serum IGF-I concentrations. Thus, patients with impaired glucose tolerance and DM had lower mean serum concentrations of IGF-I (156 ± 19 ng/ml) compared to SCLC patients showing normal glucose tolerance (279 ± 30 ng/ml), and compared with healthy subjects (231 ± 18 ng/ml). Thirdly, there was a trend towards increased fasting levels of IGFBP-1 with impaired glucose tolerance and DM. IGFBP-1 concentrations are typically <20 ng/ml using this assay, indicating a 2-fold increase in IGFBP-1 in SCLC patients with diabetic glucose tolerance. Importantly, a number of studies have shown that IGF-I and IGFBP-1 are involved in the regulation of glucose homeostasis. Recombinant IGF-I (rhIGF-I) increases glucose uptake by skeletal muscle *in vivo* following rhIGF-I adminstration [20]. RhIGF-I has also been shown to increase insulin sensitivity of target tissues through the decrease of glucose and consequently insulin levels [21,22], and the suppression of GH secretion. Importantly, it has also been demonstrated that increased IGFBP-1 can inhibit IGF-induced peripheral glucose uptake [23] and that increased IGFBP-1 present in the serum from diabetics is associated with decreased IGF-I bioactivity in a porcine cartilage IGF-I assay [9]. Such findings lend support to the hypothesis that in SCLC reduced IGF bioavailability and/or bioactivity consequent to IGFBP excess, contributes at least in part to altered insulin sensitivity and impaired glucose homeostasis. It will be important to determine whether the sera from the SCLC patients showing abnormal glucose tolerance or GH dysregulation have reduced IGF bioactivity as has been described for the serum of diabetics with rasied IGFBP-1 [9].

An important caveat to the contention that the abnormalities in GH regulation and glucose metabolism seen in the present investigation are tumour-related, derives from studies which show that smoking acutely increases GH levels and impairs insulin action in healthy habitual smokers [24]. Indeed, the finding in the present study that GH levels were elevated in some control cigarette smokers compared to control non-smoking subjects may be related to the acute effects of smoking on GH levels. In addition, impaired insulin action has also been described in chronic cigarette smokers although the plasma glucose response to oral glucose was similar in smokers and non-smokers [25]. The finding that cigarette smoking can alter GH levels and impair insulin action raises the possibility that GH dysregulation and abnormal glucose tolerance in SCLC patients reflects the smoking habits of the patients rather than the presence of malignancy. Although lack of oGTT data in an appropriately matched smoking control group for SCLC patients in the present study is a limitation of this report, no evidence for abnormal glucose tolerance was found in a study of 22 male smokers, of similar age to those in the present study, studied for the effect of smoking on lipoprotein and hormone patterns [26]. In addition, it is important to note that in the present study, GH dysregulation and abnormal glucose tolerance in SCLC patients occurred in the absence of continued smoking. Indeed, 2 SCLC patients, 1 of whom exhibited impaired glucose tolerance, the other occult diabetes, had ceased smoking for 10 years. Although there are no data on whether the effects of chronic cigarette smoking are reversible, there is evidence that the acute effects of smoking on GH and insulin action are reversible following only 2 days of abstinence from cigarette smoking.

On the basis of the data presented here, we believe that further study of the relationship between glucose tolerance, GH dysregulation and disturbance of the IGFBP axis in patients with lung cancer is warranted.

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