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Phase II study of RC-160 (vapreotide), an octapeptide analogue of somatostatin, in the treatment of metastatic breast cancer

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Summary RC-160 (octastatin/vapreotide) is a potent octapeptide analogue of somatostatin with growth inhibitory activity in experimental tumours in vitro and in vivo, including breast cancer. We evaluated the efficacy and tolerability of high-dose RC-160, 3 mg day^{-1} on week 1 increased to 4.5 mg day^{-1} for weeks 2–4 and subsequently 6 mg day^{-1} until the end of treatment, administered by continuous subcutaneous infusion in the management of 14 women with previously treated metastatic breast cancer. The age range was 37–80 years (median 58.5 years) and performance status 0–2. The treatment was well tolerated with no dose reductions being required. No grade 3 or 4 toxicities were seen. Abscess formation developed at the infusion site in eight patients and erythema and discomfort was seen in a further three patients. A significant reduction in IGF-I levels occurred by day 7 and was maintained throughout the treatment. The lowest dose of RC-160 produced the maximal IGF-I response. Although there was no reduction in prolactin levels in patients whose baseline levels were normal, elevated prolactin levels found in three patients fell to within the normal range 7 days after commencing RC-160 treatment. A small but significant rise in fasting blood glucose levels was also recorded, the highest level on treatment being 7.6 mmol l^{-1}. No objective tumour responses were observed, all patients showing disease progression within 3 months of commencing treatment. These findings demonstrate that high-dose RC-160, administered as a continuous subcutaneous infusion, can reduce serum levels of the breast growth factors IGF-I and prolactin but is ineffective in the management of metastatic breast cancer. Encouraging preclinical anti-tumour activity and the favourable toxicity profile in patients suggest the merit of future studies combining RC-160 with anti-oestrogen, cytotoxic and anti-angiogenic agents.

Keywords: somatostatin; RC-160; breast cancer; metastatic; insulin-like growth factor-I; prolactin

Somatostatin is a tetradecapeptide hormone first identified in the hypothalamus as an inhibitor of growth hormone (GH) release. The peptide has subsequently been found throughout the body, particularly in the pancreas, gastrointestinal tract and nervous system. As well as inhibiting hormone release, somatostatin functions as a neurotransmitter, immunomodulator and suppressor of angiogenesis and cell proliferation (Reichlin, 1983*a*,*b*; Schally, 1988; Patel et al, 1994; Pollak and Schally, 1998). Somatostatin acts by binding to specific receptors (sst) of which five principal subtypes have been identified: sst1, sst2, sst3, sst4 and sst5 (Bruns et al, 1994).

RC-160 (D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2) is a potent cyclic octapeptide analogue of somatostatin (Cai et al, 1986). RC-160 is up to 500 times more potent than octreotide and somatuline in inhibiting the synthesis/release of hormones from sst expressing pituitary and malignant neuroendocrine cells, although the maximal inhibitory effects are similar (Hofland et al, 1994). RC-160 inhibits the growth of sst positive colonic, pancreatic, gastric, lung and both androgen-dependent and -independent prostatic cancer and glioblastoma and osteosarcoma tumours (Schally, 1988; Qin et al, 1995; Pinski et al, 1996; Pollak and Schally, 1998). In transfection experiments, sst2 and sst5 have been shown to be the principal receptor subtypes involved in mediating the growth inhibitory effects of RC-160 (Buscail et al, 1994, 1995; Cordelier et al, 1997). RC-160 activates phosphotyrosine phosphatases (PTPs), which dephosphorylate tyrosine residues of activated type 1 growth factor receptors, including epidermal growth factor, and inhibits intracellular cAMP and cGMP accumulation and calcium mobilization (Schally, 1988; Buscail et al, 1994; Buscail et al, 1995; Qin et al, 1995; Cordelier et al, 1997; Pollak and Schally, 1998). The growth inhibitory effects of RC-160 in sst positive cancer cell lines have been directly linked to activation of PTPs and inhibition of intracellular cAMP production in vitro (Liebow et al, 1988; Qin et al, 1995).

