Endocrine profile of a long-acting somatostatin derivative SMS 201–995.
Study in normal volunteers following subcutaneous administration

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Abstract. The pharmacokinetics and the endocrine profile of a new low molecular somatostatin derivative, SMS 201–995, were investigated in a group of 35 normal subjects. Clearance studies (n = 6) for this peptide showed a prolonged half-life in plasma, 113 min, following single sc injections of 50 or 100 µg. Arginine stimulation tests (n = 6) were conducted immediately and 180 min after sc injection of 50 µg of SMS 201–995. The stimulatory effect of arginine on GH and insulin was counteracted by the peptide at the P < 0.001 and P < 0.02 significance level, respectively. Delayed arginine stimulation revealed a persistent blockade of the GH release (P < 0.02), whereas a recovery of the insulin response was observed. Plasma glucagon increments following a standard protein meal (n = 10) were significantly (P < 0.001) inhibited by previous sc injection of 50 µg of SMS 202–995. Pretreatment with 50 and 100 µg of SMS 202–995 sc (n = 9) inhibited (P < 0.001) the stimulatory effect of TRH (200 µg iv) on TSH without modifying basal levels. The injection of 100 µg/h during sleep completely abolished the nocturnal GH peak in 4 volunteers. No rebound rise after decline of the suppressive action on GH was recorded in any of the trials.

Safety chemistries and blood coagulation studies remained normal and no side-effects or untoward reactions were recorded throughout the investigation. With its high potency, slow plasma clearance, and long action SMS 201–995 may represent a valuable tool in the long-term management of states of inappropriate GH secretion.

Since the characterization in the early 1970s of a tetradecapeptide described as a growth hormone release inhibiting factor of hypothalamic origin, numerous studies have revealed a wide variety of pharmacological and potential therapeutic activities of somatostatin (SRIF). It acutely inhibits the secretion of growth hormone (GH), insulin, glucagon, and stimulated thyrotrophin (TSH), and delays glucose utilisation (for review see Gottesmann et al. 1982). In the brain and in gastrointestinal organs SRIF may play a physiological role as both a hypophysiotrophic and a gastrointestinal regulatory hormone. Its short plasma clearance, however, has recently led to the search for low molecular, long-acting, analogues for clinical use. In this line of work, Bauer et al. (1982) reported in animal studies the synthesis of an octapeptide, SMS 202–995 (D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr(ol)), 45 times more active than SRIF as GH inhibitor. This substance possesses the essential pharmacophore of natural SRIF, with a dextroisomer replacing Trp8 and a preserved cys-cys bridge. The aromatic side chain occupies the conformational space of the essential residue Phe6 in the native peptide, and the molecule is protected at the C-terminus by an aminoalcohol.

Iv administration of 5 to 10 µg/h of SMS 201–995 to humans has shown it to have a 20-fold
higher potency than SRIF as a GH inhibitor (Marbach et al. 1985). This finding and a plasma half-life of 43 min (Marbach et al. 1985), against 1–3 min for the natural peptide (Sheppard et al. 1979), prompted the investigation of other routes of administration that might facilitate its prolonged clinical use. The present report illustrates the endocrine effects of single sc injections of SMS 201–995 in normal volunteers.

Materials and Methods

Thirty-five normal male volunteers, 20 to 32 years of age, and weighing within the ± 15 weight percentile, entered the study. They were selected after basal laboratory work-up had ruled out hormonal or metabolic abnormalities. Informed consent was obtained and the subjects were allocated to five different series of experiments according to a randomized blind scheme. The tests were performed on separate days at least 1 week apart, and started at 8 a.m. following an 8 h fast, with the exception of the sleep study. A previous pilot experiment had revealed significant endocrine effectiveness for 50 µg of SMS 202–995 administered sc (Gomez-Pan et al. 1985).

Pharmacokinetics of SMS 201–995

Plasma kinetics of SMS 201–995 were studied in 6 volunteers. Blood samples were collected for peptide assay 15 min before a single sc injection of 50 or 100 µg and at frequent intervals for 465 min thereafter.

Arginine stimulation test

After cannulation of an arm vein with a heparinized needle 6 subjects received, in random order, on two occasions a sc injection of placebo or 50 µg of SMS 201–995 at time 0, followed by an arginine infusion (50% arginine HCl 0.5 g/kg/30 min) 15 min later. Blood sampling was performed before and after SMS 201–995 administration at 5, 15, 30 and 60 min intervals for 300 min. In order to investigate its length of action, the subjects were again studied under the same protocol, but arginine was infused at a 180-min interval after the administration of the same dose of SMS 201–995 or placebo at time 0. GH, insulin and glucose were measured in all samples.

Glucagon stimulation by protein meal

Following a 10-h fast, 10 subjects had an arm vein cannulated with a heparinized catheter at 9 a.m. At 10 a.m. a sc injection of placebo or 50 µg of 201–995 was followed by ingestion of a protein-rich meal (300 g of beef) within the next 30 min. Blood samples for glucagon determination were collected 30 and 15 min before and 15, 30, 60, 120, 150, 180 and 240 min after injection.

