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Increased plasma motilin concentrations in small cell carcinoma of the lung

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ABSTRACT Plasma samples from 21 patients with small cell carcinoma of the lung were screened for pancreatic polypeptide, somatostatin, motilin, and vasoactive intestinal polypeptide. One patient had severe impairment of both renal and liver function. In the 20 remaining subjects vasoactive intestinal polypeptide concentrations were normal, and only two patients had increased concentrations of somatostatin. Increases in pancreatic polypeptide were detected more commonly (7/20), but these may have been non-specific age related increases. The major finding was high concentrations of motilin (>496 pg/ml) in 17 of 20 patients. Plasma motilin was subsequently assayed in 16 more patients with lung cancer, including 10 patients with non-small cell carcinoma of the lung. At concentrations over 900 pg/ml plasma motilin appears to be a tumour marker for small cell carcinoma of the lung with acceptable sensitivity (59%) and specificity (78%). The origin of increased plasma motilin in small cell carcinoma of the lung was investigated. Bombesin (gastrin releasing peptide), a peptide known to stimulate the release of motilin in man, was, as in previous studies, detected in tumour but not in plasma, except in one patient out of 21. Immunohistochemical studies failed to detect motilin antigen in biopsy samples. Motilin tumour content was found to be low in tumour tissue from three patients with small cell carcinoma of the lung who had appreciable hypermotilinaemia and from three patients with non-small cell carcinoma of the lung who had either normal or slightly raised plasma motilin concentrations. The stimulus to motilin secretion in patients with small cell carcinoma of the lung remains unclear.

Introduction

Cells cloned from small cell carcinomas of the lung have been shown to have amine precursor uptake and decarboxylation properties.[1] Not surprisingly therefore, this tumour is able to synthesise active amines and polypeptides, and small cell carcinoma of the lung is indeed one of the neoplasms most commonly associated with an ectopic hormone syndrome. Attention has usually been focused on either antidiuretic hormone or adrenocorticotrophic hormone (ACTH), because secretion of these hormones frequently produces a clinical syndrome.[2] The association of gastrointestinal hormones with small cell carcinoma of the lung has so far been poorly investigated. Only occasional reports of single cases of small cell carcinoma of the lung associated with somatostatin secretion have been published.[3 4]

The present study was based on systematic screening of plasma concentrations of four arbitrarily chosen gastrointestinal hormones—pancreatic polypeptide, somatostatin, motilin, and vasoactive intestinal polypeptide—in a small population sample of patients with small cell carcinoma of the lung. As we found raised motilin concentrations in most of our patients, subsequent studies were performed to try to elucidate the meaning of this finding.

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Methods

STUDY DESIGN

Part 1 Twenty one patients with small cell carcinoma of the lung were screened for plasma levels of pancreatic polypeptide, somatostatin, motilin, and vasoactive intestinal polypeptide.

Part 2 In view of the results of part 1, further in-

vestigations were made retrospectively (*a*) on the plasma samples (plasma bombesin) and (*b*) on biopsy samples (immunoperoxidase method for bombesin and motilin).

Part 3 Sixteen more patients, six with small cell carcinoma of the lung and 10 with non-small cell carcinoma of the lung, were recruited prospectively to assess (*a*) plasma motilin level and (*b*) motilin tumour content in surgical samples obtained in six of these patients (three with small cell carcinoma of the lung, three with non-small cell carcinoma of the lung).

PART 1

Twenty one patients with an unequivocal histological diagnosis of undifferentiated small cell carcinoma of the lung were studied. Biopsy specimens consisted of bronchial biopsies taken during fibreoptic bronchoscopy (18/21), lymph nodes obtained during mediastinoscopy (1/21), or supraclavicular lymph node biopsy (2/21). The patients were recruited in the chest department of the Hospital Erasme during eight months. They ranged in age from 37 to 74 (mean 59·8) years and were predominantly male (18 out of 21). Patients presented with symptoms due to either thoracic or extrathoracic disease. No patient presented with diarrhoea.

All the patients were categorised as having either limited (confined to the hemithorax and ipsilateral supraclavicular lymph nodes) or extensive disease, most (16/21) having extensive disease. Staging procedures consisted of fibreoptic bronchoscopy, computed tomography of the chest, computed tomography of the head, radionuclide bone scan, bone marrow biopsy, ultrasonography, and computed tomography of the abdomen. Renal and liver function were normal in all the patients except patient 14, who had combined renal and hepatic failure.