Somatostatin analogues also inhibit the growth of sst negative tumours in in vivo studies, suggesting important indirect anti-proliferative effects. Many tumours express insulin-like growth factor-I (IGF-I) receptors and proliferate in response to exposure to IGF-I (Macaulay, 1992). Through down-regulation of the growth hormone GH/IGF-I axis, somatostatin analogues may inhibit the growth of IGF-I responsive tumours (Holly, 1998; Pollak and Schally, 1998). The growth inhibitory effects of RC-160 in sst negative tumours are associated with a decrease in serum growth hormone (GH) and IGF-I levels and in tumour IGF-I receptor expression (Pinski et al, 1994, 1996). Somatostatin analogues are also potent anti-angiogenic agents, having direct growth inhibitory effects on proliferating endothelial cells (Patel et

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al, 1994). As angiogenesis is essential for tumour growth beyond 1–2 mm in diameter, inhibition of this process may result in tumour growth inhibition (Folkman, 1995).

In the region of between 70 and 90% of breast tumours express specific high affinity binding sites for radiolabelled somatostatin analogues (Prevost and Israel, 1993, Van Eijck et al, 1994). The principal subtype detected is sst2 (Evans et al, 1997). The octapeptide somatostatin analogues, including RC-160, inhibit the growth of sst expressing breast tumours in vitro and in vivo (Setyano-Han et al, 1987; Szende et al, 1989; Szende et al, 1991; Brower et al, 1992; Szepeshazi et al, 1992; Prevost and Israel, 1993). The regressive changes in vivo are consistent with apoptosis and coagulation necrosis (Szende et al, 1989, 1991). IGF-I is a potent mitogen for breast cancer cells. The growth effects are mediated through high affinity IGF-I receptors, which have been found to be expressed by the majority of breast tumours (Klijn et al, 1993; Helle and Lonning, 1996). RC-160 therapy results in down-regulation of IGF-I receptor expression in MXT mouse mammary carcinoma (Srkalovic et al, 1989). Therefore, RC-160 therapy may inhibit breast cancer growth through modulation of the mitogenic effects of IGF-I.

A number of small studies have evaluated somatostatin analogues in the management of breast cancer. Treatment rarely induces an objective response, although disease stabilization has been reported in 20–43% of cases. When studied, only a moderate and non-durable reduction in IGF-I levels was seen (Manni et al, 1989; Morere et al, 1989; Vennin et al, 1989; Stolfi et al, 1989; Prevost and Israel, 1993; Di Leo et al, 1995; Ingle et al, 1996). Toxicity in all studies was mild, transient diarrhoea being the most common observed problem. Other side-effects of somatostatin analogue therapy include pain at the injection site, glucose intolerance and gallstone formation (Battershill and Clissold, 1989).

The work presented evaluated single agent, high-dose, continuous infusional RC-160 in the management of patients with pretreated metastatic breast cancer.

PATIENTS AND METHODS

Entry criteria

In a phase II single-centre study we evaluated the efficacy of RC-160 (Debiopharm SA, Lausanne, Switzerland) in the management of patients with relapsed breast cancer following previous systemic therapy. Inclusion criteria included cytologically and/or histologically proven breast cancer, at least unidimensionally measurable disease, age ≥ 18 years, life expectancy ≥ 3 months, performance status ≤ 2, haemoglobin ≥ 10 g dl^{-1}, white cell count ≥ 3×10^9 l^{-1}, absolute neutrophil count ≥ 2×10^9 l^{-1}, platelets ≥ 100×10^9 l^{-1}, bilirubin ≤ 2 × normal (N), aspartate and alanine transferase ≤ 3 × N unless due to metastases where values ≤ 5 × N were accepted and plasma creatinine ≤ 150 μmol L^{-1}. Patients who had received intensive chemotherapy or radiotherapy within the previous 3 weeks, pregnant women or, where appropriate, those not taking adequate contraceptive precautions were excluded from the study.

Pretreatment evaluation included a minimum of a history and physical examination, a full blood count (FBC), renal, liver and bone biochemistry, fasting blood sugar, a chest X-ray and ultrasound examination of the abdomen. Response was assessed according to standard WHO criteria. All evaluable/measurable sites of disease were recorded prior to commencing therapy employing further radiological techniques as deemed necessary. Clinical photographs and measurements of skin lesions and/or lymph nodes were also performed and utilized for response assessment where appropriate.

