Forearm Blood Flow and Local Responses to Peptide Vasodilators: A Novel Pharmacodynamic Measure in the Phase I Trial of Antagonist G, a Neuropeptide Growth Factor Antagonist[1]

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ABSTRACT

Purpose: **Arg-D-Trp-NmePhe-D-Trp-Leu-Met-NH$_2$ (Antagonist G), a substance P (SP 6-11) analogue, inhibits mitogenesis stimulated by a broad spectrum of neuropeptides and has demonstrated antitumor activity *in vitro* and *in vivo* with IC$_{50}$ concentrations of 10–20 μM in small cell lung cancer and other cell lines. Because neuropeptides are part of complex neurohumoral pathways, we have sought to develop novel pharmacodynamic approaches as part of the early clinical development of this potential anticancer drug.**

Experimental design: **A Phase I trial was performed in two stages. In stage 1, Antagonist G was administered at 3-week intervals using an accelerated dose-escalation strategy until the target maximum plasma concentration (C$_{max}$) of 10 μM was achieved. In stage 2, dose intensity was increased to weekly, and the inhibitory effect of i.v. Antagonist G was assessed by forearm blood flow (FBF) using SP as a vasodilator, as measured by venous plethysmography.**

Results: **In stage 1, dose was escalated from 2 to 300 mg/m^2 in 12 dose levels using only 15 patients. In stage 2, nine patients were entered at three dose levels (300, 350, and 400 mg/m^2) and a C$_{max}$ of 45 μM was achieved. Facial flushing was the only consistent toxicity but was not dose limiting. FBF studies demonstrated that Antagonist G consistently inhibited the vasodilatory effects of SP (mean, 62 ± 2% inhibition).**

Conclusions: **Antagonist G can be safely administered up to 400 mg/m^2, achieving C$_{max}$s >20 μM by weekly 6-h i.v. infusion. FBF studies in patients demonstrated that Antagonist G inhibits SP vasodilatory effects *in vivo* at these doses in the absence of dose-limiting toxicity.**

INTRODUCTION

Neuropeptides are small regulatory molecules that act at transmembrane glycoprotein receptors, coupled to one or more G-proteins. Neuropeptides are widely distributed, particularly in the nervous, gastrointestinal, and cardiovascular systems and are involved in functions as diverse as the regulation of neural function, fluid balance, blood pressure, digestion, allergic responses, and stimulation of cell proliferation.

The growth of a number of cancers, including SCLC[3] (1, 2) and colon cancer (3, 4), is known to be regulated in part by neuropeptides. SCLC is characterized by its ability to secrete a wide range of hormones, peptides, and neuropeptides, including bombesin, its mammalian counterpart gastrin-releasing peptide, AVP, BK, neurotensin, gastrin, cholecystokinin, galanin, and glucagon (2, 5–7). Many of these neuropeptides regulate the growth of SCLC by autocrine and/or paracrine circuits (1, 2, 6, 8, 9).

Antagonists specific to many neuropeptides have been developed (4, 10) and have demonstrated *in vitro* antitumor activity in SCLC (11–13) and breast (14), upper gastrointestinal (10), prostate (15), pancreatic (16) and colon cancers (4, 17). However, only 30–60% of cells may express receptors for any individual neuropeptide in SCLC (8, 18), whereas all or most will express receptors for multiple peptides (1). Therefore, simultaneously blocking a number of growth factors with one antagonist might produce greater antitumor activity than specific growth factor antagonists.

A family of SP analogues has been developed which have demonstrated broad-spectrum neuropeptide growth factor activity. Of these, [Arg^6,DTrp$^{7,\,9}$,NmePhe^8] SP (6–11) (Antagonist G; Ref. 19) is one of the most potent antagonists of AVP-stimulated mitogenesis. In H345 and H69 SCLC cell lines, 10 μM Antagonist G inhibits Ca^{2+} mobilization stimulated by gastrin-releasing peptide, AVP, BK, neurotensin, cholecystokinin, and galanin (2, 19) by acting specifically at neuropeptide receptors (19). It competitively inhibits basal and neuropeptide-stimulated growth of different SCLC cell lines *in vitro* with an

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[3] The abbreviations used are: SCLC, small cell lung cancer; SP, substance P; AVP, vasopressin; BK, bradykinin; MTD, maximum tolerated dose; DLT, dose-limiting toxicity; FBF, forearm blood flow; C$_{max}$, maximum plasma concentration; PK, pharmacokinetic; BP, blood pressure; CTC, Common Toxicity Criteria.

