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Increased gastrin-releasing peptide (GRP) receptor expression in tumour cells confers sensitivity to [Arg^6,D-Trp^7,9,N^{me}Phe^8]-substance P (6–11)-induced growth inhibition

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[Arg^6,D-Trp^7,9,N^{me}Phe^8]-substance P (6–11) (SP-G) is a novel anticancer agent that has recently completed phase I clinical trials. SP-G inhibits mitogenic neuropeptide signal transduction and small cell lung cancer (SCLC) cell growth in vitro and in vivo. Using the SCLC cell line series GLC14, 16 and 19, derived from a single patient during the clinical course of their disease and the development of chemoresistance, it is shown that there was an increase in responsiveness to neuropeptides. This was paralleled by an increased sensitivity to SP-G. In a selected panel of tumour cell lines (SCLC, non-SCLC, ovarian, colorectal and pancreatic), the expression of the mitogenic neuropeptide receptors for vasopressin, gastrin-releasing peptide (GRP), bradykinin and gastrin was examined, and their sensitivity to SP-G tested in vitro and in vivo. The tumour cell lines displayed a range of sensitivity to SP-G (IC_{50} values from 10.5 to 119 μM). The expression of the GRP receptor measured by reverse transcriptase–polymerase chain reaction, correlated significantly with growth inhibition by SP-G. Moreover, introduction of the GRP receptor into rat-1A fibroblasts markedly increased their sensitivity to SP-G. The measurement of receptor expression from biopsy samples by polymerase chain reaction could provide a suitable diagnostic test to predict efficacy to SP-G clinically. This strategy would be of potential benefit in neuropeptide receptor-expressing tumours in addition to SCLC, and in tumours that are relatively resistant to conventional chemotherapy.

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Despite recent advances in cytotoxic drug development, it is a universal goal to develop novel cancer therapies that are more specific to cancer cells and produce minimal damage to nonmalignant cells. One approach to realising this goal is the development of drugs that are able to block the growth-promoting effects of cancer cell mitogens.

Neuropeptides are important growth factors in a number of cancers including breast, colon, pancreatic, prostate, renal, gastric carcinoma and small-cell lung cancer (SCLC). SCLC cells proliferate in response to a range of neuropeptide growth factors, and in many cases these neuropeptides are involved in autocrine and paracrine growth loops that fuel uncontrolled proliferation (Moody et al, 1985; Sethi and Rozengurt, 1991; Sethi et al, 1992, 1993; North et al, 1997). Drugs that target specific mitogenic neuropeptides have shown little promise. Monoclonal antibodies have been developed against circulating bombesin and one such antibody, 2A11, has been shown to inhibit the growth of SCLC in vitro and also as xenografts in nude mice (Chaudry et al, 1999); however, it has limited efficacy in human trials. Thus, 'broad-spectrum' neuropeptide receptor antagonists have been the main focus of research in this drug development strategy. Synthetic analogues of substance P, for example, [Arg^6,D-Trp^7,9,N^{me}Phe^8]-substance P (6–11) (SP-G), were initially identified as antagonists of substance P-mediated cellular effects and were subsequently found to also antagonise the cellular effects of bombesin (Jensen et al, 1984). When tested in SCLC cell lines, it was found that several substance P analogues inhibited calcium mobilisation stimulated by the neuropeptides: bombesin, bradykinin, gastrin, galanin, vasopressin, cholecystokinin and neurotensin (Woll and Rozengurt, 1988; Langdon et al, 1992; Sethi et al, 1992). They were also found to inhibit mitogenesis by the same range of neuropeptides in both Swiss 3T3 cells and SCLC cells (Woll and Rozengurt, 1988; Bepler et al, 1989; Sethi et al, 1992).

In addition to the in vitro growth-inhibitory effects of substance P analogues, these compounds inhibit the growth of tumours in xenograft models in nude mice (Langdon et al, 1992; Jones et al, 1997). SP-G has recently completed a phase I clinical trial and will be entering a phase II clinical trial in SCLC in the near future (Clive et al, 2001). The exact mechanism by which these compounds exert their antitumour effects are unknown, but we have previously shown that they act as 'biased' agonists inhibiting neuropeptide-stimulated growth while directly stimulating apoptosis (Tallett et al, 1996; MacKinnon et al, 1999, 2001). Understanding the exact manner in which substance P analogues modulate neuropeptide receptor signalling will allow for the rational design of more potent analogues. Identification of biological features in tumours that confer sensitivity to SP-G will elucidate the most effective use of this group of compounds in clinical practice.