The RC-160 was supplied as a lyophilized powder in sterile vials. The powder was dissolved in sterile water for injection. The powder contained a glutamate excipient and the reconstituted solution had a pH = 4.5 (Debiopharm SA). The reconstituted solution was administered by continuous subcutaneous infusion using a Walkmed 350 pump (Medex Medical Inc., Rossendale, UK). Continuous infusion was chosen to facilitate the administration of high-dose RC-160, to maintain relatively steady-state RC-160 plasma levels (not formally tested) and because RC-160 is being developed as a slow release formulation for depot injection (unpublished data), as have the other principal somatostatin analogues octreotide and somatuline (Caron et al, 1997; Helle et al, 1998). Initially, either a 23G Butterfly needle (Venisystems, Sligo, Ireland) or a 22G Intima catheter (Becton Dickinson, Sandy, Utah, USA) was implanted into the anterior abdominal wall, alternating between the two types for each patient to investigate the efficacy and side-effects of each. The RC-160 infusion was started at 3 mg day^{-1} increasing after 1 week to 4.5 mg day^{-1} and after 4 weeks to 6 mg day^{-1}. Patients were treated on an in-patient basis for the first 2 days of therapy in order to become familiar with the pumps and to report any untoward side-effects. No problems were observed with RC-160 solubility in preclinical testing, at the time of preparation of the solution or during treatment.

Planned follow-up assessments included an FBC, biochemistry profile and fasting blood sugar, IGF-1 and PRL levels on days 7, 28 and monthly thereafter. Measurable sites of disease were to be evaluated after 28 days and subsequently every 2 months. The study was approved by the local Ethics Committee and patients were included only after giving informed written consent.

Serum insulin-like growth factor I and prolactin levels

IGF-I and PRL levels were evaluated in fasting serum samples obtained prior to commencing RC-160 treatment, and after 1, 4 and every 4 weeks thereafter until treatment was discontinued. IGF-I levels were measured using the Octeia IGF-I kit, a two site immunoenzymometric assay (IEMA) (Immunodiagnostic Systems Ltd., Boldon, Tyne and Wear, UK). The sensitivity of the assay is < 1 nmol l^{-1}. The intra-assay coefficient of variance is 2.3–3.5% and the interassay variance is 6.95–7.14%. The normal ranges are 9.5–45 nmol l^{-1} for women aged 21–40 years, 7.5–30 nmol l^{-1} for women aged 41–60 years and 5–22.5 nmol l^{-1} for women aged > 60 years. PRL levels were measured using AxSYM prolactin, a microparticle enzyme immunoassay (MEIA) (Abbott Laboratories, Diagnostics Division, Abbott Park, IL 60064, USA). The sensitivity of the assay is 15 mU/l^{-1}. The intra-assay coefficient of variance is 2.81–4.13% and the interassay variance is 0–4.97%. The normal range is 33–580 mU/l^{-1}.

Statistical methods

The paired Student's *t* test was used to determine the equality of paired means for glucose, IGF-I and PRL levels on treatment. The analysis was performed employing the Stata statistical software, release 5.0 package (Stata, College Station, TX, USA).

Table 1 Clinical characteristics of patients (pts)

Fourteen women recruited to study	
Age: median	61.3 years
range	37–80 years
ECOG performance status	0–2 (median 1)
Histological subtypes	
Invasive ductal	10 pts
Lobular	2 pts
Mixed ductal and lobular	1 pt
Other	1 pt
Previous treatments	
Prior endocrine therapy	13 pts
adjuvant	6 pts
advanced	10 pts
Prior chemotherapy	12 pts
adjuvant	7 pts
advanced	10 pts
Prior radiotherapy	
local	14 pts
ablative oophorectomy	1 pts
metastatic	3 pts
Sites of disease	
Skin	8 pts
Lymph nodes	6 pts
Bone	5 pts
Pleura	5 pts
Soft tissue	3 pts
Lung	3 pts
Liver	2 pts
Breast	2 pts
Adrenal	1 pt

RESULTS

Fourteen women, age range 37–80 years (median 58.5 years), ECOG performance status 0–2, with stage IV breast cancer were recruited to the study. Patient characteristics are summarized in Table 1. All patients had received prior radiotherapy and endocrine agents and/or cytotoxic chemotherapy on at least one occasion, either in the adjuvant setting or for advanced disease.