IC_{50} of $\sim$20 μM (9, 19). *In vivo,* Antagonist G demonstrates profound growth inhibition of H69 and WX322 SCLC xenografts when given by peritumoral injection (20) and 44% growth inhibition when given by a twice-daily i.p. schedule against H69 SCLC and HT29 colon cancer xenografts, achieving peak plasma concentrations of 14.9 μM (21). Preclinical studies of Antagonist G had estimated the MTD to be 60 mg/m^2 in mouse and 20 mg/m^2 in rat with acute severe hypotension the main DLT (22).

Therefore, preliminary PK and toxicity data from mice, using a bolus schedule, suggested that Antagonist G toxicity may be peak concentration-related, and that plasma clearance was rapid. It was likely that much larger doses could be administered to man by more prolonged infusion, and therefore, to prevent excessive treatment of patients at biologically inactive doses, a rapid dose-escalation strategy was incorporated into the study design.

In addition, we considered that a classical MTD end point was unlikely to be appropriate for Antagonist G because of its likely growth-inhibitory, rather than cytotoxic, mechanism of action. Increasingly, with new molecules under investigation for use in cancer, alternative surrogates for appropriate biological activity are being investigated (23, 24), and, therefore, a novel pharmacodynamic measure was also incorporated into the study design.

A two-stage Phase I study of Antagonist G was performed in patients with cancer. The aim of stage 1 was to achieve safely plasma concentrations in humans equivalent to those associated with antitumor activity in preclinical studies, and the rapid dose escalation strategy was used. This used single patients, dose-doubling and real-time PK analysis, enabling transfer to a more conventional dose-escalation strategy in stage 2 once target plasma concentrations were achieved.

The aim of stage 2 was to increase dose intensity and to determine whether Antagonist G was exerting activity at neuropeptide receptors in humans at these plasma concentrations. The pharmacodynamic measure used made use of the infusion of neuropeptides into the brachial artery before and during Antagonist G infusion, and FBF was measured by venous occlusion plethysmography. Because extensive published and local experience of the reproducible vasodilatory effects of SP and BK on FBF was available (25–29), these peptides were chosen for intra-arterial infusion in patients. Venous occlusion plethysmography is used widely in the clinical development of cardiac and vasoactive drugs, but we believe this to be the first time that it has been used in the study of a potential anticancer drug.

PATIENTS AND METHODS

Both stages of the Phase I study were conducted in accordance with the Declaration of Helsinki, under the auspices of the Cancer Research Campaign Phase I/II Committee. Approval was obtained from the local research ethics committee, who also approved all protocol amendments (Lothian Research Ethics Committee 1702/95/4/128), and written informed consent was obtained from each patient before participation in any part of the trial.

Patient Eligibility

Patients with refractory solid tumors were recruited from the Western General Hospital (Edinburgh, United Kingdom) oncology outpatient department. Eligibility criteria included: (*a*) patients with a histologically proven diagnosis of a malignant disease for which no satisfactory treatment existed, or against which established treatments had failed, but with a life expectancy of at least 3 months; (*b*) patients capable of understanding the nature of the trial and giving written informed consent; (*c*) patients aged $\geq$18 years with WHO Performance Status $\leq$2; (*d*) patients with hemoglobin $\geq$10 g/dl, neutrophils $\geq$1.5 $\times$ 10^9/liter, and platelets $\geq$100 $\times$ 10^9/liter; (*e*) patients with adequate hepatic (normal bilirubin and alanine transaminase <2 $\times$ upper limit normal), renal (creatinine clearance, $\geq$60 ml/min), and cardiac (no cardiac history and normal blood pressure and electrocardiogram) functions.

Exclusion criteria were chemotherapy within the last 4 weeks; radiotherapy, nitrosoureas, or mitomycin C within the last 6 weeks; primary brain tumors or symptomatic brain metastases; severe preexisting medical conditions; concurrent use of other experimental agents or anticancer therapy; hematological malignancies; and women who were pregnant or breast-feeding.