A blood sample was taken from each fasting patient and placed in a heparinised tube containing trasylol (0·5 ml for each 10 ml blood) at 0°C. After centrifugation the plasma samples were stored frozen at −20°C. Plasma concentrations of pancreatic polypeptide, somatostatin, and motilin were measured by radioimmunoassay with methods previously described.[5–7] The pancreatic polypeptide antibody, porcine pancreatic polypeptide (for labelling), and human pancreatic polypeptide (standard) were a gift from Dr RE Chance (Eli Lilly, Indianapolis, USA). The somatostatin radioimmunoassay used a commercial label (tyr-^{125}I-somatostatin, NEN, Dreiech, FRG) and our own antibody. For the motilin radioimmunoassay rabbit antiserum and porcine motilin obtained from Dr J Brown (Vancouver, Canada) were used. In our hands, the sensitivity (smallest amount of peptide distinguishable from dose zero with 95% confidence, duplicate determinations being used) was 16 pg/ml for pancreatic polypeptide, 8 pg/ml for somatostatin, and 2·4 pg/ml for motilin. The vasoactive intestinal polypeptide radioimmunoassay was developed recently according to the methods described by Long and Bryant,[8] on the basis of commercial reagents (UCB, Bioproducts, Braine L'Alleud, Belgium). The sensitivity of the vasoactive intestinal polypeptide determination was 12 pg/ml. Reference ranges for the different peptides were established from determinations in plasma samples from 20 healthy volunteers (13 male, 7 female; mean age 31, range 14–52), obtained after an overnight fast, by calculating the mean and the 99% probability interval. The results are summarised in table 1. It has been shown that the distribution of motilin and pancreatic polypeptide concentrations in fasting subjects is non-Gaussian, and non-parametric statistics should be used to delineate reference intervals.[9] In view of the small size of the reference group this seems inappropriate, but the results obtained by our approximate method correspond rather well with those published in an extensive study on 196 subjects.[9]

Table 1 *Reference intervals for the different peptides*

Peptide	*Mean**	*SD**	*Reference interval*
Motilin	202	103	0–496
PP	176	107	0–482
Somatostatin	33	15	0– 76
VIP	47	31	0–135

*Mean and t SD as determined in a group of 20 healthy volunteers, where *t* is the value from the Student's distribution delineating the 99% probability interval.
PP—pancreatic polypeptide; VIP—vasoactive intestinal peptide.

PART 2

(*a*) Plasma concentrations of bombesin were measured six months later in stored samples from the same patients. The measurements were performed with tracer and antibody supplied by Amersham (Buckinghamshire) and according to the methods suggested by the manufacturer. Bombesin standard was obtained from UCB Bioproducts. In our hands, the sensitivity of the assay system was 10 pg/ml after an incubation period of two days. The highest detectable level in normal subjects was found to be 30 pg/ml. The concentration of gastrin was also measured in stored sera, which were available in 11 cases (Nos 3, 5, 6, 7, 10, 11, 13, 15, 18, 20, 21), the normal range being 0–115 pg/ml.

(*b*) In 12 patients (cases 1, 2, 3, 5, 6, 9, 11, 13, 14, 18, 20, 21) biopsy material of sufficient size and free from crush artefact was available for immunohistochemical studies. The specimens were those used initially for diagnosis, after they had been fixed in

Bouin's liquid, dehydrated, and embedded in paraffin. An indirect peroxidase method was used. Paraffin sections were first incubated with rabbit antiserum for three hours at room temperature. Bombesin antibody was obtained from Merseyside (England) and was used according to the recommendations of the manufacturer; motilin antibody was the same as that used for plasma radioimmunoassay and was used at a 1:400 dilution. The slides were then washed. In the second step swine antirabbit immunoglobulin serum coupled with horseradish peroxidase was incubated for one hour. The presence of the enzyme was detected by the precipitation of diaminobenzidine in the presence of 0·01% H_2O_2 according to the method of Graham and Karnovsky.[10] Negative controls were achieved by omitting the antiserum, while the reactivity of the antisera used was confirmed by positive staining of normal human tissues, including fetal lung, for bombesin[11] and jejunal mucosa for motilin.[12]