In this study, we measured the expression of the mitogenic neuropeptide receptors for vasopressin (V_{1A}), gastrin-releasing peptide (GRP), bradykinin (BK_{2}) and gastrin by reverse
transcriptase–polymerase chain reaction (RT–PCR), and show that GRP-receptor expression predicts sensitivity to SP-G in a variety of tumours (SCLC, non-small-cell lung cancer (NSCLC), colorectal, ovarian and pancreatic) in vitro and in vivo. Our results suggest that an antineuropeptide growth factor strategy may be effective in a wider range of tumours and may also be of benefit in these neuropeptide-expressing tumours, which have acquired relative resistance to conventional chemotherapeutic agents.

MATERIALS AND METHODS

Materials

Rat-1a cells and rat-1a cells stably expressing the mouse bombesin/GRP receptor (GRPR) (BOR-15) were established by the Imperial Cancer Research Fund (ICRF, London, UK). RPMI-1640, Dulbecco’s essential Eagle’s medium (DMEM), bombesin and vasopressin were from Sigma (Poole, UK). SP-G was a kind gift from Peptec (Copenhagen, Denmark). All other reagents were of the purest grade available.

Cell culture

Stocks were maintained in RPMI-1640 (tumour cell lines) or DMEM (nontumour cell lines) supplemented with 10% (v/v) fetal bovine serum (heat-inactivated at 57°C for 1 h) 50 U ml−1 penicillin, 50 μg ml−1 streptomycin and 5 μg ml−1 l-glutamine in a humidified atmosphere of 5% CO2:95% air at 37°C. For experimental purposes, cells were either grown in SITA medium (RPMI-1640 medium supplemented with 30 nM selenium, 5 μg ml−1 insulin, 10 μg ml−1 transferrin media supplement and 0.25% bovine serum albumin (tumour cell lines)) or with 0.1% (v/v) fetal bovine serum in DMEM (nontumour cell lines). Rat-1A fibroblasts stably transfected with the mammalian bombesin receptor (BOR-15 cells) were cultured in the presence of 400 μg ml−1 G418-sulphate.

Growth assays

Liquid growth was determined in SITA medium in the presence or absence of mediators. Cell number was determined using a Coulter Counter (model Z1, Beckman Coulter, Bucks, UK). Colony growth was determined in 0.3% agarose in SITA medium for 21 days as described by Sethi and Rozengurt (1991).

Determination of intracellular Ca2+ concentration

Intracellular Ca2+ concentration was determined using the fluorescent indicator Fura-2-tetraacetoxymethylester AME (FURA-2-AM 1 μM) as described (Sethi et al, 1993) Ratiometric fluorescence was monitored in a Perkin-Elmer Fluorometer Spectrophotometer with dual excitation wavelengths of 340 and 380 nm and emission wavelength of 510 nm.

Xenograft activity

Female nu/nu mice (6 weeks old) (ICRF, London, UK) were maintained in negative pressure isolators (Moredun Animal Research Unit, Edinburgh, UK). All xenografts used throughout were initially established by subcutaneous injection of 106 cells from their respective cell line and then maintained by serial passage of fragments of viable tumour as described previously (Langdon et al, 1992).

Mice were allocated into control and treatment groups containing six to eight mice. Treatment started when the xenografts reached a diameter of 3–10 mm with the first day of peptide administration designated as day 0. SP-G was dissolved in sterile distilled water at a concentration of 10 mg ml−1 and administered by i.p. injection in a volume of 0.1 ml per 20 g of body weight to yield a dose of 50 mg kg−1. The dose schedule employed was twice daily injections with an 8 h gap for a total of five injections. This schedule was chosen based largely on the pharmacokinetics of SP-G in nu/nu mice (Cummins et al, 1995). Controls received the same dose schedule of vehicle. Xenografts were measured either two or three times a week by means of calipers and tumour volume (V) was then calculated by the formula V = π/6L2W, where L is the longest diameter and W the diameter perpendicular to L. Results were expressed as a relative tumour volume (RTV) which is defined simply as: V on day x/V on day 0, with RTV = 1 on day 0. Xenograft experiments were repeated on two or three separate occasions.

