Immunoreactive Gastrin-releasing Peptide as a Specific Tumor Marker in Patients with Small Cell Lung Carcinoma

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ABSTRACT

Gastrin-releasing peptide (GRP) is now known to be a very common product of small cell lung carcinoma (SCLC). With the aim of investigating the possible role of this peptide as a tumor marker of SCLC, we have developed a sensitive radioimmunoassay system for plasma immunoreactive GRP using immune-affinity chromatography for plasma extraction. Plasma immunoreactive GRP levels in control subjects were determined by using 15 ml of plasma as the starting material (minimum concentration detectable, 0.8 pg/ml). The levels in 10 control subjects were (mean ± SD) 1.2 ± 0.27 pg/ml; range, 0.86–1.7 pg/ml. This assay system was applied for the clinical use by using 3 ml of plasma as the starting material (minimum concentration detectable, 4.0 pg/ml). Plasma immunoreactive GRP levels were elevated in SCLC patients at frequencies of 71% in patients with limited disease and 80% in those with extensive disease. Furthermore, a change in the level showed excellent correlation with the therapeutic response. In six patients with complete response who had had elevated levels before treatment, the levels decreased to an undetectable range when the tumor disappeared, and they remained undetectable until 1 month later, when the patients were judged to have achieved complete response. In the partial response group, plasma immunoreactive GRP levels had decreased to an undetectable level in two of three patients, when the patients achieved partial response. In four patients with progressive disease, plasma immunoreactive GRP levels were elevated at the time of the progressive disease judgment, when compared with levels before treatment. The levels in 21 patients with non-SCLC (10 with adenocarcinoma, seven with squamous cell carcinoma and four with large cell carcinoma) were not elevated. These results indicate the plasma immunoreactive GRP level as a useful tumor marker in SCLC patients. It is now believed that GRP can function as an autocrine growth factor for SCLC. The present study suggests that the possible autocrine growth factor could serve as a reliable tumor marker for cancer patients.

INTRODUCTION

SCLC is a subtype comprising approximately 15% of all primary lung carcinomas occurring each year in Japan. Since in general SCLC is highly sensitive to chemotherapeutic agents and frequently has metastasized by the time of clinical presentation, systemic chemotherapy plays a major role in the management of these patients. Accordingly, reliable tumor markers as well as imaging diagnostic techniques could yield valuable information for the treatment of these patients.

GRP is a bombesin-like peptide present in mammalian tissues (1–4). The production of GRP/bombesin by SCLC has been reported by several investigators including ourselves (5–7), and we have shown that GRP/bombesin is a very common product of SCLC when compared with other peptide hormones, such as calcitonin and adrenocorticotropic (8). This fact suggests the possibility that the plasma GRP level could serve as a tumor marker in SCLC patients, and several investigators have measured plasma GRP levels in patients with SCLC, but only rarely found them to be elevated (9–13). In the present study, we developed a highly sensitive RIA system which detects plasma IR-GRP by using immune-affinity chromatography for plasma extraction. With this system, we measured plasma IR-GRP levels in SCLC patients, and found that the peptide is a reliable tumor marker for SCLC.

MATERIALS AND METHODS

Materials. Activated CH-Sepharose 4B and Sephadex G-50 superfine were purchased from Pharmacia Fine Chemicals AB (Uppsala, Sweden); NaCl was from New England Nuclear (Boston, MA); Trasylol was from Bayer Co. (Leverkusen, FRG); bovine serum albumin (Cohn Fraction V) was from Dai-ichi Pure Chemicals (Osaka, Japan); [125I]-human albumin and moniodoiodinated porcine GRP (1–27) were from Amersham International Public Limited Company (Buckinghamshire, UK); and a rabbit antisera against bombesin (R-1078) (14) was kindly provided by Professor John H. Walsh, Center for Ulcer Research and Education, School of Medicine, University of California, Los Angeles, CA.