Study Design

The Phase I trial of Antagonist G was a nonrandomized study performed in two stages. In stage 1, Antagonist G was given by three weekly infusions with rapid dose escalation to achieve an end point of C_{max} $\geq$10 μM. In stage 2, dose intensity was increased to weekly therapy, dose escalation was more conservative, and FBF studies were introduced as a pharmacodynamic end point.

Dose Escalation Strategy

In stage 1, single patients were entered at each dose level if no drug-related toxicities greater than CTC grade I were identified, and dose escalation was guided by the incidence of toxicity. In the absence of toxicity, dose doubling was allowed. If systemic drug-related toxicities greater than or equal to CTC grade II were observed, then subsequent dose escalation was to be reduced to 20–50% increments. If CTC grade II toxicities were observed, three patients were to be entered at that and every subsequent dose level. If any patient developed greater-than-or-equal-to CTC grade III toxicity (excluding nausea and vomiting or hematological toxicity, unless considered dose limiting), five patients were to be entered at that level. Once the target C_{max} (10 μM) had been achieved, patients were entered into stage 2, with three patients at each dose level and a more cautious dose escalation strategy.

In this study, DLT was defined as CTC grade III or IV myelosuppression of >7 days duration and/or associated with neutropenic sepsis. For nonhematological toxicities, DLT was defined as the incidence of CTC grade III or greater toxicity (excluding alopecia, nausea, and vomiting). Nausea and vomiting were only considered dose limiting if uncontrolled by antiemetic therapy. The MTD was defined as the dose associated with an incidence of DLT in $\geq$3 of five patients.

Antagonist G Formulation and Administration

Antagonist G (supplies were organized by Peptech Ltd., North Ryde, New South Wales, Australia) was lyophilized without any additives/excipients. The bulk drug was dissolved in water for injection to a final drug concentration of 10 mg/ml. After membrane filtration (0.22 μm), 5-ml aliquots of this solution were placed into 10-ml glass vials, which were then lyophilized. Antagonist G was reconstituted in 5 ml of sterile 5% dextrose (Baxter Healthcare Ltd., United Kingdom) to a final drug concentration of 10 mg/ml. The reconstituted vials were then placed in an ultrasonic bath (Clifton, Avon, England) for 15 min. Antagonist G prepared in this way was added to 500 ml of 5% dextrose.

Then Antagonist G was infused via a skin-tunneled catheter sited in a central vein over 6 h under medical supervision. In stage 1, each cycle was repeated every 3 weeks, and all treatments were given on an inpatient basis. In stage 2, cycles were repeated weekly, provided any drug-related nonhematological toxicities had resolved and neutrophils were $\geq 1.5 \times 10^9$/liter and platelets $\geq 100 \times 10^9$/liter. Only the first cycle in stage 2 was given as an inpatient and all other cycles were given as outpatient treatment. Toxicity assessments were made weekly in both stages, and disease reassessment was performed every 6 weeks (two cycles) in stage 1 and every 6 weeks (six treatments) in stage 2, unless indicated sooner. Treatment was continued until DLT or disease progression, up to six cycles in stage 1 and up to 12 weeks treatment in stage 2.

Investigations and Patient Monitoring

Before each course of treatment, clinical examination, complete blood count, sodium, potassium, urea, creatinine, calcium, phosphate, calculated creatinine clearance, alanine aminotransferase, alkaline phosphatase, bilirubin, albumin, γ-glutamyl transferase and lactate dehydrogenase were performed. During infusion and for 2 h after the end of infusion, blood pressure and heart rate were measured every 15 min by an automated monitor (Life Sign, Welch Allyn, United Kingdom) during all cycles in stage 1 and during the first infusion of stage 2. In subsequent cycles in stage 2, blood pressure and heart rate were measured hourly, unless symptoms arose. All patients had a baseline 12-lead electrocardiogram performed before the first cycle of Antagonist G, and continuous cardiac monitoring (Life Pulse; HME Ltd., St. Albans, Hertfordshire, United Kingdom) during all cycles. For all patients receiving Antagonist G at doses of ≥ 300 mg/m^2, a 12-lead electrocardiogram was performed the day before Antagonist G, during the last 2 h of Antagonist G infusion and 24 h after infusion of the first cycle of Antagonist G.

In patients who had assessable disease, tumor measurements made by means of clinical assessment, radiological examination, or blood tumor markers were recorded. Tumor measurements were made before starting Antagonist G and after every two cycles (6 weeks) in stage 1 and every six cycles (6 weeks) in stage 2.