PART 3

(*a*) A further 16 patients with lung cancer were recruited over two months. Six of them (four men, two women, aged 57–66) had small cell carcinoma of the lung; 10 patients (six men, four women, aged 52–74) had non-small cell carcinoma of the lung. The histological diagnosis was based, as in part 1, on analysis of bronchial biopsies (12/16), lymph nodes obtained at mediastinoscopy (3/16), or supraclavicular (1/16) lymph node samples. In the non-small cell carcinoma of the lung group four patients underwent thoracotomy, and examination of the surgical specimen confirmed the diagnosis of non-small cell carcinoma of the lung. The non-small cell carcinoma of the lung group comprised five patients with squamous cell carcinoma, three patients with adenocarcinoma, one patient with undifferentiated large cell carcinoma, and one with alveolar cell carcinoma. Five patients with small cell carcinoma of the lung and two with non-small cell carcinoma of the lung had extensive disease. Blood sampling and motilin radioimmunoassay were performed as in part 1.

(*b*) Surgical samples were obtained from three patients with small cell carcinoma of the lung (mediastinal lymph nodes from two and subcutaneous metastasis from one) and three with non-small cell carcinoma of the lung (mediastinal lymph nodes from one patient with a large cell undifferentiated carcinoma, a thoracotomy specimen from one patient with adenocarcinoma, and bronchial biopsies and a thoracotomy specimen from one patient with a squamous cell carcinoma). The presence of tumour in each sample was confirmed independently by a histopathologist. The presence of motilin was assessed by using the radioimmunoassay that was used for the plasma samples.

Results

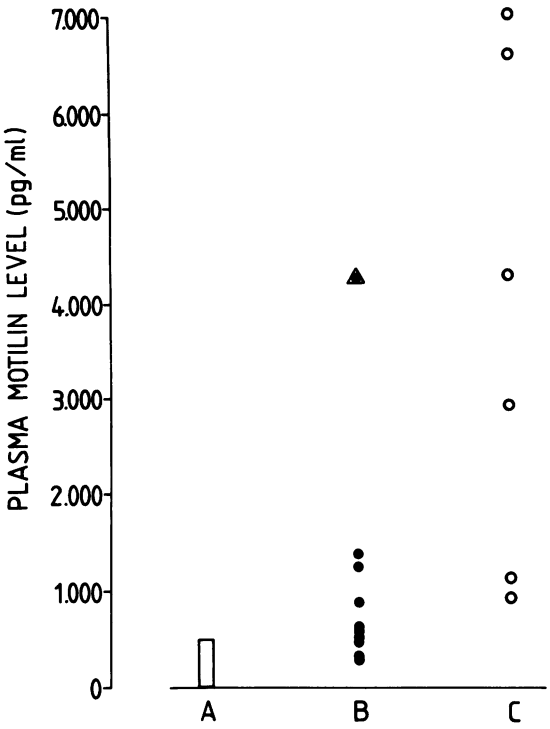
PART 1

Table 2 summarises the results of the hormonal assays. One patient (case 14) had increased concentrations of somatostatin, motilin, and vasoactive intestinal polypeptide of equivocal significance because of combined renal and liver failure. Of the 20 remaining patients, none had an increased vasoactive intestinal polypeptide concentration, and a significantly increased somatostatin concentration was detected in the plasma of only two patients. In both of these patients, however, appreciable hyperglycaemia of recent onset was present and increased concentrations of 8 am serum cortisol and 24 hour urinary excretion of cortisol suggested ectopic ACTH secretion. An increased concentration of pancreatic polypeptide occurred more commonly (7/20). The most prominent finding was an increase of motilin concentration in 17/20 patients. The increase was substantial in 10 out of the 17 patients, three patients having an extremely high level, ranging from 3094 to 6718 pg/ml—that is, from 6 to 13 fold the upper limit of our reference interval. The 15 patients with extensive disease had only slightly greater motilin concentrations (mean 1797 pg/ml) than the five patients with limited disease (mean 801 pg/ml), with a wide overlap between the two groups.

Table 2 *Plasma concentrations (pg/ml) of gastrointestinal hormones in 21 patients with small cell carcinoma of the lung*

Patient No	*Somatostatin*	*PP*	*Motilin*	*VIP*
1	52	624**	525*	26
2†	12	122	342	20
3	18	656**	645*	37
4	56	410	6718**	7
5	16	122	1096**	33
6	70	908**	1637**	13
7	21	796**	92	38
8†	26	462	765*	0
9	769**	230	1833**	22
10†	18	578*	940**	30
11	36	234	882*	12
12	47	280	6005**	26
13†	11	220	1581**	16
14	168**	440	590*	155*
15	17	420	539*	16
16	79	230	1576**	8
17	20	200	1005**	17
18†	0	83	376	70
19	324**	120	745*	24
20	86	840**	558*	13
21	53	560*	3094**	21

*Values exceeding $\bar{X} + t$ (1%) SD.
**Values exceeding $\bar{X} + t$ (0·1%) SD.
†Patients with limited disease.
PP—pancreatic polypeptide; VIP—vasoactive intestinal polypeptide.