Subjects. Plasma samples were obtained from 17 patients with untreated SCLC and from four with PD at the time they were examined. In these patients, the stage of disease (limited versus extensive) was decided according to the Veterans’ Administration Lung Study Group criteria (15), and the clinical stage (I to IIIM) according to the American Joint Committee on Cancer criteria (16). Clinical response was evaluated by an imaging diagnostic technique and the overall response to systemic chemotherapy was analyzed according to the World Health Organization criteria (17). Plasma samples were further obtained from 10 control subjects and 21 patients with non-SCLC. All non-SCLC patients were in the advanced clinical stages of IIIMO and IIIM1, and consisted of 10 with adenocarcinoma, seven with squamous cell carcinoma, and four with large cell carcinoma. Among these patients, 12 had not been treated, and the remaining nine had been treated with systemic chemotherapy but had not responded. Venous blood samples were drawn into chilled heparinized tubes containing Trasylol at a final concentration of 500 KIU/ml of blood, and centrifuged at 1,500 × g for 15 min at 4°C. After centrifugation, the plasma samples were stored at −20°C until extraction. The addition of Trasylol at the time of blood collection is for the purpose to inhibit the degradation of all IR-GRP components, although we have no data indicating that Trasylol is absolutely necessary for blood collection. In the case of bombesin, Tateishi et al. reported that there was no difference in the stability of bombesin added to plasma with and without Trasylol (18).

RIA. Porcine GRP RIA was performed as previously described (7),
with slight modifications. A rabbit antiserum against bombesin (R-1078) was used at a final dilution of 1:140,000. Monoiodinated porcine GRP(1–27) was used as a tracer. The specificity of the antiserum (R-1078) was reported previously (14).

Affinity Column. For the preparation of an immune-affinity column, rabbit anti-GRP antiserum, R-6902 (3), was used. A 50-µl sample of antiserum was coupled to 1 g of activated CH-Sepharose 4B according to the manufacturer's instructions (19), and a portion of the gel was packed in a small column (0.7 x 1.5 cm). At room temperature, the plasma samples (15 or 3 ml) were applied to the column and washed with 5 ml of assay buffer followed by 5 ml of distilled water. The assay buffer used in this system was a solution of 0.1 M phosphate buffer, pH 7.4, containing 0.1 M NaCl, 0.06% (v/v) monoethanolamine, 1% bovine serum albumin (Cohn Fraction V), and Trasylol (250 KIU/ml). IR-GRP was eluted from the column with 4 ml of 1 M acetic acid. [125I]humanalbumin and [125I]humanalbumin and containing porcine GRP at the concentration of 500 pg/ml (high dose) described above, and the recovery rates were determined. Furthermore, synthetic GRP(1–27) and GRP(18–27) were extracted by the method described previously (20). Samples tested were GRP-free plasma containing 60 or 3000 pg of these peptides were also examined. These recovery experiments were performed at least five times at each concentration.

The recovery of affinity columns was examined by the method described previously (20). Samples were extracted to GRP-free plasma (3 ml) containing porcine GRP at the concentration of 500 pg/ml (high dose) or 10 pg/ml (low dose) and pooled plasma. Five samples containing different doses of porcine GRP as well as samples of pooled plasma were consecutively applied to the column as reported previously (20).

Gel Filtration Studies. Extracted samples were chromatographed on a Sephadex G-50 superfine column (1.0 x 50 cm) which had been equilibrated and eluted with 1 M acetic acid. [125I]human albumin and Na[125I] were added to the samples which were then eluted at a speed of 6 ml/h from the column by the fraction collector-pump control system (7). Fractions of 0.8 ml each were collected, lyophilized, and reconstituted in the assay buffer. The column was also calibrated with porcine GRP(1–27) and porcine GRP(18–27).

Changes in Plasma IR-GRP Level following Treatment. Seven SCLC patients who achieved CR, three patients who achieved PR, and four patients with PD were examined for correlations between changes in plasma IR-GRP levels and responses to therapy. The responses to therapy were evaluated by imaging diagnostic techniques including plain and tomographic chest X-ray, and computed tomographic scan. The patients who achieved CR and PR had had no prior therapy. Plasma samples were obtained sequentially after treatment from these patients and the plasma IR-GRP levels were determined.

RESULTS

Recovery and Reuseability of Affinity Column. When 15 ml of plasma were used as the starting material, the minimum detectable amount was 0.8 pg/ml. It was 4.0 pg/ml when 3 ml of plasma were used. When 15 ml of GRP-free plasma containing 15 or 150 pg of synthetic porcine GRP(1–27) were extracted, the recovery rate was (mean ± SD) 100 ± 7.3% and 100 ± 1.5%, respectively. In the case of synthetic porcine GRP(18–27), the recovery rate was (mean ± SD) 98 ± 6.2% and 100 ± 11%, respectively. When 3 ml of GRP-free plasma containing 60 or 3000 pg of synthetic porcine GRP(1–27) were extracted, the recovery rate was (mean ± SD) 94 ± 8.4% and 96 ± 2.4%, respectively, and in the case of synthetic porcine GRP(18–27), the rate was (mean ± SD) 110 ± 24% and 100 ± 3.1%, respectively.

