A somatostatin analogue inhibits chondrosarcoma and insulinoma tumour growth

J. C. Reubi

Preclinical Research, Sandoz Ltd., CH 4002 Basel, Switzerland

Abstract. The in vivo effects on tumour growth of a potent somatostatin analogue, SMS 201-995 [H-(D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-(ol)], were measured in two characterised transplantable tumours:
a) the Swarm rat chondrosarcoma, known to be insulin-, growth hormone (GH)-, somatomedin- and corticosteroid-dependent,
b) a hamster insulinoma, bearing specific high affinity somatostatin receptors.
SMS 201-995 (1.25 mg/kg/day) given for 25 days to rats bearing freshly transplanted chondrosarcomas inhibited tumour volume by 48%. A significant tumour growth inhibition was measured also in well developed tumours treated with high doses of SMS 201-995 (1.25 mg/kg/day) for 7 days. In the treated animals, GH was significantly inhibited.
In hamsters bearing a freshly transplanted insulinoma, the daily application of SMS 201-995 (200 μg/kg/day, sc) for 33 days could significantly inhibit the growth (as measured by tumour volume) of the tumour. A moderate inhibitory effect of SMS 201-995 on the growth of well grown insulinomas could also be observed.
This study shows that SMS 201-995 under the present experimental conditions has a moderate but significant growth inhibitory effect in two different transplantable tumour models. In the rat chondrosarcoma, the effect of SMS 201-995 is probably indirect, due to inhibition of GH, somatomedin and insulin. In the hamster insulinoma, the effect is possibly due to a more direct action of SMS 201-995 on specific somatostatin receptors present in this tumor.

Somatostatin (SS), a tetradecapeptide first characterized as GH release inhibiting factor (Brazeau et al. 1973), has been found to exert a mainly inhibitory action on a wide variety of cellular processes in multiple tissues (Vale et al. 1977). It is well documented that SS is particularly powerful in inhibiting the release of various hormones such as GH, insulin, glucagon and most gut hormones (Vale et al. 1977). But it can also inhibit cellular activity in various tissues (Renaud et al. 1975) and it has been suggested to inhibit various growth processes (Wagner et al. 1978; Mascardo & Sherline 1982). Such a wide inhibitory action of SS lets speculate that it could play a more general role as inhibitor of cell proliferation. It has been shown recently for instance to inhibit the growth of some well differentiated, hormone dependent tumours (Redding & Schally 1983; De Quijada et al. 1983). Such a growth inhibitory process could be the result of a lowering of plasma levels of growth-promoting substances such as GH, somatomedin, insulin and prolactin, a hormonal situation very well achieved by SS, but it could alternatively be mediated through specific SS receptors located on the various tumour tissues.

Recently a stable octapeptide analogue of SS, SMS 201-995 [H-(D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-(ol)], has been synthesized and shown to be very potent in inhibiting GH, insulin and glucagon in animals (Bauer et al. 1982) and man (Plew et al. 1984). It also shows a high affinity towards SS receptors located in normal and tumorous tissue (Reubi 1984; Reubi & Landolt 1984). We therefore investigated whether such a potent and long-acting SS analogue, given chronically, is able to significantly inhibit the growth of two different kinds of transplantable tumours, at different developmental stages:
a) a rat chondrosarcoma, known to be somato-
medin-, insulin- and corticosteroid-dependent (Choi et al. 1971; McCumbee & Lebovitz 1980; McCumbee et al. 1980),

b) a hamster insulinoma, known to possess a high density of high affinity SS receptors (Shapiro et al. 1975; Reubi et al. 1982).