Plasma motilin concentrations in 16 patients with lung cancer. A—normal range; B—10 patients with non-small cell carcinoma of the lung (closed circles, except for the one patient with alveolar cell carcinoma, who is represented by a triangle); C—six patients with small cell carcinoma of the lung (open circles).

PART 2

(*a*) *Plasma bombesin* was normal in all patients except patient 14, who had a very large concentration (1200 pg/ml). A second assay for bombesin performed two months later yielded identical results. *Serum gastrin* concentration was normal (range 51–113 pg/ml) in the 11 patients studied.

(*b*) *Immunoperoxidase studies* on biopsy samples detected bombesin in 11 out of 12, including that from the patient with the increased plasma concentration. Motilin was not detected in any biopsy specimens, although the group investigated included five patients with moderate and five more patients with substantial increases in plasma concentrations.

PART 3

(*a*) As shown in the figure, *plasma motilin* concentration exceeded 900 pg/ml in all patients with small cell carcinoma of the lung (range 910–7000 pg/ml). In the non-small cell carcinoma of the lung group, the one patient with alveolar cell carcinoma had a plasma motilin concentration of 4290 pg/ml. Plasma motilin concentrations exceeded 900 pg/ml in two other patients from the group, while the remaining seven had either normal (n = 4) or moderately increased (n = 3) levels.

(*b*) *Tumour motilin* content was respectively 0·5, 0·5 and 2 pg/mg in the three patients with small cell carcinoma of the lung (plasma concentrations 910, 1130, 6580) and 0·5 and 0·6 pg/mg in the first two patients with non-small cell carcinoma of the lung (plasma concentrations 280 and 495). In the third patient (plasma concentration 630) it was 1 pg/mg in the lung tumour sample and 34 pg/mg in the bronchial biopsy sample.

Discussion

The present study was initially designed as a systematic screening of plasma concentrations of gastrointestinal hormones in patients with small cell carcinoma of the lung. Our inability to detect any increases in vasoactive intestinal polypeptide, as well as only rare occurrence of hypersomatostatinaemia, was not surprising in view of previous reports.[3 4 13–15] Indeed, vasoactive intestinal polypeptide secreting tumours appear to be limited to the pancreas and sympathetic chain. Long *et al*, who screened 12 patients with lung cancer (cell type not given) and diarrhoea for plasma vasoactive intestinal polypeptide concentration, did not find a single increased concentration.[13] Vasoactive intestinal polypeptide tumour content was found to be undetectable in patients with small cell carcinoma of the lung by Wood *et al*.[14] In contrast, these authors reported the presence of somatostatin in tumour tissue, and this peptide was also detected in cell cultures from patients with small cell carcinoma of the lung.[15] Symptomatic hypersomatostatinaemia, including hyperglycaemia and steatorrhoea of recent onset, has been described as the presenting manifestation of small cell carcinoma of the lung.[4]

The detection of increased concentrations of pancreatic polypeptide, which occurred in seven of our 20 patients, was somewhat more unexpected. Increased plasma concentrations of this peptide have been reported once in a patient with a bronchial carcinoid tumour,[16] but not in small cell carcinoma of the lung. It is known that pancreatic polypeptide concentrations increase with age. Our reference values relate to a group of volunteers with a mean age of 31 years, but all seven abnormal patients were men aged from 49 to 63. In the 20–40 year age group an extensive study[9] determined the upper limit of normal for men to be 306 pg/ml and for women to be 444 pg/ml, but for the 40–60 year age group the upper limit was 738 pg/ml. If the latter limit is applied to our experimental

group, only three patients should be considered as having slightly raised pancreatic polypeptide concentrations.