In the reusability study, the values of five samples consecutively extracted ranged from (mean ± SD) 480 ± 8.6 to 520 ± 11 pg/ml in the case of a high dose of plasma (500 pg/ml), and from (mean ± SD) 9.2 ± 0.07 to 10 ± 0.55 pg/ml in the case of a low dose of plasma (10 pg/ml). These results show that it is possible to use these affinity columns repeatedly.

Plasma IR-GRP Levels in Control Subjects. Plasma IR-GRP levels in the 10 control subjects were determined, with 15 ml of plasma used as the starting material. Plasma IR-GRP was measurable in all the subjects and the level was (mean ± SD) 1.2 ± 0.27 pg/ml; range, 0.86–1.7 pg/ml.

Plasma IR-GRP Levels in Lung Cancer Patients. Plasma IR-GRP levels in control subjects and lung cancer patients were measured with 3 ml of plasma used as the starting material. The plasma IR-GRP levels and the frequency of plasma IR-GRP elevation in these patients are shown in Fig. 1. All control subjects and non-SCLC patients had levels less than 4.0 pg/ml. Levels were elevated in 13 of the 17 untreated SCLC patients (76%), with values ranging from 4.6 to 58 pg/ml.

The relation between plasma IR-GRP level and stage of disease was investigated in these 17 SCLC patients. In the seven patients with LD, five had elevated levels, and these were in clinical stages I, IIIM0, IIIM1, IIIM0, and IIIM1. The other two patients, with undetectable levels, were in stages I and IIIM1. In the 10 patients with ED in clinical stage IIIM1, eight had elevated levels.

Gel Filtration Studies. Gel filtration patterns of plasma extracts prepared from a control subject and two SCLC patients are presented in Fig. 2. IR-GRP in these three plasma extracts showed two peaks. One peak eluted at the position corresponding to that of porcine GRP(1–27); the other eluted at the position of porcine GRP(18–27).

Change in Plasma IR-GRP Level following Treatment. As shown in Table 1, six of the seven patients had had elevated levels before treatment; these decreased to an undetectable range in five patients, when the tumor shadows showed a 50% regression. Levels were undetectable in all these patients when the tumor disappeared, and they remained undetectable until 1 month later, when the patients were judged to have achieved CR. In the PR group, as shown in Table 2, plasma IR-GRP levels had decreased to an undetectable range in two of the three patients, when the tumor shadows showed a 50% regression, and they remained undetectable when the patients achieved PR. In the remaining patient of the PR group, the plasma IR-GRP level decreased from 52 to 10 pg/ml after the initial course of treatment. There was a 50% regression of the tumor shadow, and, after 1 month, the clinical response was judged as PR. At this time, however, the plasma IR-GRP level
began to increase, and soon a new bone metastasis appeared, when the disease was judged to be PD. In four PD patients, as shown in Table 3, plasma IR-GRP levels were determined and compared with the levels before treatment. It was found that plasma IR-GRP levels had increased at the time of PD judgment (25% or more increase in tumor size or the appearance of a new lesion).

Table 1 Correlation between change in plasma IR-GRP level and response to therapy in SCLC patients showing CR

<table>
<thead>
<tr>
<th>Patient</th>
<th>Stage by VALSG criteria</th>
<th>Stage by AJCC criteria</th>
<th>Plasma IR-GRP (pg/ml)</th>
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<tr>
<td></td>
<td>Before treatment</td>
<td>At 50% regression</td>
<td>At disappearance</td>
</tr>
<tr>
<td>1</td>
<td>ED</td>
<td>III1</td>
<td>54</td>
</tr>
<tr>
<td>2</td>
<td>LD</td>
<td>III0</td>
<td>22</td>
</tr>
<tr>
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<td>ED</td>
<td>III1</td>
<td>8.3</td>
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<td>5</td>
<td>ED</td>
<td>III1</td>
<td>7.9</td>
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<tr>
<td>7</td>
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</table>

Table 2 Correlation between change in plasma IR-GRP level and response to therapy in SCLC patients showing PR

<table>
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<th>Patient</th>
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<th>Stage by AJCC criteria</th>
<th>Plasma IR-GRP (pg/ml)</th>
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<td>At 50% regression</td>
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</tr>
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<td>LD</td>
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<tr>
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Table 3 Correlation between change in plasma IR-GRP level and response to therapy in SCLC patients showing PD