Semiquantitative RT–PCR

RNA was extracted using Tri-Reagent (Sigma, Poole, UK) from exponentially growing cells, which had been in SITA medium for 3–5 days and was subsequently treated with DNaseI to remove any DNA contamination. cDNA was produced from this RNA using a first-strand synthesis kit (Boehringer Mannheim, Roche, UK). PCR primers used were: GRPR sense 5’-ATCTTTCTGTA-CAGTCAAAGTC-3’, antisense 5’-GCTTTCCATCGGAAAGGTATA-3’; V1αR sense 5’-ATCTTGCAGTTGCT-3’, antisense 5’-ACAGAGAGAGGAGATTG-3’; BK-R sense 5’-CTGTACTTACCATCCTTG-3’, antisense 5’-TTGATACAGCGCGAC-3’; receptor for gastrin (gastrinR) sense 5’-CTACCTTCTCCTCATCTGC-3’, antisense 5’-AGTGTGCTGATGTGTGATGCG-3’; γ-actin sense 5’-ATGGCATCGTCAC-3’, antisense 5’-ATGACATGGCAGTG-3’. Polymerase chain reaction products were run on a 1.5% agarose gel containing 1 μg ml−1 ethidium bromide. Stored images of the gels were analysed by densitometry using Gel Base/Gel Blot software. γ-Actin levels were measured as an internal control. Each product was expressed relative to the levels of γ-actin for the same cDNA batch and for each PCR reaction.

RESULTS

Neuropeptide sensitivity in the longitudinal cell lines GLC14, 16 and 19

The GLC14, 16 and 19 cell lines are SCLC cell lines established from one patient during longitudinal follow-up (Berendsen et al, 1989). During this period, the tumour changed from sensitive to resistant to chemotherapy, and the in vitro sensitivity to chemotherapeutic agents reflected the clinical resistance to treatment (de Vries et al, 1989 and T.S. observations (results not shown)). Initially, neuropeptides (at a concentration of 100 nM) in each of the three cell lines were screened for their ability to increase [Ca2+]i. Vasopressin and neurotensin caused large increases in [Ca2+]i in all three cell lines of 100–150 nM, suggesting that the mobilisable Ca2+ pools were equivalent in each line; however bombesin, neuromedin B and CCK showed an increased responsiveness in the GLC19 cells. Typical concentration response curves in GLC16 and 19 cells are shown in Figure 1B. Thus, during the tumour progression from GLC14, 16 to 19 cell lines, there is an increase in...
Neuropeptide Ca^{2+} mobilisation in the SCLC GLC longitudinal cell line series

| Ca^{2+} mobilising neuropeptides | GLC14 | GLC16 | GLC19 |
|---------------------------------|------|------|------|
| Bombesin                        | 6 ± 3 (n = 7) | 25 ± 59 (n = 7) | 100 ± 15 (n = 7) |
| Bradykinin                      | 66 ± 8 (n = 8) | 105 ± 7 (n = 8) | 98 ± 12 (n = 10) |
| Carbacholcholine                | 83 ± 11 (n = 3) | 104 ± 18 (n = 5) | 113 ± 25 (n = 7) |
| Cholecystokinin                 | 31 ± 9 (n = 4) | 32 ± 4 (n = 3) | 56 ± 5 (n = 4) |
| Neurenomed B                    | 7 ± 4 (n = 4) | 25 ± 3 (n = 3) | 150 ± 20 (n = 4) |
| Neurenomensin                   | — (n = 3) | 43 ± 12 (n = 5) | |
| Substance P                     | — (n = 3) | 39 ± 6 (n = 3) | 72 ± 10 (n = 3) |
| Vasopressin                     | — (n = 3) | 28 ± 8 (n = 7) | 42 ± 9 (n = 7) |
| Serum                           | 105 ± 8 (n = 8) | 109 ± 15 (n = 8) | 119 ± 19 (n = 10) |