PK Sample Collection and Analysis

The timing of plasma PK sampling was based on simulations using preclinical mouse PK data and the computer pro-

gram ADAPT II (30), and it was designed to maximize information during the elimination phase. Seven ml of blood were drawn from an indwelling cannula (BOC Ohmeda AB, Helsingborg, Sweden) into a heparinized tube preinfusion and 60, 300, 360, 365, 370, 380, 390, 405, 420, 450, 540, 720, and 1440 min after the start of infusion in course 1, preinfusion and 60, 300, 360, 390, 420, and 480 min in course 2, and preinfusion and end of infusion (360 min) in course 3 and subsequent courses.

As Antagonist G is unstable in plasma at room temperature (31), blood samples were taken, placed on ice, and immediately centrifuged at $3000 \times g$ for 5 min at 4°C (Heraeus Instruments, Hanau, Germany). The plasma supernatant was aspirated, labeled, and then rapidly frozen at -20°C until analysis.

High-performance liquid chromatography was used to quantify Antagonist G and its metabolites in plasma. Initially, a method using electrochemical detection was developed in the laboratory, predominantly for analysis of *in vitro* and *in vivo* animal samples (32). Sensitivity at low concentrations of Antagonist G was of particular importance, and this was also an appropriate method for the "early" patients in the Phase I trial, whose plasma concentrations were low. Once plasma concentrations > 10 μM had been achieved in the Phase I trial, an UV detection technique was developed that was less sensitive at the lower concentrations, but which allowed automated analysis and increased sample throughput (33). This method was more sensitive at detecting the metabolites of Antagonist G and avoided the difficulties of variable electrode responses, more limited detection range, the need for frequent cleaning of electrodes, and poor detector stability at higher sensitivity associated with the electrochemical method. The UV detection method was used in stage 2 of the Phase I trial. Both methods gave highly correlated results ($R^2 = 0.94$) when 23 random patient plasma samples were compared (data not shown).

Pharmacodynamic Studies

FBF Studies. Each patient participating in stage 2 (weekly Antagonist G administration) was asked to participate in two FBF studies (one control and one at the time of Antagonist G infusion). The baseline study was always before the first cycle of Antagonist G, but the second FBF study was during the second or third Antagonist G infusions, depending on laboratory availability. Refusal to participate in the FBF studies would not have jeopardized patient care, and patients were permitted to participate in the Phase I trial of Antagonist G without FBF studies. Separate written informed consent was obtained for the FBF studies. Patients were asked to abstain from vasoactive drugs for at least 1 week, from alcohol for 24 h, and from food, caffeine-containing drinks, and cigarettes for 3 h before each study. On the day of each vascular study, patients attended at 8 a.m. and were positioned on the study bed in the quiet, draft-free environment of the Clinical Research Center at a constant temperature between 22 and 24°C. Intravenous fluid (5% dextrose or Antagonist G in 5% dextrose) was commenced at 83 ml/h through the central venous catheter. After 90 min, the patient was allowed to rest comfortably in the supine position, cuffs and mercury-in-silastic strain gauges were applied to both arms for measurement of FBF, and the intra-arterial cannula was sited. On completion of the study, the FBF set-up was dismantled, and either the dextrose was discontinued and the patient

Table 1 Characteristics of patients in both stages of the Phase I trial of Antagonist G

Characteristic	Stage 1 (3 weekly) $n = 15$	Stage 2 (weekly) $n = 9$
Gender		
Male	12	5
Female	3	4
Age	59 (36–65)[a]	54 (34–75)[a]
WHO Performance Status	1 (0–2)[a]	1 (1–2)[a]
Cancer		
Renal	4	0
Colon	2	2
Non-small cell lung	2	2
Small cell lung	0	3
Mesothelioma	2	0
Other	5	2
Previous chemotherapy	9	8
Previous radiotherapy	5	4
Previous immunotherapy	1	0
No. of treatments Antagonist G	2 (1–6)[a]	6 (3–12)[a]

[a] Median (range).