Toxicity

Toxicities are summarized in Table 2. No grade 3 or 4 toxicities were seen. Haematological toxicities were virtually absent, while no alterations were seen in renal or liver function. A significant increase was seen in fasting blood glucose levels, the mean rising from 5.3 mmol l^{-1} to 6.2 mmol l^{-1} ($P = 0.0012$). The most common side-effects included mild fatigue and diarrhoea. The diarrhoea was associated with steatorrhoea in five cases. Two patients reported a marked increase in borborygmi. Regular anti-emetics were required for mild nausea by seven patients. Both patients with liver metastases experienced nausea which, although present at baseline, worsened on treatment

The most troublesome side-effect was inflammation at the infusion site seen in 11 patients associated with the development of subcutaneous abscesses in eight. Two cases were lanced. These and two others were treated with oral antibiotics. In two cases swab cultures grew *Staphlococcus aureas*. However, it became apparent with time that the abscesses probably represented local reactions either to the cannulas or to the RC-160 infusion itself. This led to a policy of resiting the cannula site weekly, whether or not there was a problem. Subsequently, abscess formation became less of a problem without the need for therapeutic intervention. Regarding the assessment of cannula type, the Intima cannula proved more user friendly than Butterfly needles, being subjectively more comfortable for the patient and easier to secure.

Table 2 Number of patients developing grade 1 and 2 toxicities based on CALGB common toxicity criteria (total number of patients in study = 14)

	Grade	
Toxicity	**1**	**2**
Diarrhoea	9	3
Vomiting	4	1
Nausea	4	3
Fatigue	7	5
Headache	6	1
Constipation	2	1
Abdominal pain	3	2
Inflammation at infusion site	7	4

No grade 3 or 4 toxicities were seen in any of the patients. Four patients with inflammation at the infusion site were treated with antibiotics until we determined that the problem was due to sterile abscess formation. Borborygmi were recorded in two patients and steatorrhoea in five. Haematological toxicities were rare with two patients developing grade 1 neutropenia and one a grade 2 leucopenia.

Serum IGF-I and PRL levels

IGF-I levels fell in all patients treated with RC-160. The mean level for the 14 patients decreased by 45% by day 7 ($P < 0.0001$). No subsequent significant change was seen between days 7, 28 and 56, despite increasing doses of the medication (Figure 1). Only three patients were sampled on day 84. Again the values were similar to those on day 7. Samples were obtained from two patients off treatment and doubled from the last on treatment sample. PRL levels were within the normal range in 11 of the 14 patients evaluated and

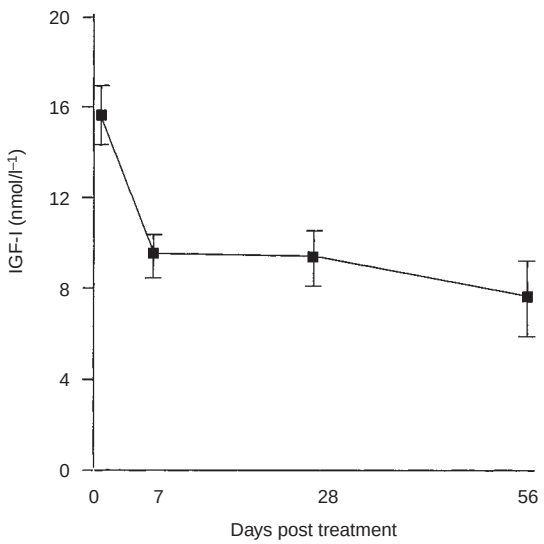

Figure 1 Serum IGF-1 (mean±SE). Paired test: pre vs. day 7, $P < 0.00001$ ($n = 14$); pre vs. day 28, $P < 0.0002$ ($n = 11$); pre vs. day 56, $P < 0.0009$ ($n = 8$); day 7 vs. day 28, P = NS ($n = 11$); day 28 vs. day 56, P = NS ($n = 8$)

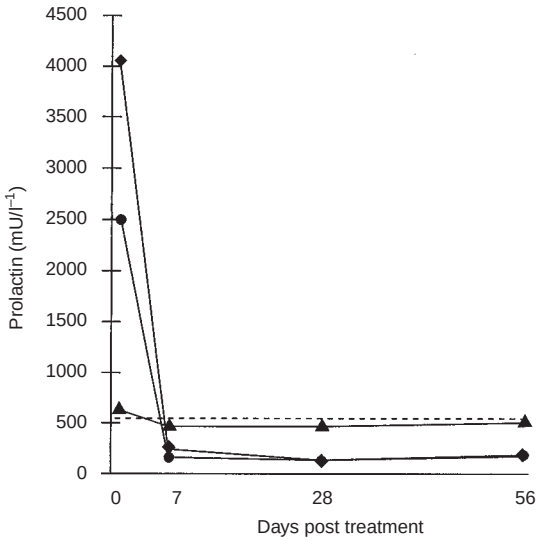

Figure 2 RC-160 therapy resulted in normalization of serum prolactin in all 3 patients with elevated levels pretreatment

were not affected by RC-160 therapy. However, elevated levels seen in three patients fell to normal within 7 days of commencing the RC-160 infusion (Figure 2).