Stimulation of TSH release with TRH

Nine subjects received an iv bolus injection of 200 µg of TRH (TRF Roche, Basle, Switzerland) under basal conditions and after receiving 50 or 100 µg SMS 201–995 sc immediately before administration of the releasing factor. Blood for PRL and TSH measurements was collected at 30-min intervals for 210 min.

Sleep study

To assess the efficacy of SMS 201–995 in inhibiting the physiological sleep-related GH increase, 4 healthy male volunteers were studied over 2 sleep periods: a) a basal sleep after a sc injection of saline following an adaptation night; and b) a treatment night following sc administration of 100 µg of SMS 201–995.

Occipital and precentral EEG profiles throughout sleep were recorded on an Elema-Schönander electroencephalograph, and sleep stages were scored according to Rechtschaffen & Kales (1968). Initiation of stage 2 of sleep was taken as the starting point of blood sampling at 20-min intervals through and indwelling cubital catheter connected to the experimenter’s room. During the treatment night, SMS 201–995 was administered approximately 1 h before the expected time for onset of sleep.

Safety tests

Routine blood cell counts, blood chemistries, and urinalysis were conducted in all subjects before initiation of the study and on its completion. In addition, platelet count, platelet aggregation, partial thromboplastin and prothrombin times, and fibrinogen were estimated in 10 probands.

Assay methods and statistical analysis

Growth hormone, PRL, insulin and glucagon were measured by previously reported methods (del Pozo et al. 1977; Hwang et al. 1971; Yallow & Berson 1960; Unger et al. 1962) and TSH was determined with reagents supplied by Serono, Italy. Blood glucose was measured by a standard oxidase method.

A specific radioimmunoassay was used for determination of SMS 201–995 in plasma. Antibodies were raised in rabbits immunized with SMS 201–995 in complete Freund's adjuvans. The Tyr-I-analogue of SMS 201–995 was iodinated by the chloramine-T method and purified in HPLC. The original SMS 201–995 was used as the standard. The system did not cross-react with natural somatostatin. The assay procedure involved incubation of 50 µl of standard or sample with 200 µl of antiserum (1:150 000) and 100 µl of tracer (6–10 000 cpm) for 24 h followed by separa-
tion with dextran-coated charcoal. All steps were performed at 4°C. The sensitivity of the assay is 20 pg/ml.

Statistical significances were established by integration of the plasma hormone profiles and comparison by means of the paired t-test or by one way variance analysis in the case of multiple dose application.

Results

Pharmacokinetics of SMS 201–995
Pharmacokinetic analysis was conducted according to a monocompartmental system by the exponential function \( C_p = C_e \cdot e^{-kt} \). Results revealed a rapid invasion with SMS 201–995 peak values of 1.9 and 2.7 ng/ml at 30–60 min for the 50 and 100 µg dose, respectively. Clearance was slow as calculated from the area covered by the serum profiles (AUC 0–465 min: 298 ± 17 SE ng/ml h-1) with an elimination half-life \( (t_{1/2}) \) of 113 min for both doses (Fig. 1).

Effect of SMS 201–995 on plasma GH, glucagon, insulin, and glucose after arginine stimulation
The results of arginine stimulation are depicted in Fig. 2. The GH increment expressed as the area under the plasma profiles (ng/ml/time) was markedly suppressed by 50 µg of SMS 201–995 injected sc just before arginine stimulation (1488 ± 607 vs 203 ± 82 SE: \( P < 0.001 \)) (left upper panel). Insulin was also significantly inhibited (1144 ± 466 SE vs 454 ± 32 SE \( P < 0.02 \)) and induced a significant \( (P < 0.01) \) elevation in plasma glucose as compared with the placebo effect (left middle and lower panels). The glucose elevation recorded during the first 15 min is interpreted as induced by the rapid glucagon increase elicited by the arginine stimulus (peak values of 510 and 593 pg/ml from a baseline of 280 pg/ml at 15 and 30 min, respectively; not shown in the figure).

Arginine stimulation 3 h after injection of 50 µg of SMS 201–995 revealed a persistent blockade of the GH release (1449 ± 591 vs 236 ± 96 SE \( P < 0.02 \)), whereas a recovery of the insulin response was observed (Fig. 2 right upper and middle panels) indicating a different duration of action for SMS 201–995 on GH and insulin suppression. The blood glucose profile showed an initial elevation corresponding to the insulin suppression and a steep fall coinciding with the insulin peak elicited by arginine (right lower panel).

Glucagon stimulation test
The plasma glucagon increment induced by a protein meal was significantly antagonized by pre-

![Fig. 1.](image-url)

Plasma kinetics of SMS 201–995 in normal volunteers following single sc administration of 50 and 100 µg.
The calculated half-life for both doses is 113 min.
Effect of a single sc injection of 50 µg of SMS 201–995 at 0 and 180 min on arginine-stimulated growth hormone and insulin, and on blood glucose. Both hormones are inhibited in the early test (left panel) but despite persistent GH suppression, insulin is no longer sensitive to SMS 201–995 at 180 min (right panel). There is an elevation in blood glucose coinciding with the blockade of insulin secretion. This effect is more apparent on delayed arginine stimulation.

