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# *IN VITRO AND IN VIVO* INHIBITION OF HUMAN SMALL CELL LUNG CARCINOMA (NCI-H69) GROWTH BY A SOMATOSTATIN ANALOGUE

John E. Taylor*, Arthur E. Bogden*, Jacques-Pierre Moreau*, and David H. Coy**

*Biomeasure Inc, 11-15 E. Ave, Hopkinton, MA 01748

** Peptide Research Laboratories, Department of Medicine, Tulane University School of Medicine, New Orleans, LA 70112

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SUMMARY: An endocrinologically-potent octapeptide analogue of somatostatin (SRIF), 3-(2-naphthyl)-D-Ala-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-$NH_2$ (BIM-23014 C), was examined for its ability to inhibit the *in vitro* and *in vivo* growth of the human small cell lung carcinoma (SCLC) line, NCI-H69. When cultured cells were implanted into athymic nude mice, treatment (500 μg/injection, twice daily) resulted in a prolongation of lag time for the appearance of measurable tumors, and there was a marked inhibition of the growth rate. Indeed, peptide injection in the region of the tumor resulted in a complete regression of the NCI-H69 tumors. Withdrawal of BIM-23014 C treatment resulted in an acceleration of tumor growth indicating an antiproliferative rather the oncolytic action. A similar inhibition of tumor growth was also observed when solid tumors obtained from the first implantion were used as the donor tissues. In cell culture, the proliferation in the presence of a low concentration (10nM) of BIM-23104 C was also significantly retarded suggesting a direct mechanism of action. 

The tetradecapeptide, SRIF, initially described as an inhibtor of pituitary growth hormone secretion, also exhibits a diversity of other biological activities ranging from the inhibition of thyrotropin releasing hormone and prolactin secretion, to the regulation of endocrine and exocrine pancreatic function. SRIF is also inhibitory to gastrointestinal secretory activity and may function as a neurotransmitter or neuromodulator in the central nervous system (1-4). In view of these physiological actions of SRIF, it is not surprising SRIF analogues have been employed to either inhibit the growth or retard the secretory activity of endocrine tumors from tissues that are responsive to the biological actions of SRIF (5-11). SRIF receptors have also been reported to be expressed in tumors derived from tissues not normally considered as targets for SRIF, and there are indications that SRIF may have direct antitumor activity independent of its known endocrine activity (12-17). We have recently observed that human small cell lung carcinoma cells (SCLC), which exhibit neuroendocrine properties (18,19), are highly enriched with SRIF receptors (20), suggesting that SRIF may also have role in the regulation of

Address correspondence to John E. Taylor, Ph.D.

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SCLC function. It seemed logical, therefore, to test the hypothesis that SRIF could be a growth inhibitory factor for SCLC, similar to that observed for endocrine tumors, and in the present report, we show for the first time that a potent SRIF analogue, BIM-23014 C (21), has antiproliferative activity for SCLC tumor growth *in vitro* and *in vivo*.

## METHODS

### *In Vitro Experiments*

Human SCLC (NCI-H69), an *in vitro* maintained cell line, was obtained from the American Type Culture Association, and grown in RPMI-1640 medium containing 10% fetal bovine serum. The cells were cultured in a humidified atmosphere of 10% $CO_2$ and 90% air (37° C). For the *in vitro* growth experiments, approximately 1 x $10^6$ were seeded into T-flasks containing 20 ml of the culture medium or medium which had been supplemented with 10 nM BIM-23014 C. At 24, 36, and 72 hours aliquots (1 ml) were removed from each flask and the number of viable cells (trypan blue exclusion) was determined using a hemocytometer.