The GLC19, 16 and 14 SCLC cell lines were loaded with 1 μM Fura2-AMe washed and resuspended in 2 ml electrolyte solution and the cell suspension placed in a quartz cuvette and stirred continuously. Neuropeptides were added at 100 nM concentration and fluorescence was recorded continuously as described in Materials and Methods. Results are expressed as change in intracellular calcium (nM). The following peptides were also tested at 100 nM–1 μM concentrations and no increase in [Ca^{2+}]-i was ever observed: adrenocorticotrophin hormone, angiotensin I, atrial natriuretic peptide, calcitonin, choriocionic gonadotropin, dynorphin, β-endorphin, endothelin, epinephrine, galanin, growth hormone-releasing hormone, gastric-inhibitory peptide, glucagons 5-hydroxytryptamine, Leu-enkephalin, neuropeptide-Y, parathyroid hormone, substance K, thyrotrophin-releasing hormone.

Figure 1 Effects of bombesin, neuromedin B, bradykinin, cholecystokinin and serum on [Ca^{2+}]-i in SCLC cell lines GLC14, GLC16 and GLC19. Cells loaded with FURA-2-AMe were resuspended in electrolyte solution and placed in a quartz cuvette. Fluorescence was monitored and basal and peak [Ca^{2+}]-i calculated as described in Materials and Methods. Panel A: All peptides were added at a final concentration of 100 nM. Abbreviations used: BN, bombesin; NmB, neuromedin B; BK, bradykinin; CCK, cholecystokinin. Serum was added at a final concentration of 1% v:v/CO. Panel B: Dose-dependent effects of bombesin, neuromedin B, bradykinin and CCK in SCLC cell lines GLC16 and GLC19, open and closed circles, respectively. Peptides were added at the concentrations indicated. Representative concentration–response relations of three to five experiments are shown. Panel C: mRNA encoding the GRP receptor was detected by semiquantitative RT–PCR. γ-Actin mRNA levels were measured as an internal control for each PCR reaction. The results show a representative PCR reaction for the GLC14,16 and 19 cell lines. A bar chart showing relative receptor expression, calculated as density of PCR product/actin for each cell line. The results represent the mean of two independent experiments.
Increased Ca^2+ lines was approximately 1.5%. Hence, the GLC19 cell line develops was consistently less than that seen in the GLC16 cell line. Serum growth in both GLC16 and 19 cell lines. The ability of these (Figure 2). Bradykinin was equally effective in stimulating clonal growth in the GLC19 SCLC cell line compared to the GLC16 cell line bombesin, neuromedin B and CCK to stimulate clonal growth in and 19 cell lines. There was a significant increase in the ability of concentration-dependent increase in clonal growth in the GLC16 and 19 cell lines. SP-G was still effective in inhibiting the growth of the GLC19 SCLC cell line (IC_{50} for the GLC14, 16 and 19 SCLC cells was 25, 25 and 15 μM, respectively, for cloning efficiency, Figure 3B). Interestingly, SP-G was still effective in inhibiting the growth of the GLC19 SCLC line despite the development of resistance to conventional chemotherapeutic agents (de Vries et al, 1989). These findings suggest that increased neuropeptide receptor expression results in increased sensitivity to SP-G.

Sensitivity to SP-G in a panel of tumour cell lines

A panel of tumour cell lines was selected that represents a spectrum of tumour types, NSCLC, ovarian, pancreatic and colon cancer, which express neuropeptide receptors in addition to SCLC. The characteristics of these cell lines and their sensitivity to SP-G are shown in Table 2. Growth inhibition by SP-G was not confined to tumour type. The two most sensitive cells were the H69 SCLC cell line (IC_{50} = 10.5 μM) and the HT29 colorectal cell line (IC_{50} = 18 μM). Four out of five SCLC cell lines had IC_{50} values below the mean IC_{50} value of 44 μM. Both the ovarian carcinoma and the non-small-cell carcinoma were sensitive to growth inhibition by SP-G, with IC_{50} values of 31 and 33.5 μM, respectively. During the phase 1 clinical trial of SP-G, plasma levels of up to 40 μM were achievable with no dose-limiting toxicity (Clive et al, 2001). A total of 72% of the cancer cell lines tested had IC_{50} values below or near this concentration. This suggests that 'broad-spectrum' neuropeptide antagonists such as SP-G may be effective antitumour agents in a variety of cancers other than SCLC.