Methods

1. Chondrosarcoma

The rat chondrosarcoma has been kindly provided by Dr. R. Swarn (Hoffman La Roche, Nutley) and Dr. W. Bollag (Hoffman La Roche, Basel) and served as the source of malignant chondrocytes. The tumours were removed from donor rats and washed in buffer solution (Walker solution: 9 g NaCl, 0.42 g KCl, 0.24 g CaCl₂, 0.15 g NaHCO₃, 10 g glucose, 1 g streptomycin and 10 mio IU penicillin per litre water; the solution was sterilized at 150°C for 12 h (pH 7.4)). Capsular material was carefully removed and slices of the tumour were passed through a nylon mesh into a beaker containing buffer at 37°C. The resulting slurry was kept in suspension by mechanical stirring, and male rats were injected sc in the neck with 0.5 ml portions of it.

Two types of experiments were performed:

a) Young rats (120-150 g) were used. At day 10 post-transplantation (pt) they were treated with sc injections of 0.2 ml SMS 201-995 (1.25 mg/kg/day, 5 days a week). Control rats bearing the tumours were similarly injected daily with 0.2 ml vehicle solution (10% polyvinylpyrrolidone and 0.01 m acetic acid dissolved in saline). The transplanted rats (6-8 per group) were treated for 40 days. In all animals the tumours were regularly monitored with a caliper (three-dimensionally). At the end of the experiment (50 days after transplantation) they were sacrificed 1 h after the last injection and trunk blood was collected. The blood was centrifuged and serum was carefully removed. Tumours were cleaned of any adhering tissue, weighed and their volume was measured with a caliper. Tumour tissue was stored in formalin for later histological evaluation. Some tissue was processed to a membrane preparation used to measure SS receptors (Reubi et al. 1982).

Serum levels of GH (RIA: Marbach et al. 1978) and glucose (glucose-oxidase method: Beckman Glucose analyzer 2) were measured at the end of the experiment (trunk blood). Statistical evaluation of tumour weight, volume, and its percentual increase as well as weights of animals and of the pituitary gland were made by using Student's t-test with or without logarithmic transformation of the data.

b) Adult rats bearing a tumour which had reached a size of at least 2000 mm³ were treated for 1 week with SMS 201-995 (1.25 mg/kg/day, dissolved in saline) or saline alone. Volume and weight of the tumours were then measured as described under a).

2. Insulinoma

The transplantable hamster insulinoma was kindly provided by Drs. N. Fleischer and D. Schubart (New York) and served as a source of malignant ß-cells. Tumour transplantation to Syrian hamsters was performed as described for rat chondrosarcoma.

a) A first group of 8 to 12 hamsters were treated daily from day 3 pt either with SMS 201-995 (200 µg/kg/day sc, 5 days a week) or with vehicle (NaCl 0.9% w/v, 0.2 ml sc/animal/day). For each group of treatment a glucose-rich diet (4.5 g/l water) was provided for one half of the animals to compensate for the hypoglycaemic effect of the insulin producing insulinoma.

In all animals the tumours were regularly monitored with a caliper (three-dimensionally). At the end of the experiment (36 days pt) they were sacrificed 1 h after the last injection of the experimental substance and trunk blood was collected. The blood was centrifuged and serum was removed. Tumours were cleaned of any adhering tissue and weighed. Tumour tissue was stored in formalin for later histological evaluation. Some tissue was used for SS receptor binding (Reubi et al. 1982).
Table 1.

Effect of chronic application of SMS 201-995 (1.25 mg/kg/day sc) for a period of 18 and 25 days on chondrosarcoma tumour volume and weight as well as rat weight. Therapy is started 10 days after transplantation (day 10 pt).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tumour volume (mm³)</th>
<th>Tumour weight (g)</th>
<th>Rat weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st measurement</td>
<td>2nd measurement</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(day 28 pt)</td>
<td>(day 35 pt)</td>
<td></td>
</tr>
<tr>
<td>SMS 201-995, 1.25 mg/kg</td>
<td>917 ± 301</td>
<td>2746 ± 595*</td>
<td>11.87 ± 2.3</td>
</tr>
<tr>
<td>(n = 7)</td>
<td>(42%)</td>
<td>(48%)</td>
<td>(32%)</td>
</tr>
<tr>
<td>Control (n = 8)</td>
<td>1588 ± 390</td>
<td>5237 ± 906</td>
<td>17.33 ± 2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>361 ± 8</td>
</tr>
</tbody>
</table>

Control: 10% polyvinylpyrrolidone 0.2 ml/day. In parenthesis: per cent inhibition of growth since beginning of treatment as compared to controls. Mean ± SEM. n = number of experiments.