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<th>Stage by AJCC criteria</th>
<th>Plasma IR-GRP (pg/ml)</th>
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<td>Before treatment</td>
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<tr>
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DISCUSSION

To develop a highly sensitive assay system to measure plasma IR-GRP levels, we applied an immune-affinity chromatography for extraction of IR-GRP in plasma. This procedure made it possible to concentrate IR-GRP in plasma and to remove substances that interfered in the GRP RIA. Since plasma IR-GRP levels in control subjects have not yet been reported, we have examined them and found that plasma IR-GRP levels in control subjects were 1.2 ± 0.27 pg/ml; range, 0.86–1.7 pg/ml. When compared with the levels of several gut peptides (21–24), the plasma IR-GRP levels in control subjects are very low, presumably due to the possible physiological role of GRP as a neurotransmitter (25). Gel filtration studies revealed that IR-GRP in plasma was composed of two molecular forms similar to those present in the tissue extracts, as reported previously (7).

We have shown that GRP is the most frequent hormonal product of SCLC (8). With the aim to investigate the clinical usefulness of this peptide as a tumor marker of SCLC, plasma IR-GRP levels were determined by using 3 ml of plasma as the starting material. Plasma IR-GRP levels were found to be elevated in patients with SCLC at frequency of 76%. In contrast, none of the patients with non-SCLC had elevated levels. The frequency of elevated plasma IR-GRP levels in SCLC patients was approximately equal to that of elevated carcinoembryonic antigen levels in patients with colorectal carcinoma and to that of elevated α-fetoprotein levels in patients with hepatocellular carcinoma, indicating that plasma IR-GRP levels could be a useful tumor marker in SCLC patients. Furthermore, the fact that the plasma IR-GRP level was elevated in SCLC patients with LD as well as those with ED at almost the same rate of occurrence suggests that the plasma IR-GRP level could be elevated at a relatively early stage of this disease. It is worth noting, however, that two patients with LD and two with ED had undetectable IR-GRP levels; one of them was in an early disease stage (stage I), but the disease in the other three was rather advanced. These findings agree with our previous observation (7) that, when tissue IR-GRP concentrations in a large number of SCLC tumors were determined, about 26% of the tumors did not produce IR-GRP, indicating that plasma IR-GRP levels reflect the tumor IR-GRP production.

As far as the other tumor markers for SCLC patients are concerned, it has been demonstrated that NSE is useful (26, 27). When compared to NSE, however, several factors favor GRP. GRP is considered to be secreted actively from SCLC cells into the general circulation. It is probably because of these characteristics that plasma IR-GRP levels have a tendency to be elevated in patients with LD and in those with ED at similar rates. In the case of NSE, serum levels are elevated at frequencies of 38% in patients with LD and 82% in those with ED (28). Additionally, the results presented in this report show that the plasma IR-GRP level is specifically elevated in SCLC patients, not in non-SCLC patients. In contrast, serum NSE levels have been shown to be elevated frequently in non-SCLC patients as well as in SCLC patients (29).

The correlation between changes in plasma IR-GRP levels and responses to therapy was investigated. In CR patients, the levels decreased to an undetectable range when the tumor disappeared, and they remained undetectable when the patients were judged to have achieved CR. In the PR group, plasma IR-GRP levels had decreased to an undetectable level in two of three patients, when the patients achieved PR. In all PD patients, plasma IR-GRP levels were elevated at the time of the...
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PD judgment. These results indicate that changes in the level showed excellent correlation with the therapeutic responses. SCLC has a tendency to metastasize early and widely to various organs. In such a case it is sometimes difficult to evaluate the response to the treatment by imaging diagnostic techniques. Accordingly, the determination of plasma IR-GRP level may yield valuable information for the treatment of these patients.

Recent reports have indicated that GRP/bombesin is able to function as an autocrine growth factor for SCLC in vitro and in vivo (30–33). In other types of cancer, several factors are considered to be possible autocrine growth factors; these are TGF-α (34), TGF-β (34), insulin-like growth factor I (35), platelet-derived growth factor (34), interleukin 2 (36), and granulocyte-macrophage colony-stimulating factor (37). Although there is no information that they could serve as a tumor marker for cancer patients, the present study reveals that GRP, a possible autocrine growth factor, could serve as a reliable tumor marker for cancer patients.

ACKNOWLEDGMENTS

The authors thank Dr. John H. Walsh, Center for Ulcer Research and Education, School of Medicine, University of California at Los Angeles for the generous supply of rabbit antisem against bombesin (R-1078). They also thank the physicians of the National Cancer Center Hospital for collecting the samples.

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