Improved Analogs and Novel Delivery Systems for Somatostatin Octapeptides

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Appropriate N-terminus modification can result in somatostatin (SRIF) octapeptide analogs that are both more potent and more selective in vitro for the human SRIF receptor type 2 ($hsst_2$). In addition, these modifications can improve the duration of action and bioavailability of SRIF analogs following parenteral administration, as shown by both pharmacological and distribution studies in vivo with BIM-23190 and BIM-23197 in the rat.

SOMATOSTATIN (SRIF) octapeptide analogs such as lanreotide and octreotide are now commonly used in the symptomatic treatment of carcinoid syndrome, and as a second- or third-line therapy in acromegaly. Although these analogs are longer-acting than the natural SRIF tetradecapeptide ($SRIF_{1-14}$), their biological half-life of less than 2 hours requires either frequent administration or the use of a sustained-release formulation. This regimen of administration is clearly required to achieve a sustained suppression of growth hormone (GH), as well as downstream insulin-like growth factor-1 (IGF-1). Better delivery systems, and longer-acting analogs with improved receptor affinity and selectivity over the first-generation products, have been investigated.

EVALUATIONS

Pharmacokinetic profiles on long-acting sustained-release formulations for both octreotide (Sandostatin® LAR®; Sandoz Pharma Ltd, Basel, Switzerland),[1] and lanreotide (Somatuline LP®; Pharma Biotech, Signes, France)[2] have been published. Even though both formulations are able to modulate GH release, neither can be considered optimal in view of an initial "burst" release in one case (Somatuline®) and of an initial delay in release in the other (Sandostatin®). These release profiles are only acceptable clinically in view of the safety of both products, which allows peak supramaximal drug levels for GH inhibition without side effects. Although a circulating drug concentration less than 1 nmol/L is achieved throughout the period of efficacy, these systems could clearly be improved in terms of efficiency during both manufacture and release.

Some of the approaches under investigation include poly lactic-glycolic acid microcapsules optimized for 1-month delivery, and novel biodegradable polymer complexes such as the peptide-polymer ionic conjugate (PPIC); the latter is specially designed to provide a better control of release through the optimization of polymer-peptide ionic interaction.[3] This is achieved by increasing the carboxylate content of the polymer by incorporation of polybasic carboxylic acids during synthesis. These new modified polymers have been found to be beneficial in that they yield tighter complexes with the peptide drug species, which are typically monobasic or polybasic.

With regard to the discovery of more potent, longer-acting analogs, efforts were initially focused on the design of compounds with improved receptor binding affinity and an optimal selectivity profile, based on the known expression of SRIF receptor type 2 (sst_2) and type 5 (sst_5) in the pituitary. Our initial objective was to identify an octapeptide "platform" with optimal receptor affinity in vitro, which was suitable for N-terminus modification. Compounds were evaluated in vitro for affinity to human sst_2 ($hsst_2$) and $hsst_5$ receptors expressed in CHO-K1 cells. Analysis of the somatostatin analog database at Biomeasure indicated that while it was relatively easy to obtain compounds with in vitro potencies approximately equal to $SRIF_{1-14}$, there were no examples of analogs that were significantly more potent than the parent hormone. Consequently, we concluded that it would be difficult to extract significantly greater potency from the octapeptide pharmacophore, and decided to select a platform for N-terminus modification from our existing compound database (Table 1). We chose two compounds: (1) BIM-23060 (cyclo D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Cys-Nal-NH_2), which was approximately equivalent to $SRIF_{1-14}$ in potency, and (2) BIM-23023 (cyclo D-Phe-Cys-Tyr-D-Trp-Lys-Abu-Cys-Thr-NH_2), which was about twofold less potent than the tetradecapeptide.

BIM-23060 was of particular interest, as it was three to five times more potent in terms of $hsst_2$ receptor binding affinity than lanreotide and octreotide. BIM-23060 and BIM-23023 showed about the same selectivity for the $hsst_2$ receptor over the $hsst_5$ receptor as lanreotide and octreotide.