In vivo sensitivity to SP-G in a panel of tumour cell lines

Four members from the original panel of tumour cell lines were established as xenografts from their respective cell lines: H69 SCLC, HT29 colon carcinoma, PEO4 ovarian cancer and PANC-1 pancreatic cancer. Typical growth inhibition curves (Figure 4) are shown for each xenograft after i.p. treatment with 50 mg/kg SP-G. The HT29 xenograft showed a growth inhibition of 70.4%, 7 days after the commencement of treatment. In addition, this growth...
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Correlation of neuropeptide receptor expression with sensitivity to SP-G in a panel of tumour cell lines

The expression of GRP, gastrin, BK2 and V1A receptors were examined by RT-PCR, in nine cell lines from the original panel representing a spectrum of sensitivity to SP-G. The results are shown in Table 2 and Figure 5 (n = 4–5 independent experiments). Receptors for vasopressin (V1A) were detected in four out of four lung cancer cell lines (three SCLC and one NSCLC). The Wx330 SCLC cell line was the highest expresser of V1A. The pancreatic and ovarian cancer lines expressed low levels of the V1A receptor and in the colorectal cancer cell lines only HT29 cells expressed V1A. GRPRs were detected in three out of four lung cancer cell lines (two SCLC and one NSCLC). In the colorectal cell lines, the HT29 cell line showed high levels of GRPR expression and HCT116 cell line showed no detectable GRPR expression with intermediate expression in the HT18 cell line. The pancreatic cell line Panc1 had very low levels of GRPR expression. The PE04 ovarian cancer cell line and H69 SCLC cell lines showed high expression of the GRPR. Receptors for bradykinin were detected in all cells of the cell panel. Other studies have found an almost ubiquitous expression of bradykinin receptors in human lung cancers (Bunn et al., 1992). Gastrin receptors were detected in three out of four lung lines (two SCLC and one NSCLC) and low expression was detected in the ovarian line PE04. The highest expression was in the SCLC cell line HT10. Interestingly, none of the colorectal cell lines expressed gastrin receptors (Table 2). This finding confirms other studies, which show that the gastrin receptor is rarely expressed in colorectal cancer cell lines (Reubi et al., 1997). These results show that sensitivity to SP-G in a variety of tumours appears to correlate with the level of expression of neuropeptide receptors. In particular, all of the neuropeptide receptors examined, the correlation between relative GRP receptor expression (receptor/β-actin) and sensitivity to SP-G was the most apparent (Spearman’s correlation R = −0.73; P = 0.026, Figure 5).

Effect of GRP receptor expression on SP-G sensitivity in fibroblasts

The importance of neuropeptide receptor status for cell sensitivity to SP-G was further tested in a rat-1 fibroblast system in which only one neuropeptide receptor (the mouse GRPR) was expressed at high levels (Kd = 1 nM, Bmax = 106 receptors per cell) (Charlesworth et al., 1996). In native rat-1a fibroblasts, bombesin and other Ca2+-mobilising neuropeptides, vasopressin, neurotensin, bradykinin and gastrin, failed to mobilise intracellular Ca2+, suggesting absence of receptors in the parent cell line (Figure 6A). Rat-1a fibroblast cells stably expressing the mammalian bombesin receptor (B0R15) respond normally to bombesin stimulation in terms of signal transduction and stimulation of DNA synthesis (Charlesworth et al., 1996; MacKinnon et al., 2001). Bombesin stimulated a marked and rapid increase in [Ca2+]i in B0R15 cells, which was blocked by SP-G (Figure 6B). The effect of SP-G on the growth of rat-1a fibroblasts and B0R15 cells was therefore determined. The parent rat-1a cells were comparatively resistant to SP-G-mediated growth inhibition with only partial inhibition observed at the highest concentration of SP-G (41 ± 12% inhibition at 100 μM, IC50 > 100 μM), whereas B0R15 cell growth was completely inhibited by 80 μM SP-G (IC50 44 μM, Figure 6). These results show that the presence of the bombesin receptor leads to increased sensitivity to growth inhibition by SP-G in fibroblasts.