TSH response to TRH stimulation
Sc injection of 50 or 100 µg of SMS 201–995 significantly ($P < 0.001$) inhibited the stimulatory effect of TRH on TSH without modifying basal
levels at both doses (Fig. 4). Values of integrated TSH plasma profiles were 21.8 ± 3.1 (se) for the control and 11.8 ± 2.2 (se) and 12.3 ± 2.5 for the 50 and 100 µg tests, respectively, both at the P < 0.001 significance level. SMS 202–995 did not modify the Prl increment induced by TRH.

**Effect of SMS 201–995 on GH secretion during sleep**

The sc injection of 100 µg of SMS 201–995 practically abolished the sleep-induced GH surge (Fig. 5). The difference between the integrated GH profiles (1132 ± 180 se vs 260 ± 40 se ng/ml/360 min for placebo and SMS 201–995, respectively) was significant at the P < 0.01 level. It is worth noting that the typical GH rebound observed after administration of natural somatostatin (Hall et al. 1973) was missing in this study and in the previous trials.

**Safety chemistries and tolerability**

Blood cell counts, chemistries and coagulation studies were reported normal. No side-effects or changes in vital signs were recorded.

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**Fig. 3.**

Inhibition of protein induced hyperglucagonaemia by a single sc injection of 50 µg of SMS 201–995.

**Fig. 4.**

Plasma TSH profiles recorded in normal volunteers after TRH (200 µg iv) under basal conditions and subsequent to single sc injections of 50 and 100 µg of SMS 201–995. There is a significant inhibition of the TSH surge at both dosage levels, but basal secretion is not modified.
Plasma GH profiles during monitored sleep under basal conditions and after a sc injection of 100 µg of SMS 201–995. The nocturnal GH elevation is completely blocked and there is no rebound hypersecretion.

Discussion

The rapid clearance of natural somatostatin has rendered this substance unsuitable for prolonged clinical use. The recent availability of a low molecular derivative with high potency and prolonged action has opened new perspectives in the long-term management of conditions characterized by inappropriate GH secretion. As illustrated in this paper, SMS 201–995 in single sc administration counteracted the stimulatory effect of exogenous arginine 3 h post injection without the typical rebound phenomenon observed when the native peptide is injected (Unger et al. 1962; Peracchi et al. 1974). GH rebound was also missing in the sleep trial in which the nocturnal GH surge was completely abolished by SMS 201–995. These findings are interpreted as evidence for high potency and prolonged plasma clearance. Recently, Althoff et al. (personal communication) compared the activity of SMS 201–995 and the natural peptide by the iv and sc routes. They found a 10-fold higher activity for the octapeptide and a prolongation of its GH-suppressing effect for 6 h beyond that of native somatostatin. Absence of rebound was also observed. As found in the present study too, these authors reported a shorter inhibitory effect on insulin secretion than on GH. This asynchronous effect may turn advantageous in the management of conditions with intact beta-cell function preventing excessive postprandial hyperglycaemia by timely administration of the peptide.

Apart from its high potency, prolonged action, and time difference in the GH and insulin inhibitory actions, the endocrine profile of SMS 201–995 does not essentially differ from that of the natural peptide. Suppression of provoked hyperglucagonaemia and of stimulated TSH has also been described with native somatostatin (Carr et al. 1975; Siler et al. 1974). In addition, a screening of the actions of SMS 201–995 on endocrine and exocrine functions in the gastrointestinal tract had revealed a broad spectrum of inhibitory actions similar to endogenous somatostatin (Kränzlin et al. 1985).

Initial reports of a possible effect of natural somatostatin on platelet function (Koerker et al. 1975; Besser et al. 1975) have not been confirmed (Mielke et al. 1975; Rasche et al. 1976). The coagulation screening conducted in the present trial also failed to demonstrate an effect of SMS 201–995 on such mechanisms.

SMS 201–995 may represent a suitable tool for the management of acromegaly and other conditions in which a modification of the interplay between insulin and glucose counterregulation might result in better metabolic control. Plewe et al. (1984) recently reported that single sc administration of 50 µg of SMS 201–995 was followed by a long-lasting reduction in circulating GH levels to normal values in 6 out of 7 patients with active acromegaly. Their results have been corroborated by other investigators (Ching et al. 1985; Lamberts et al. 1985). Furthermore, Lamberts et al. (1985) showed potentiation of the GH lowering effect by combined administration of SMS 201–995 and a dopamine agonist in an acromegalic subject previously resistant to either agent. A beneficial action of this peptide on insulin hypersensitivity has been reported in brittle in diabetes too, presumably consisting in delaying glucose resorption from the gut an dampening counterregulatory mechanisms (Gomez-Pan et al. 1985; Spinas et al. 1985). However, a number of problems can result from the variety of actions exerted by a single peptide. Only long-term clinical experience will define the place of this new somatostatin in the management of such hormonal disturbances.
References


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