Response

No objective tumour responses were seen, all patients showing objective evidence of progressive disease within 3 months of commencing RC-160 treatment.

DISCUSSION

Breast cancer is the most prevalent malignant disease among women in developed countries. When the disease has spread beyond the breast and axilla, it is essentially incurable and therapy is palliative, being directed at prolonging survival and improving symptom control (Hayes et al, 1995; Goldhirsch and Gelber, 1996). Somatostatin analogues are among the novel hormonal agents being investigated for the treatment of breast cancer.

Although RC-160 represents the most potent of the principal somatostatin analogues in clinical development and is well tolerated when administered in high dose as a continuous subcutaneous infusion, the agent proved to be ineffective in the treatment of relapsed, previously treated, metastatic breast cancer. This is in keeping with the results reported for the somatostatin analogues, octreotide and somatuline (Manni et al, 1989; Morere et al, 1989; Stolfi et al, 1989; Vennin et al, 1989; Prevost and Israel, 1993; Di Leo et al, 1995; Ingle et al, 1996). Similar results are also seen in inoperable pancreatic cancer where, although tumour growth was stabilized and symptoms improved in a proportion of patients, it was felt that RC-160 alone, even in large doses, is insufficient to produce effective palliation in these patients (Poston and Schally, 1993).

One of the principal side-effects seen was the development of skin inflammation in 11 patients, associated with cutaneous abscesses at the cannulation site in eight. These were initially treated as being infected. However, with experience the suspicion increased that the abscesses were most probably sterile and represented a reaction to either the cannula or to the infusion itself. Similar findings have been documented in patients treated at other centres with infusional RC-160. In a number of cases the material from the abscesses has been studied microscopically, revealing florid chronic inflammation with infiltration by multinucleated giant cells. In a study in the Yucatan minipig, prolonged subcutaneous catheter implantation induced focal ulceration in the epidermis in one of six animals, fibroblast proliferation in the dermis and local inflammation centred around the catheter. Vapreotide with or without glutamate excipient at a concentration of 1.5 mg ml^{-1} induced granulomas surrounded with fibrosis, associated sometimes with local oedema (Debiopharm SA; unpublished data). This led to a policy of resiting the cannula on a weekly basis, which eased the problem considerably. A small but significant increase in fasting blood glucose levels, and mild gastrointestinal side-effects were also seen in keeping with known side-effects of somatostatin analogue therapy (Battershill and Clissold, 1989). The only therapeutic intervention required was the use of anti-emetics in seven patients.

A significant reduction in serum IGF-I levels was seen, levels falling in all patients as compared with baseline. Unlike previous studies in breast cancer, where lower doses of octreotide and somatuline were used, the high dose continuous infusion of RC-160 induced a 45% reduction in serum IGF-I levels which was sustained over time (Manni et al, 1989: Vennin et al, 1989; Di Leo et al, 1995). This is likely to be a result of the dose used as in a recent phase I study in 14 patients with gastrointestinal tract and pancreatic cancer, microencapsulated octreotide pamoate 90 mg i.m. every 4 weeks or 160 mg i.m. every 2 weeks (> 3 mg day^{-1}) produced an equivalent sustained reduction in plasma IGF-I levels of 49–53% (Helle et al, 1998).

The lack of efficacy of single agent somatostatin analogue therapy in breast cancer may in part be due to the antagonistic effects of oestrogen (Setyano-Han et al, 1987). Recent in vitro and in vivo studies indicate that anti-oestrogens may potentiate the anti-tumour activity of somatostatin analogues in breast cancer (Weckbecker et al, 1994; Candi et al, 1995; Pollak, 1996; Xu et al, 1996). In 33 post-menopausal women with untreated breast cancer the combination of depot injections of somatuline with oral tamoxifen resulted in an objective response rate of 50% (95% CI: 35–69%) (Canobbio et al, 1995). The potential therapeutic benefits of combined manipulation of the GH/IGF-I and gonadotropin/sex steroid axes in sex hormone-associated tumours is supported by a recent study combining anti-androgen therapy with RC-160 in 19 men with hormone refractory prostate cancer. After 3 months, 14 patients showed a decrease in serum prostate-specific antigen levels, reduction in bone pain and improvement in Karnofsky performance status (Gonzalez-Barcena et al, 1998).