* P < 0.02. ** P < 0.01.

Plasma levels of glucose were measured at the end of the experiment.

b) Another group of hamsters were transplanted and left untreated until the tumours had reached a measurable size. They were then divided into 3 groups: one group was treated with SMS 201-995 (25 µg/kg/day, sc. 5 days a week), one group with SMS 201-995 (2 µg/kg/day) and one group with vehicle only (10% polyvinylpyrrolidone). After 16 days of treatment, tumour volume was measured as previously described.

Results

a) Chondrosarcoma

Among the rats transplanted on the same day, tumours could be detected on each of them between day 20 and 24. Variability of tumour growth within each experimental group was small for this type of study; the individual tumour volume values for the control group are shown as example in Fig. 1. An optimal phase of growth (phase I) could be observed between the 24th and 38th day. In a later phase, the frequency of animal deaths increased and tumour ulceration and necrosis were more pronounced. In the evaluation of treatment efficiency, the phase I was usually taken for analysis.

Effect of SMS 201-995 on tumour growth. As seen in Table 1 daily injections of SMS 201-995 (1.25 mg/kg/day, 5 days a week) starting the 10th day pt, showed an inhibition of tumour growth of 42% already at day 28 pt and 48% at day 35 pt, when compared to controls. Tumour weight was decreased by 32% at day 50 pt.

Effect of SMS 201-995 treatment on plasma GH and glucose. Table 2 shows that at the end of the experiments, SMS 201-995 inhibits GH 1 h after application. This is accompanied, in the same group, by a moderate hyperglycaemia.

Effect of SMS 201-995 given for 8 days on the growth of advanced chondrosarcoma. The group with well developed tumours was treated for 8 days starting at day 29 pt with 1.25 mg/kg SMS 201-995 daily. The per cent increase in tumour volume was significantly lower in the SMS 201-995-treated group already at day 28 pt and 48% at day 35 pt, when compared to controls. Tumour weight was decreased by 32% at day 50 pt.

Table 2.

GH and glucose levels in rats bearing a chondrosarcoma. Rats were treated for 30 days with SMS 201-995 (1.25 mg/kg/day) or with vehicle alone (control: 10% polyvinylpyrrolidone). Trunk blood was taken 1 h after the last drug injection.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GH (ng/ml)</th>
<th>Glucose (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMS 201-995, 1.25 mg/kg</td>
<td>11 ± 1.0*</td>
<td>187 ± 6**</td>
</tr>
<tr>
<td>(n = 7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 8)</td>
<td>112 ± 46</td>
<td>151 ± 5</td>
</tr>
</tbody>
</table>

Mean ± SEM. n = number of experiments.

* P < 0.05. ** P < 0.001.
Effect of chronic application of SMS 201-995 (1.25 mg/kg/day) for 7 days in rats bearing an advanced chondrosarcoma tumour. Initial volume was measured 29 days after transplantation. Therapy was started at that day. Final volume was measured 37 days after transplantation.

Table 3.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tumour volume (mm³)</th>
<th>% increase (log) in volume*</th>
<th>Growth inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
</tr>
<tr>
<td>SMS 201-995, 1.25 mg/kg (n = 4)</td>
<td>2040 ± 421a</td>
<td>6609 ± 1611</td>
<td>2.30 ± 0.133*</td>
</tr>
<tr>
<td>Control (n = 6)</td>
<td>2714 ± 352a</td>
<td>9579 ± 2492</td>
<td>2.67 ± 0.098</td>
</tr>
</tbody>
</table>

* Per cent increase (log) represents the mean of individual increase (in %) in tumour volume for the period between both measurements expressed as a logarithm.

a Tumour volume in control animals was measured at day 30 pt. For the calculation of per cent increase (log) and per cent inhibition, initial tumour volume of treated animals was extrapolated to day 30, assuming linear growth.