Table 2 Dose levels, doses, and C_{max}s of Antagonist G in stage 1 of the Phase I clinical trial

Dose Level	Dose (mg/m^2)	Patient No.	No. of Cycles	C_{max} (μM)
1	2	1	2	0.1
2	4	2	4	0.2
3	8	3	2	0.3
4	10.5	4	2	0.5
5	14	5	1	0.3
		6	2	0.4
6	21	7	4	0.6
7	30	8	3	0.8
8	45	9	3	1.0
9	67.5	10	2	1.1
10	135	11	2	2.1
11	200	12	6	3.1
12	300	13	2	15.9
		14	1	12.6
		15	1	23.7

discharged (control study) or the Antagonist G infusion was run to completion with the usual cardiovascular monitoring and PK sampling.

Intra-arterial Administration and Drugs. The brachial artery of the nondominant arm was cannulated with a 27-standard wire gauge steel needle (Cooper's Needle Works Ltd., Birmingham, United Kingdom) attached to a 16-gauge epidural catheter (Portex Ltd., Hythe, Kent, United Kingdom) under lidocaine 1% local anesthesia (Antigen Pharmaceuticals, Southport, United Kingdom). Patency was maintained by infusion of saline via an IVAC P1000 syringe pump (IVAC Ltd., Basingstoke, Hants, United Kingdom), which was set at a constant rate of 1 ml/min throughout the study. All intra-arterial drugs were pharmaceutical grade and were dissolved in 0.9% physiological saline (Baxter Healthcare Ltd., Thetford, Norfolk, United Kingdom) under aseptic conditions on the day of the study. Different concentrations were prepared by serial dilution and infused intra-arterially at a constant rate of 1 ml/h. Physiological saline (Baxter Healthcare Ltd.) was infused initially for 30 min, with baseline FBF measurements to allow a run-in period after the insertion of the arterial needle. This preceded three incremental doses of SP (Clinalfa, Calbiochem-Novabiochem Ltd., Nottingham, United Kingdom) at 0.5, 1, and 2 pmol/min, each for 6 min, with FBF measurements at each dose. A 30-min saline washout separated the infusion of SP from that of BK (Clinalfa) which was infused at 10, 30, and 100 pmol/min, each for 6 min.

FBF Measurement. FBF was measured simultaneously in both infused and noninfused arms by venous occlusion plethysmography using calibrated mercury-in-silastic strain-gauges applied to the widest part of the forearm (34, 35). The hands were excluded from the circulation during each measurement period by inflation of wrist cuffs to 220 mmHg using E20 Rapid Cuff Inflators (D. E. Hokanson, Inc., Washington, DC). Upper arm cuffs were intermittently inflated to 40 mmHg for 10 s every 15 s to temporarily prevent venous outflow from the forearm and thus obtain plethysmographic recordings. Recordings of FBF were made over 3 min at 6-min intervals. Venous

occlusion plethysmography was performed using a dual-channel strain-gauge plethysmograph (D. E. Hokanson, Inc.), and calibration was achieved using the internal standard of the Hokanson plethysmography unit. Analogue voltage output from this was processed by a MacLab analogue-to-digital converter and Chart version 3.3.8 software (both from A D Instruments Ltd., Castle Hill, Australia) and recorded to a MacIntosh computer (Classic II; Apple Computers, Inc., Cupertino, CA). Blood flow in both forearms was obtained from the mean of the last five consecutive recordings of each measurement period. Because of the instability in blood flow that wrist-cuff inflation causes (35), recordings made in the first 60 s after wrist-cuff inflation were not used for analysis.

BP. BP was monitored throughout the FBF study period using a validated semiautomated noninvasive oscillometric method (Takeda UA 751; Takeda Medical, Inc., Tokyo, Japan) in the noninfused arm (36). BP was measured immediately after each FBF measurement, thereby avoiding any effect of venous congestion caused by this procedure on blood flow, and was measured every 10 min during saline infusions and with every new dose of drug.

Data Analysis and Statistics. Basal blood flow was taken to be the final recording of the saline equilibration phase before drug infusion. Plethysmographic data listings were extracted from data files and FBFs were calculated for individual venous occlusion cuff inflations using a template spreadsheet (Excel *v* 5.0; Microsoft). The ratio of flows in the two arms was calculated for each time point, thus using the noninfused arm as a contemporaneous control for the infused arm (37), and when appropriate this was expressed as a percentage change from baseline. Data were examined by repeated measures ANOVA and paired Student's *t* tests as appropriate (Excel *v* 5.0; Microsoft). Statistical significance was taken at the 5% level, and all *P*s are two-sided. All results are expressed as mean $\pm$ SE.