### *In Vivo Experiments*

For the initial *in vivo* experiments, NCI-H69 cells, which had been cultured *in vitro,* were collected by low speed centrifugation, resuspended in the culture medium, and injected (~ 5 x $10^6$ cells) subcutaneously (s.c.) into the right flank of female, athymic nude mice (nu/nu/CR, 5-6 weeks of age). The xenografted mice were randomized into vehicle treated control and two test groups, and treatment was initiated in the p.m of the implantation day according to the following protocol:

| Group No. | Treatment (s.c.=subcutaneous; i.p.=intraperitoneal) | No. Animals |
|---|---|---|
| 1 | saline vehicle, 0.2 ml/inj. | 10 |
| 2 | BIM-23014 C, 500 µg/inj., i.p. | 5 |
| 3 | BIM-23014 C, 500 µg/inj., s.c. around tumor | 5 |

Injections were continued on a twice daily schedule Monday through Friday and as a single total daily dose on weekends. Tumors were measured with Vernier calipers and size recorded as the average of two diameters in mm.

Since human SCLC's grow as solid tumor masses it was also desirous to test BIM-23014 C against xenografts orginating from an *in vivo* growing tumor as contrasted to the cell aggregates implanted from *in vitro* culture. Therefore, NCI-H69 tumors growing s.c. as first transplant generation xenografts were used as donor tissues and transplanted s.c. into athymic nude mice as a 2 mm cube fragment. These animals were randomized into one control and three test groups and treated as follows:

| Group No. | Treatment | No. Animals |
|---|---|---|
| 4 | saline vehicle control, 0.2 ml/inj. | 10 |
| 5 | BIM-23014 C, 500 µg/inj., s.c. | 5 |
| 6 | BIM-23014 C, 500 µg/inj., s.c. around tumor | 5 |
| 7 | BIM-23014 C, 500 µg/inj., s.c., i.p | 5 |

Treatment frequency and tumor measurements were made as described above.

## RESULTS

Figure 1 illustrates the tumor growth curves obtained with the cultured NCI-H69 cells that had been implanted into athymic nude mice. All vehicle treated controls developed progressively growing s.c. tumors with a mean lag time (days to first appearance of measurable

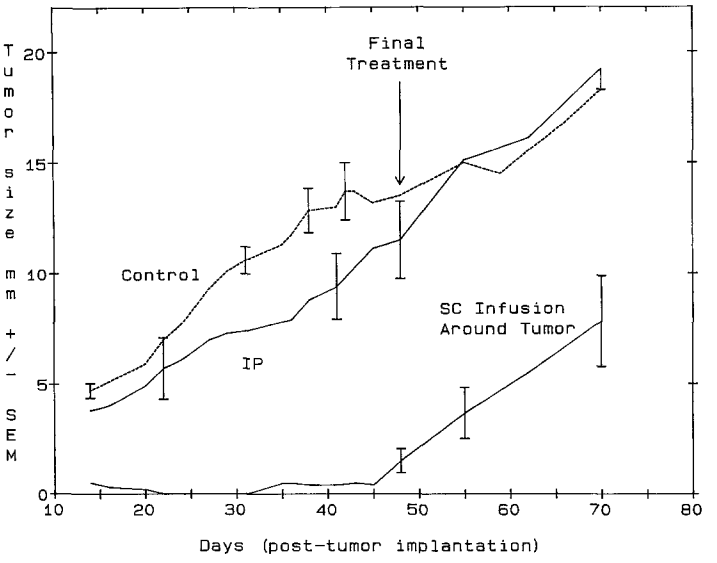


Fig. 1 The effect of SRIF analogue, BIM-23014 C on the *in vivo* growth of cultured SCLC (NCI-H69) cells implanted into female, athymic nude mice. Some SEM bars have been omitted for clarity.

tumors) of 12.4 days. Appearance of measurable tumors in animals treated i.p. was delayed, with a mean lag time of 14 days. Though progressive, tumors grew at a slower rate while under i.p treatment. One tumor regressed after 15 days, regrowing only after termination of treatment. Animals administered BIM-23014 C as a s.c. infusion aroung the tumor developed small palpable tumor nodules which regressed, being almost compeletely inhibited during the 48 day treatment period. There was an apparent acceleration of tumor growth in both groups following termination of treatment. However, two of the five tumors that had regressed in the s.c. treated group failed to regrow.