DISCUSSION

The GLC14, 16 and 19 are classic type SCLC cell lines derived from a 55-year-old female with SCLC. The GLC14 cell line was established from a supraclavicular lymph node before treatment.
After chemotherapy, the patient was in complete remission, 4 months later she relapsed. Further chemotherapy resulted in a partial response and the GLC16 line was established from a recurrence in the lung. After radiotherapy, the lung appeared tumour free; however, 3 months later tumour recurred in the lung from which the GLC19 cell line was derived. This was resistant to any further treatment and the patient died 2 months later (Berendsen et al, 1988; de Vries et al, 1989).

In vitro sensitivity to chemotherapeutic agents reflected the clinically observed development of resistance to treatment (de Vries et al, 1989). We were also able to confirm these findings in the GLC cells used in this study (results not shown). One percent serum caused a rapid and equivalent increase in $[Ca^{2+}]_i$ in all three GLC SCLC cell lines suggesting that the mobilisable $Ca^{2+}$ pools were equivalent in each of the three cell lines. However, we show that in the progression to chemoresistance the GLC cells are able to respond to a greater

### Table 2

| Cell line | IC50 (µM) | GRPR* | V1AR | BK2R | Gastrin R |
|-----------|-----------|-------|------|------|-----------|
| H69 (SCLC, Moody et al, 1985) | 10.5 | 0.91 ± 0.15 | 0.8 ± 0.18 | 0.65 ± 0.31 | 0.56 ± 0.49 |
| HT29 (colorectal, Brattain et al, 1981) | 18 | 0.55 ± 0.12 | 0.02 ± 0.01 | 0.35 ± 0.04 | — |
| H510 (SCLC, Moody et al, 1985) | 29 | 0.3 ± 0.11 | 1.10 ± 0.21 | 0.51 ± 0.17 | 1.51 ± 40.61 |
| PEO4 (ovarian, Libian et al, 1975) | 31 | 1.31 ± 0.25 | 1.10 ± 0.02 | 0.24 ± 0.10 | 0.2 ± 0.05 |
| NKO02 (NSCLC, Stark et al, 2001) | 33.5 | 1.01 ± 0.21 | — | 0.65 ± 0.23 | 0.56 ± 0.28 |
| HRT18 (colorectal, Fogh et al, 1977) | 37 | 0.18 ± 0.04 | — | 0.80 ± 0.21 | 0.03 ± 0.01 |
| W X330 (SCLC, Hay et al, 1991) | 42 | 0.09 ± 0.01 | 3.71 ± 0.91 | 0.22 ± 0.12 | 0.27 ± 0.18 |
| PANC1 (pancreatic, O'Hara et al, 1986) | 58 | 0.09 ± 0.01 | 0.10 ± 0.02 | 0.38 ± 0.11 | — |
| HCT116 (colorectal, Langdon et al, 1988) | 129 | — | — | 0.65 ± 0.25 | — |

RT-PCR products were analysed by densitometry and the relative expression level of four neuropeptide receptors are summarised. These values have been expressed as a ratio of the level of $\gamma$-actin expression for each cDNA batch and PCR run. Numbers represent the mean of four to six experiments. Spearman’s rank correlation between IC50 value and receptor expression level was performed. A significant correlation between sensitivity to SP-G and GRPR expression was confirmed (Spearman’s $R$ value = −0.75, $P = 0.026$).
range of neuropeptides with increased potency, and are consequently more sensitive to growth inhibition by SP-G. The GLC19 cell line was more sensitive to SP-G both in liquid culture and in semisolid medium than the GLC16 and 14 cell lines. The Ca\(^{2+}\)-mobilisation and the clonal growth results suggest that this cell line may have greater neuropeptide dependence and this is reflected in its greater sensitivity to neuropeptide growth factor blocking agents such as SP-G. This led to the hypothesis that other neuropeptide-expressing tumours may also be sensitive to the growth-inhibitory effect of SP-G.