RESULTS

A total of 24 patients were entered into this Phase I trial, 15 patients in stage 1 and 9 patients in stage 2, the characteristics of which are shown in Table 1. In stage 1, the target C_{max} was

Table 3 Dose levels, doses, and first-cycle C_{max}s of Antagonist G in stage 2 of the Phase I clinical trial

Dose Level	Dose (mg/m²)	Patient No.	No. of Cycles	C_{max} (µM)
12	300	16	6	20.8
		17	4	30.2
		18	6	17.0
13	350	19	3	20.9
		20	12	17.0
		21	12	26.0
14	400	22	6	27.8
		23	3	45.5
		24	5	24.5

Fig. 1 Plasma concentration *versus* time curve in a patient receiving 300 mg/m² Antagonist G over 360 min. *Ant G*, Antagonist G.

Fig. 2 Plasma C_{max} of Antagonist G during the first cycle of Antagonist G *versus* dose of Antagonist G in all 24 patients. A trend line has been drawn through the points representing patients at doses <300 mg/m² and extended to the higher doses.

achieved at the 12th dose level (13th patient) at a dose of 300 mg/m² (Table 2), which compared well with the dose predicted (200 mg/m²) by PK simulations performed during trial design based on preclinical PK data. This C_{max} was confirmed in two additional patients at this dose, thus completing the first stage of the Phase I trial. Dose increments varied between 1.3 and 2 times the previous dose, according to the toxicities and C_{max} encountered at the previous dose level. At the fifth dose level, two patients were entered because the first patient developed symptoms caused by early progression of his disease and was withdrawn from study.

In stage 2, patients received between 3 and 12 weeks of Antagonist G, and plasma concentrations of over 20 µM were consistently achieved at the highest dose of 400 mg/m² (12 of 14 treatments), with a highest C_{max} of 45.5 µM (Table 3).

Toxicities. At doses ≤200 mg/m², there were no side effects attributed to Antagonist G. At doses >200 mg/m², all patients described similar side effects relating to facial flushing, which was never dose limiting. Symptoms usually started around 90 min into the 360-min infusion and lasted until ~2 h after completion. In most cases, symptoms had resolved completely 24 h later, and there was no delayed or cumulative toxicity. In particular, there was no significant cardiovascular toxicity throughout the study.

PKs. Antagonist G plasma concentrations increased during infusion, often reaching a plateau, and then declined rapidly after the end of each infusion. The mean duration of plasma concentration >10 µM in patients receiving doses of Antagonists G >200 mg/m² was 330 ± 21 min, highlighting the rapid decline after infusion and probably explaining the short duration of adverse events. A representative example, the concentration-time curve from a patient who received 300 mg/m² Antagonist G, is shown in Fig. 1. The C_{max} achieved during the first cycle of Antagonist G for each patient in stage 1 and stage 2 is shown in Tables 2 and 3, respectively. There was some variability in C_{max} for subsequent cycles (mean, −15% to +14% of first cycle), but this did not follow any pattern (data not shown). At doses ≤200mg/m², C_{max} increased linearly with dose, but at doses >200mg/m², there was an apparent nonlinear increase in C_{max} with dose, as shown in Fig. 2. This prompted caution in dose-escalation increments in stage 2.

Preliminary PK modeling using the WinNonlin computer program (Pharsight Corporation, Mountain View, CA) suggested that th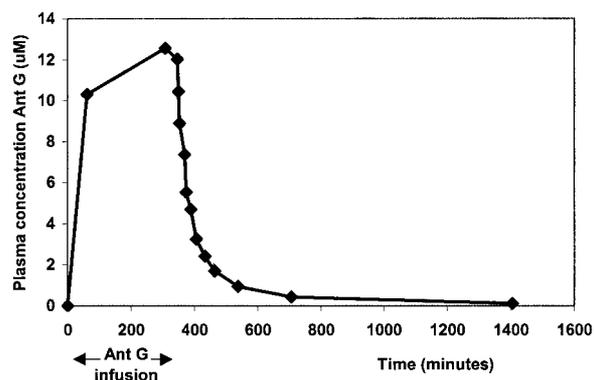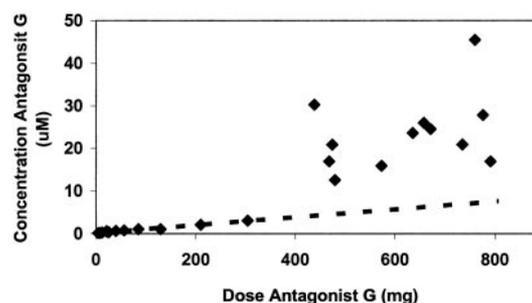e kinetic data were best fitted by a two-compartment open linear model. However this model under-predicts concentrations at the higher doses of Antagonist G and more complex population PK modeling should be considered when additional PK data are available for this compound.