In confirmatory studies, BIM-23014 C was tested against the *in vivo*-propagated human SCLC tumors, thus directing antitumor activity against s.c. tumor xenografts prepared from solid tumors (Fig. 2). Except for the inclusion of an additional s.c. group (away from the tumor transplant), the same dose level and treatment regimen were followed. Growth of NCI-H69 tumors was most effectively inhibited when BIM-23014 C was administered as a s.c. infusion around the tumor compared to s.c. and i.p. injections.

The proliferation of NCI-H69 cells in culture was significantly decreased by incubating the cells in the presence of low concentrations (10 nM) of the SRIF analogue, BIM-23014 C (Fig. 3). and after 72 hours the average cell concentration was 59% of that observed in the control cultures.

## DISCUSSION

It is well established that SRIF and analogues inhibit the growth and hypersecretory activity of SRIF-responsive endocrine tumors (i.e., acromegaly, insulinoma, glucagonoma,

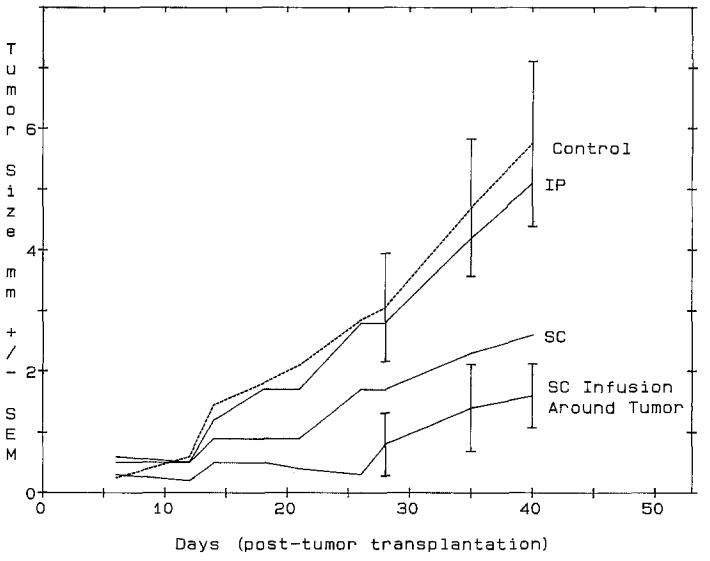


Fig. 2 The effect of SRIF analogue, BIM-23014 C on the growth of the *in vivo*-propagated SCLC (NCI-H69) tumors transplanted into female, athymic nude mice. Some SEM bars have been omitted for clarity.

carcinoid syndrome, VIPoma) which are derived from target tissues for SRIF (5-11). Antitumor activity at non-target tissues may also result from an inhibition of pituitary growth hormone and a subsequent decline in the circulating levels of somatomedins. In addition, SRIF may have more direct receptor-mediated antiproliferative actions. The present observation that SCLC proliferation is inhibited *in vitro* , and an earlier report (20) that SCLC NCI-H69 cells expresses a high density of SRIF receptors would certainly support this hypothesis. Similar