Experimental evidence suggests that PRL, a growth factor for normal breast tissue, is an autocrine factor for breast cancer. Tumour cells have been demonstrated to express both PRL and PRL-receptors (Clevenger et al, 1995; Das and Vonderhaar, 1996). The prolactin receptor, of which three subtypes have been identified, belongs to the superfamily of cytokine receptors. Binding of PRL to the PRL-receptor results in activation of cytoplasmic signal transducers and transcriptional activators through phosphorylation of JAK2 kinases. Recent work has shown that activation of breast cancer cell line PRL receptors results in stimulation of the *ras-raf*-MEK(MAP kinase kinase)-MAP kinase mitogenic pathway. The activation of ras is mediated via tyrosine phosphorylation of SHC, recruitment of GRB2 and the guanine nucleotide exchange factor SOS (Erwin et al, 1995; Das and Vonderhaar, 1996).

RC-160 reduced serum PRL to within the normal range in all three patients with elevated levels prior to commencing treatment. Somatostatin analogues have little or no effect on primary pituitary hyperprolactinaemia (Lamberts et al, 1991). These findings suggest that the elevated PRL levels seen in the patients in our study are due to synthesis and release of the peptide from breast cancer cells. The reduction of elevated PRL levels to within the normal range and activation of PTPs in tumours expressing sst2 suggest that RC-160 may counteract the tumour growth stimulating effects of PRL in some breast cancer patients. A recent study of triple therapy with tamoxifen, the anti-prolactin agent CV 205–502 and octreotide gave an objective response in five of nine evaluable patients. A significant reduction in IGF-I levels was seen similar to that documented in the present study. Furthermore, a strong significant reduction in circulating PRL levels was documented in patients with normal PRL secretion (Bontenbal et al, 1998). RC-160 may have a role to play in this setting.

In experimental models octreotide has been shown to enhance the efficacy of cytotoxic agents in the treatment of solid tumours while ameliorating their side-effects. The drugs studied include doxorubicin, paclitaxel, mitomycin C and 5-fluorouracil all of which are routinely used in the management of metastatic breast cancer (Lee et al, 1993; Weckbecker et al, 1996). The enhanced anti-tumour activity may in part be explained by the inhibitory effects of somatostatin analogues on angiogenesis, as known anti-angiogenic agents such as TNP-470 and minocycline have been shown to increase the anti-tumour activity of cyclophosphamide (Patel et al, 1994; Teicher et al, 1994). In keeping with these findings, RC-160 has been shown to increase the effectiveness of 5-fluorouracil in vivo (Szepeshazi et al, 1991). These results suggest the merit of evaluating the combination of somatostatin analogues with cytotoxic agents in breast cancer therapy, initially in animal models and subsequently in phase I studies. Cytotoxic analogues of somatostatin containing potent anthracyclines have recently been developed and have shown promising anti-tumour activity in vitro and in vivo in a number of sst positive solid tumours including breast cancer. The possibility of specifically targeting sst-rich tumours in patients with such agents is an exciting prospect for the future (Nagy et al, 1998).

IGF-I is a potent trophic and survival factor for many normal cells including those of the breast and prostate gland. Overexpression of growth hormone and IGF-I receptor agonists is associated with the development of breast cancer in transgenic mice (Tornell et al, 1992; Bates et al, 1995). In two recently published prospective studies, high normal IGF-I levels were associated with an increased relative risk for the development of prostate cancer in men (4.3) and breast cancer in premenopausal women (7.28 in premenopausal women aged < 50 years, when adjusted for IGF binding protein-3 levels) (Chan et al, 1998; Haankinson et al, 1998). Given the sustained suppression of IGF-I levels documented in the present study, RC-160 alone or in combination with tamoxifen or LHRH analogues or antagonists may have a role in the chemoprevention of breast and prostate cancer (Holly, 1998).

In conclusion, although ineffective as single agent therapy, high-dose RC-160 is well tolerated, induces a significant and sustained reduction in serum IGF-1 levels and normalizes hyperprolactinaemia in patients with pretreated metastatic breast cancer. Based on the favourable toxicity profile and the encouraging preclinical findings, further experimental and clinical studies combining RC-160 with anti-oestrogens, antiprolactins, cytotoxic and anti-angiogenic agents are warranted.

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