Pharmacodynamic Studies. All nine patients in stage 2 agreed to participate in FBF studies. All patients had a control study, but one patient withdrew from additional FBF studies after a difficult arterial cannulation. All other patients had two studies performed, allowing comparison of the effects of SP on forearm arterial vasculature before and during Antagonist G infusion to be made in eight of nine patients. In two patients, the arterial cannula became dislodged before the first dose of BK, so that comparison of the effects of BK are described for only six of nine patients. The procedure was well tolerated by all patients, and there were no significant changes in blood pressure or heart rate throughout any study.

SP caused a dose-dependent increase in FBF in all eight subjects during the control study. When repeated in the presence of Antagonist G, the FBF response to SP was attenuated in all eight patients at all three doses, and this was statistically significant at all doses (Fig. 3). This constituted a 62, 59, and 66% inhibition of SP-induced vasodilatation by Antagonist G for each dose of SP, respectively.

BK caused a dose-dependent increase in FBF in all six subjects during the control study. When repeated during Antag-
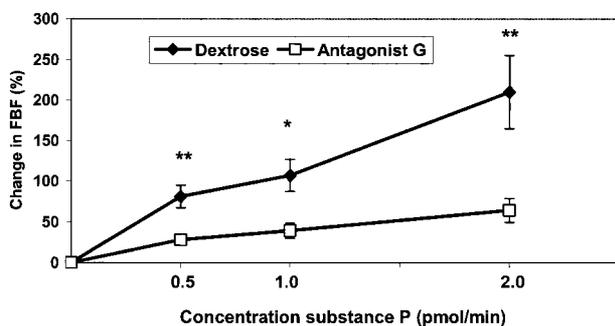
Fig. 3   Mean ± SE percentage change in FBF in eight of eight patients with three increasing concentrations of SP during dextrose (♦) and Antagonist G (□) infusions. Significant: ∗, $P < 0.05$; ∗∗, $P < 0.01$.

Fig. 4   Mean ± SE percentage change in FBF in six of six patients with three increasing concentrations of BK during dextrose (♦) and Antagonist G (□) infusions. Significant: ∗, $P < 0.05$; ∗∗, $P < 0.01$.

onist G infusion, the vasodilatory response was attenuated in five of six patients at all three doses, which was significant at all but the highest dose (Fig. 4). This constituted a 19, 33, and 23% inhibition of BK-induced vasodilatation by Antagonist G for each respective dose of BK. Plasma concentrations of Antagonist G at the start of SP infusions were always >10 μM (range, 13.1–20.9 μM), and there was no correlation between this or C_{max} (range, 22.7–41.3 μM) and the ability of Antagonist G to inhibit the effects of SP or BK (data not shown).

Antitumor Activity.   Of 24 patients treated with Antagonist G, 7 were not evaluable, 14 developed progressive disease while on treatment (5 were early progression), and 3 had stable disease (mean duration, 8 ± 2 weeks).

DISCUSSION

This Phase I trial demonstrated that plasma concentrations of Antagonist G equivalent to those associated with antitumor activity in animal models can be achieved safely in patients. It also showed that at these concentrations Antagonist G has an inhibitory effect at two different neuropeptide receptors *in vivo*, consistent with its proposed mechanism of antitumor action.