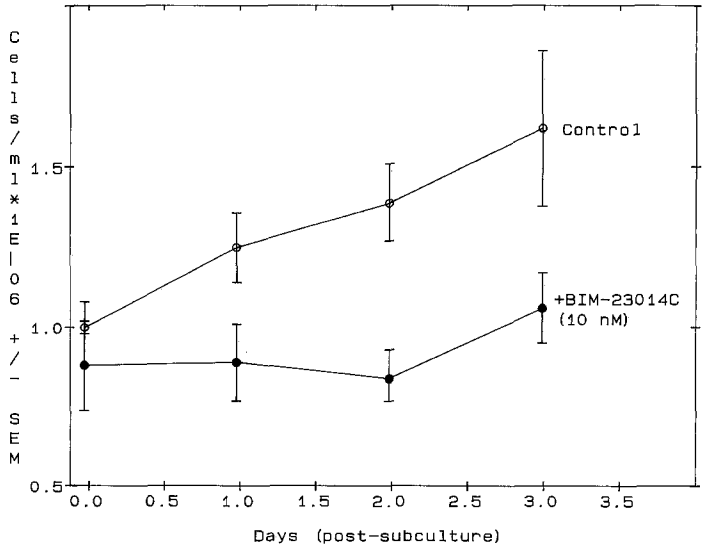


Fig. 3 The effect of SRIF analogue, BIM-23014 C on the proliferation of the SCLC (NCI-H69) cells in culture.

observations have been made for MCF-7 breast cancer cells grown in cell culture (17). While the cellular basis for this putative direct antiproliferative action is not known, the secretion of paracrine or autocrine growth factors which promote tumor proliferation may be inhibited by SRIF (12-17). SCLC secrete several neuroendocrine peptides, including bombesin, a potent and selective growth factor for SCLC *in vitro*. (22) In addition to bombesin, it is not known, however, if other peptide growth factors ( e.g., somatomedin C, epididermal growth factor) are secreted by SCLC cells. It is important to note in this regard, that SRIF analogues have been reported to inhibit the vasoactive intestinal peptide (VIP)-stimulated bombesin secretion from the SCLC cell line, NCI-H345, and it was proposed that the secretion of bombesin by SCLC tumor cells may be under the regulation of VIP and SRIF (23). Alternatively, SRIF could also have direct receptor-mediated effects on cell division mechanisms and act as functional growth factor antagonist at the cellular level (24,25). Irrespective of the mechanism, withdrawal of peptide treatment resulted in an acceleration of tumor growth indicating that the acitivty of BIM-23014 C, was antiproliferative as opposed to a generalized oncolytic effect against SCLC cells.

The concept that tumors may escape from physiologic control by the excessive production of autocrine growth factors (26) suggests that tumors may be responsive to the antiprofilerative action of SRIF or analogues at doses only exceeding the normal physiological levels. This phenomonen appeared to be evident in the present study, where the *in vivo* inhibition of SCLC growth was obtained at BIM-23014 C doses approximately 30 times that required to inhibit pituitary GH release (21). However, *in vitro*, the inhibitory concentration employed in these experiments was more closely related to its *in vitro* binding affinity (20 ), and furthermore, s.c. infusion around the tumor was the most effective route of administration, indicating that the high dose required *in vivo* may be related in part to an inability of the peptide to gain access to the tumor.

It has been reported that desensitization to pituitary GH suppression occurs with prolonged SRIF analogue administration *in vivo* (25,27). This phenomenom also occurs with respect to pancreatic insulin secretion (12,28), and some patients with VIPoma have been shown to escape the therapeutic response to SRIF analogue treatment (29). The density of SRIF receptors on cultured MCF-7 cells has also been shown to be diminished after chronic exposure to the peptide (17). In spite of a very lengthy treatment in the present experiments, we did not note any trend towards an escape from the antitumor activity of the peptide, an observation which may have important therapeutic advantages in clinical treatment of SCLC.

In conclusion, the growth of human SCLC cells (NCI-H69) implanted into nude mice and *in vivo*-propagated tumors was inhibited by chronic treatment with the potent SRIF analogue, BIM-23014 C. Growth of the *in vitro*-propagated NCI-H69 cell line was also effectively inhibited. Termination of treatment resulted in an acceleration of SCLC growth indicating antiproliferative action rather than a cytotoxic effect. The mechanism of the antiproliferative effect is not known, but the presence of high-affinity SRIF receptors and *in vitro* activity

indicates a direct action on the tumor cells. These results suggest that SRIF or SRIF analogues may be useful in the clinical treatment of SCLC.

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