Mean ± SEM. n = number of experiments. *P < 0.05.

(Table 3). Although tumour volume and weight showed a reduction in the SMS 201-995-treated group, there was no significant difference with controls, due partly to the large variability of tumour size. The per cent of growth inhibition in treated vs control rats was 28%.

b) Insulinoma

These experiments were characterized by a noticeable variability of tumour size and shape. Since it is the first time that such a quantitative study on growth parameters is performed with this tumour it is not known whether this variability is tumour-linked i.e. whether hamster insulinoma show high variability in general for this tumour growth or whether it is due to technical problems: indeed the very loose subcutis of hamsters makes a spread of the transplanted material over a large area inevitable. This variability affords the use of a high number of animals to reach statistical significance.

Table 4 shows the insulinoma tumour volumes and weights measured at day 36 pt. There is no difference in controls injected daily with saline compared to non-injected animals. However, a 50% inhibition in tumour volume is observed in animals treated with SMS 201-995 for 33 days. However, significance is only reached if control animals (injected and non-injected) are pooled. Tumour weight is inhibited by 38%.

Table 5 shows the blood glucose values among the various treatment groups at the end of the above experiment. Compared to normal hamsters,
Table 5.
Glucose levels in hamsters under various experimental conditions (insulinoma experiment). Hamsters without tumour. Hamsters with tumour, with and without glucose diet (4% glucose solution as drinking water), treated with vehicle alone (saline) or SMS 201-995 for 33 days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Glucose (mg/ml)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamsters without tumour</td>
<td>105 ± 1.8</td>
<td>5</td>
</tr>
<tr>
<td>Hamsters with tumour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment: SMS 201-995 (200 µg/kg/day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ glucose diet</td>
<td>157.1 ± 2.7*</td>
<td>7</td>
</tr>
<tr>
<td>- glucose diet</td>
<td>150.1 ± 6.5*</td>
<td>7</td>
</tr>
<tr>
<td>Control: Vehicle only</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ glucose diet</td>
<td>102.2 ± 7.0</td>
<td>5</td>
</tr>
<tr>
<td>- glucose diet</td>
<td>57.5 ± 8.5*</td>
<td>4</td>
</tr>
</tbody>
</table>

Mean ± SEM. n = number of experiments.
* P < 0.001 vs control (+ glucose diet).

tumour-bearing hamsters fed with a normal diet showed very low glucose levels which are normalized in hamsters with glucose-rich diet. Application of SMS 201-995 produced a highly significant hyperglycaemia in all hamsters, either fed normally or with glucose-rich food.

Table 6 shows the results obtained in the second type of experiment. Low doses of SMS 201-995 given for 16 days to hamsters bearing well grown tumours resulted in a growth inhibition of 52% for the 25 µg/kg dosis and 33% for the 2 µg/kg dosis as compared to controls. These results are, however, not statistically significant, due to tumour size variability, except for the per cent increase in volume which is significantly reduced in hamsters treated with 25 µg/kg SMS 201-995.

c) Measurement of SS receptors
Specific high affinity receptors for SS were measured in the insulinoma tumours, as reported previously (Reubi et al. 1982; Reubi 1984). However, under similar conditions no specific SS receptors could be detected in the chondrosarcoma tumours (data not shown).

Table 6.
Effect of chronic treatment with SMS 201-995 (25 or 2 µg/kg/day) for 16 days on tumour volume of advanced insulinomas grown in hamsters.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tumour volume (mm³)</th>
<th>% increase</th>
<th>Growth inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
</tr>
<tr>
<td>SMS 201-995, 25 µg/kg/day (n = 10)</td>
<td>697 ± 20</td>
<td>1622 ± 230</td>
<td>131 ± 37*</td>
</tr>
<tr>
<td>SMS 201-995, 2 µg/kg/day (n = 9)</td>
<td>467 ± 19</td>
<td>1309 ± 210</td>
<td>185 ± 47</td>
</tr>
<tr>
<td>Control (n = 13)</td>
<td>562 ± 20</td>
<td>1892 ± 208</td>
<td>275 ± 44</td>
</tr>
</tbody>
</table>

Control: 10% polyvinylpyrrolidone. Mean ± SEM. n = number of experiments. * P < 0.02.
Discussion

This study describes a moderate effect of SMS 201-995 on tumour growth at early or advanced stages, in two different types of transplantable tumours.