It is well known that neuropeptides can stimulate the growth of many types of cancers including pancreatic, colorectal, prostate, ovarian, breast and NSCLC (Bologna et al, 1989; Halmos et al, 1995; Aprikian et al, 1996; Ferris et al, 1997). A panel of 11 tumour cell lines, which represented a spectrum of sensitivity to SP-G, showed that sensitivity to SP-G differed across the panel with a range of >1 log order (10 \(\mu\)M to >100 \(\mu\)M). The sensitive cell lines (four SCLC, two colorectal carcinoma, one NSCLC and 1 ovarian) had IC\(_{50}\) values that were in the range of physiologically obtainable plasma concentrations. Moreover, in the cell lines tested \textit{in vitro} sensitivity correlated with sensitivity to SP-G \textit{in vivo}. The results of this screen suggest that SP-G could be used therapeutically against several tumour types in addition to SCLC.

We have previously shown that SP-G augments etoposide-induced growth inhibition and apoptosis in SCLC cells and suggested that SP-G may be of increased benefit in patients following relapse or in conjunction with conventional chemotherapy (MacKinnon et al, 1999). It is of interest to note that the cell lines SCLC GLC19, ovarian cancer PEO4 and NSCLC NX022, which \textit{in vitro} and \textit{in vivo} are resistant to standard chemotherapeutic agents such as etoposide, are sensitive to the growth-inhibitory effects of SP-G. This raises the possibility that SP-G may be an effective anticancer agent in patients with neuropeptide-expressing tumours which are intrinsically resistant or have acquired resistance to conventional chemotherapy. A plausible extension of this hypothesis is that clinically aggressive drug-resistant SCLC...
cells that emerge after chemotherapy might have a more extensive network of neuropeptide regulation and therefore display increased sensitivity to neuropeptide antagonists. Further studies in longitudinal cell lines are required to investigate this hypothesis in further detail.

Although our data cannot indicate the expression of fully functional receptors, the RT-PCR approach was taken as it could be used as a possible diagnostic test in cancer patients to determine the potential tumour sensitivity to neuropeptide growth factor antagonist therapy. Of the four neuropeptide receptors tested, the most apparent correlation was between high expression of the GRP receptor and increased sensitivity to SP-G. This is in some ways surprising given that SP-G is more selective for the neuromedin B receptor to assess its role in substance P-analogue-induced growth. It would be interesting to look at the expression of other bombesin-like peptide receptors such as the GRPR in particular, as it could be used as a possible diagnostic test in cancer patients to determine the potential tumour sensitivity to neuropeptide antagonists. Further studies in longitudinal cell lines are required to investigate this hypothesis in further detail.

The GRPR has been shown to be oncogenic when transfected into the nonmalignant NCM460 colon epithelial cell line (Ferris et al, 1995). The increased proliferation was shown to be a consequence of constitutive activation of the GRPR in that the receptors tonically coupled to $G_q$ in the absence of ligand. This gives a potential mechanism whereby the GRPR may act as an oncogene. In addition, many tumour types such as breast cancer (Halmos et al, 1995) and prostate cancer (Bologna et al, 1989; Aprikian et al, 1996) have also been shown to aberrantly express GRPRs. We and others have previously shown that the expression of GRPRs in fibroblasts increases the ability of SP-G and other substance P analogues to activate the extracellular-signal-regulated kinase (ERK) and c-jun-N-terminal kinase (JNK) pathways leading to growth arrest and apoptosis (Jarpe et al, 1998; MacKinnon et al, 2001). Together, these data demonstrate that not only can GRPR expression transform cells, but these cells then become more sensitive to substance P-analogue-induced cell death.

These findings have important implications for the design of more advanced phase human clinical trials using substance P analogues. SP-G is currently entering a phase II clinical trial where its effectiveness will be tested in SCLC patients, but ultimately, compounds of this type may also be suitable for the treatment of a wide range of other tumour types and neuropeptide-expressing tumours that have become resistant to standard conventional chemotherapeutic agents. Screening tumour biopsy samples for neuropeptide receptor expression may provide insight into the likelihood of patients responding to treatment with substance P analogues, analogous to oestrogen receptor expression conferring efficacy to tamoxifen in breast cancer. It is suggested that tumours should be biopsied to select patients for substance P-analogue trials based on the expression of the GRPR and another neuropeptide receptor. Our results suggest that these tumours should show growth inhibition regardless of intrinsic or acquired resistance to standard chemotherapeutic agents. It is therefore proposed that in the first instance, patients with tumours that express the GRPR and another neuropeptide receptor who have failed conventional treatment should be randomised into two groups – best supportive care and treatment with substance P analogues.
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