The most striking feature of this study was the detection of increased motilin concentrations in most (17/20) of the patients, concentrations exceeding 900 pg/ml in the plasma from 10 patients. In the fasting state motilin concentrations are known to fluctuate in accordance with the so called migrating motor complex (a specific pattern of motor activity that originates in the gastroduodenal area, migrates down the small intestine, and begins again as the complex reaches the terminal ileum).[17] These fluctuations, however, can be revealed only by careful statistical analysis.[7] The maximum difference between the highest and the lowest value during one interdigestive cycle may be estimated at around 100 pg/ml,[7 17] so that a fortuitous timing of blood sampling in the patient group cannot explain our findings. Furthermore, age does not affect plasma motilin in normal subjects.[9] Hypermotilinaemia has been reported in patients with renal insufficiency[18] and in patients with acute diarrhoea[19] but, to the best of our knowledge, it has not been previously described in association with small cell carcinoma of the lung.

The hypothesis that plasma motilin could be a sensitive and specific diagnostic marker for small cell carcinoma of the lung was tested by measuring plasma concentrations in six more patients with small cell carcinoma of the lung and in a somewhat larger control group of patients with non-small cell carcinoma of the lung. If our patient with alveolar cell carcinoma is excluded from the analysis, it is necessary to consider 900 pg/ml as the threshold value for specificity to reach an acceptable (78%) level. This, however, results in a loss of sensitivity, which falls to 59% when all 27 patients with small cell carcinoma of the lung studied are pooled. As a comparison, the reported sensitivity for neurone specific enolase, now considered as one of the best markers for small cell carcinoma of the lung, ranges in large series from 69%[20] to 85%,[21] with a specificity of about 75%.[21]

The significance of the increase in plasma motilin concentrations in small cell carcinoma of the lung is unclear. As a first hypothesis we considered the possibility that it was associated with circulating gastrin releasing peptide. The latter peptide is the mammalian counterpart of amphibian bombesin and is therefore usually called simply bombesin. Detected in a large majority of small cell carcinoma of the lung cell cultures[22-24] and tumour biopsy specimens,[14] it appears to be an intracellular marker of small cell carcinoma of the lung combining a high sensitivity with a high specificity.[24] The ability of bombesin to stimulate the release of motilin in man has been well documented.[25] Furthermore, as we are not aware of any report of a motilin containing tumour, even among gut and pancreatic neoplasms, the hypothesis that motilin was derived from a non-neoplastic source did not seem unreasonable. Plasma bombesin concentrations were, however, normal in all but one of our patients. Although the bombesin measurements were performed later, the suggestion that false negative results were obtained owing to degradation of the peptide during sample storage can be discounted because we obtained high values from the stored plasma of one patient, assessed by a reproducible duplicate determination after a two month interval. It might be speculated that circulating bombesin is either rapidly broken down in plasma or present in a form not recognised by conventional radioimmunoassay. Normal serum gastrin levels, however, which were found in 19 patients, make the presence of circulating bombesin extremely unlikely.[25] In tumour tissue immunoreactive bombesin was detected in samples from 10 patients out of the 12 who were investigated. Our results compare well with the findings of other investigators. Sorenson *et al*, studying 27 patients with small cell carcinoma of the lung, detected increased plasma bombesin in only two patients despite detection of tumour bombesin in all of them.[24] In another study by the same group of authors on 31 patients none had hyperbombesinaemia.[26]

The second possibility is that motilin was derived from a neoplastic source. Immunoperoxidase studies failed, however, to detect motilin antigen in the biopsy samples. In a subsequent study, motilin tumour content was assessed by radioimmunoassay of surgical samples obtained from three patients with small cell carcinoma of the lung with a plasma motilin concentration ranging from 910 to 6580 pg/ml. The values found in those patients, as well as in three patients with non-small cell carcinoma of the lung who had plasma motilin concentrations below 630 pg/ml, used as controls, were much less than those found by us (130–200 pg/mg, TL Peeters, unpublished data) and by others[12] in motilin rich human tissue such as the first or the second part of the duodenum. These results effectively exclude the hypothesis that small cell carcinoma of the lung might be a motilinoma.

In conclusion, we have measured increased plasma motilin concentrations in samples of blood obtained from patients with small cell carcinoma of the lung. Concentrations higher than 900 pg/ml are required for the estimation to have sufficient specificity as a diagnostic marker. As tumour immunoreactivity is absent and tumour content assessed by radioimmunoassay is low, production of motilin by the neoplasm itself is excluded. Motilin might originate from a non-neoplastic source but, as circulating bombesin is undetectable, the stimulus to the release of motilin from motilin containing cells is unknown.

but may be an as yet unidentified mediator produced by the tumour.

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