The rapid dose escalation strategy used allowed dose escalations up to dose doubling as well as single patients at each dose level. Apparent nonlinearity in PK (Fig. 2) led to <100% dose increments early in the trial, but, despite this, only 12 dose levels (13 patients) were required to achieve the target C_{max} of 10 μM (at 300 mg/m^2), from the starting dose of 2 mg/m^2 (Table 2). Had a standard modified Fibonacci dose escalation strategy been used, 45 (compared with 12) patients would have been treated at doses that were not biologically meaningful. The use of single patient dose levels was the most significant factor in reducing this patient number. Despite recent recommendations for the incorporation of more rapid dose escalation strategies into all Phase I trials (38, 39), few of these designs are being put into practice (40). We have shown that incorporation of such a design in the Phase I trial of a novel "biological" therapy reduced the number of patients treated at suboptimal doses, without compromising patient safety. The additional inclusion of real-time PK analysis enabled transfer to a more conventional dose-escalation strategy when "relevant" plasma concentrations were achieved. This was particularly important in the Phase I trial of Antagonist G, because its preclinical toxicity profile
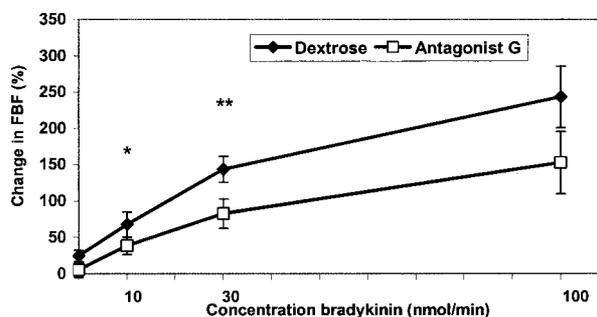
indicated that no conventional indicators of biologically active concentrations *e.g.,* myelosuppression, would be seen before significant hypotension occurred.

The main toxicity observed in the patients treated with Antagonist G was facial flushing. This was seen consistently in patients treated at doses >200 mg/m^2. Possible explanations for this include the release of inflammatory mediators such as histamine or prostaglandins, partial agonism of inflammatory neuropeptides in the skin, and localized vasodilatation. Thorough cardiovascular monitoring during Antagonist G infusion revealed no significant changes at the doses given, even at the highest C_{max} (45.5 μM) despite concerns regarding the preclinical toxicology of acute hypotension in rodents (22). This enables a degree of confidence in the safety of Antagonist G at the doses used by this route and schedule of administration.

Stage 1 was completed after demonstration that C_{max} >10 μM was achieved in three of three patients with no DLT at 300 mg/m^2. Therefore, in addition to increasing dose intensity in stage 2, FBF studies using intra-arterial neuropeptide infusions and venous plethysmography were performed as a pharmacodynamic measure. This is the first time that such studies have been performed in a Phase I trial in patients with cancer, and the studies were designed specifically to enable demonstration of activity by Antagonist G against individual neuropeptides *in vivo*.

Antagonist G was shown to antagonize SP in eight of eight patients and BK in six of six patients tested, which is consistent with its broad-spectrum neuropeptide receptor activity, and this is thought to be of particular importance for its antitumor effect (1, 19, 20). The magnitude of the effect against BK was smaller, but comparison of the inhibitory effect of Antagonist G on SP and BK is not necessarily meaningful, inasmuch as doses of BK 2,000–50,000 times the dose of SP were given at equivalent time periods to produce a similar degree of vasodilatation of the forearm vasculature.

In these studies, a relationship between Antagonist G plasma concentrations and the extent of inhibition of the effects of SP and BK was not demonstrated, but the study was not designed to show this. It had been decided that it was too invasive to perform FBF studies in patients at low doses of Antagonist G to determine fully whether a dose-effect relationship was present. As a result of this, the plasma concentrations

of Antagonist G during FBF studies were all within a similar range (>10 μM).

The aims of this Phase I trial were fulfilled by the demonstration of inhibition of neuropeptide-induced vasodilatation at plasma concentrations >10 μM Antagonist G. However, it should be noted that the duration of these plasma concentrations subsequent to discontinuation of the Antagonist G infusion was brief, and whether such short exposure to an agent which might be considered to be cytostatic is optimal will need to be considered in the design of future studies. The rapidity of clearance of Antagonist G meant that large doses of peptide were needed in this study, and therefore continuous protracted infusions would not be feasible for reasons of practicality and cost. The design of more potent and more stable analogues of Antagonist G is ongoing.

However, we believe that we demonstrated proof of principle in patients, demonstrating inhibition of SP induced peripheral vasodilation at tolerable plasma concentrations of Antagonist G, but we also accept that no surrogate is conclusive and that the maximum inhibition demonstrated (66%) may not be adequate for antitumor activity. Therefore, additional mechanistic studies are planned to optimize the schedule of administration of Antagonist G before proceeding with activity testing.

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