On one hand, we show that the growth of the GH-, somatomedin- and insulin-dependent chondrosarcoma can be significantly inhibited by SMS 201-995. This is in agreement with a recent report by Redding & Schally (1983) showing that an analogue of the tetradecapeptide SS is also able to inhibit the growth of a chondrosarcoma. We furthermore show that SMS 201-995 has an effect both in freshly transplanted or in advanced tumours. The mechanism of action of SMS 201-995, as a powerful GH (and therefore somatomedin) as well as insulin inhibitor, is likely to be an indirect one, the inhibition of growth being a consequence of generalised low GH, somatomedin and insulin. The fact that no specific SS-receptors could be found in this chondrosarcoma supports this hypothesis.

On the other hand, the SS analogue SMS 201-995 is also able to significantly reduce the size of a pancreatic β-cell tumour, the hamster insulinoma. This tumour has been shown to possess high affinity binding sites for both SS (Reubi et al. 1982) and SMS 201-995 (Reubi 1984). Earlier studies in man have shown that SS and various SS analogues can inhibit the release of insulin in such insulinomas (Adrian et al. 1981). However, these authors did not investigate whether the inhibition of hormone release was accompanied by a decrease in the size of the tumour. A good example that such an effect can indeed occur in other systems is given by the well documented shrinkage of prolactinoma after therapy with bromocriptine, a strong prolactin inhibitor (Thornor et al. 1980). Considering these data we could possibly extrapolate that, in our case, the inhibition of tumour growth by SMS 201-995 is likely to be due to a direct mechanism of action on the tumour, through specific SS receptors. However, since it is unknown whether this insulinoma is hormone-dependent, i.e. GH-, somatomedin-, prolactin- or insulin-dependent for its growth, an indirect action of SMS 201-995 on this tumour growth is not completely excluded.

It is not clear whether the moderate growth inhibition seen in the present experiments can be improved under different conditions. The decreased GH levels as well as increased plasma glucose values after chronic SMS 201-995 application suggest that in both experiments the targets are still sensitive to SMS 201-995 and that a complete desensitisation does not occur at this dosage. However, we recently observed (Reubi, unpublished results) that high doses of SMS 201-995 given for a period of 3 days twice a day produce a significant down-regulation of insulinoma SS receptors. In addition, it is uncertain, despite the long-lasting inhibitory effect of SMS 201-995 on GH (Plewe et al. 1984), whether its effect on tumour growth inhibition is similarly long-lasting in order to compensate for single daily application. It is therefore not excluded that a different schedule of application and/or dosage would be able to improve the reported effects.

We can conclude that SMS 201-995 is able to inhibit the growth of two different endocrine tumours. However, no clues about the molecular mechanism of such growth inhibition can be provided at the present time. For this, and for assessing the respective importance of each mechanism of action in these tumours, further experiments will be necessary. These results suggest, however, potential therapeutical consequences for SMS 201-995 as growth-inhibitory factor for certain types of tumours. Preliminary clinical trials of SMS 201-995 in a vipoma patient seems indeed to agree with such a suggestion (Wood et al. 1983).

Acknowledgments

We would like to thank Dr. R. Swarm (Nutley) and Dr. Bollag (Basel) for providing us generously with rats bearing chondrosarcoma as well as Drs. D. Schubart and N. Fleischer (New York) for the gift of hamsters bearing insulinoma tumours. We thank Dr. P. Marbach for performing GH radioimmunoassay. We acknowledge R. Brun and B. Thomi for excellent technical assistance.

References


Received on October 1st, 1984.