We next considered desirable characteristics for the nature of the N-terminus modification. An important aspect limiting the length of action of SRIF analogs has been the rapid biliary secretion of octapeptide analogs.[4] Lanreotide is a relatively lipophilic peptide, and we hypothesized that manipulation of this parameter should influence pharmacokinetic behavior; consequently, we decided to increase "local" hydrophilicity in the region of the N-terminus. We evaluated several types of N-terminus modification and

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Table 1. Binding Affinities of SRIF Analogs

Peptide	$hsst_2$ (K^1, nmol/L)	sst_5 (K^1, nmol/L)	2/5 Ratio	GH Inhibition* IC_{50} nmol/L	GH Inhibition* Potency Relative to $SRIF_{1-14}$
$SRIF_{1-14}$	19 ± 0.01	0.88 ± 0.1	5	0.47	(1)
Lanreotide	87 ± 0.01	5.2 ± 1.3	6	0.40	1.2
Octreotide	0.57 ± 0.08	7.0 ± 1.6	12	0.52	0.9
BIM-23023	0.42 ± 0.13	4.2 ± 1.09	10	0.11	4.3
BIM-23060	0.16 ± 0.02	1.5 ± 0.39	10	0.06	8.3
BIM-23190	0.32 ± 0.12	11.1 ± 0.1	35	0.24	2.0
BIM-23197	0.19 ± 0.08	9.8 ± 0.4	52	0.23	2.0

*From male rat anterior pituitary cells in vitro in primary culture.

concluded that an N-terminus N-(2-hydroxyethyl) piperazine substituent was most suitable for our purposes. We also speculated that these polybasic moieties would improve complexation of the peptide with the PPIC matrix. We decided to attach these modifications to the peptide platforms by several linkages, including an amide linkage [eg, BIM-23190, the N-(N′-(2-hydroxyethyl) piperazinyl)-2-acetyl derivative of BIM-23023], and a sulfonamide linkage [eg, BIM-23197, the N-(N′-(2-hydroxyethyl) piperazinyl)-2-ethylsulfonyl derivative of BIM-23023]. While these N-substituents had little effect on binding affinity at the $hsst_2$ receptor compared with BIM-23023, there was a marked improvement in selectivity over the $hsst_5$ receptor, with the new analogs BIM-23190 and BIM-23197 showing a 30- to 50-fold selectivity. Therefore, a considerable improvement in both potency and selectivity had been achieved over the existing family of SRIF octapeptides. It is worth noting that modification of the N-terminus of BIM-23060 by Amadori rearrangement[5] with maltose led to a fourfold reduction in receptor affinity.[6] We may speculate that this more bulky N-terminus modification interferes with receptor interaction in potent analogs such as BIM-23060.

The compounds were also evaluated for their ability to block growth hormone–releasing factor (GRF)-stimulated GH release in male rat anterior pituitary cells grown in primary culture. We have shown that there is an excellent correlation between the affinity at the rat (r)sst_2 receptor,[5] and inhibition of GRF-induced GH release in the rat pituitary cells (correlation coefficient, $r = .93$). This result adds further weight to the association of the sst_2 receptor with GH inhibition.[7] However, other than indicating that a reasonable range of substitution is allowed at the N-terminus of SRF peptides without compromising either the affinity or efficacy of the parent at sst_2 receptors, these data provide little assistance in the choice of a SRIF analog with improved potency in vivo. We therefore decided to evaluate the better compounds in the rat, measuring the inhibition of (D-Ala^2-GRF)-stimulated GH release in vivo. BIM-23023, -23190, -23197, and octreotide (BIM-23198) were selected for study. In this study, ED_{50} values for the inhibition of circulating GH were calculated for the compounds of interest at 2, 4, 6, and 8 hours following subcutaneous administration of test peptide in the D-Ala^2-GRF–primed rat. These data are shown in Fig 1. N-terminus modification does appear to have an influence on the length of action of the BIM-23023 series, with the most potent analogs (BIM-23190 and BIM-23197) being approximately three times more potent than the parent BIM-23023 at the 8-hour time point. The BIM-23023 series, in general, are more potent than octreotide; the most potent compounds, BIM-23190 and BIM-23197, typically display approximately 10 times the potency of octreotide (Fig 1).

This finding of improved length of action with analogs such as BIM-23190 and BIM-23197 was supported by the results of distribution, metabolism, and excretion studies using the corresponding iodine-labeled peptides. These studies in male and female Sprague-Dawley rats showed that both these compounds were stable to degradation in vivo, providing a high level of intact drug in the plasma 1 hour following subcutaneous administration, with little evidence of binding to plasma proteins or red blood cells. There was a similar pattern of plasma pharmacokinetics for both compounds, with a terminal elimination half-life of 2 to 4 hours. In both cases, it was apparent that the highest levels of peptide were distributed to the pituitary and adrenals, peaking at 2 to 4 hours postinjection. Excretion data showed that the drug was concentrated in the urine and gastrointestinal tract at 8 hours.

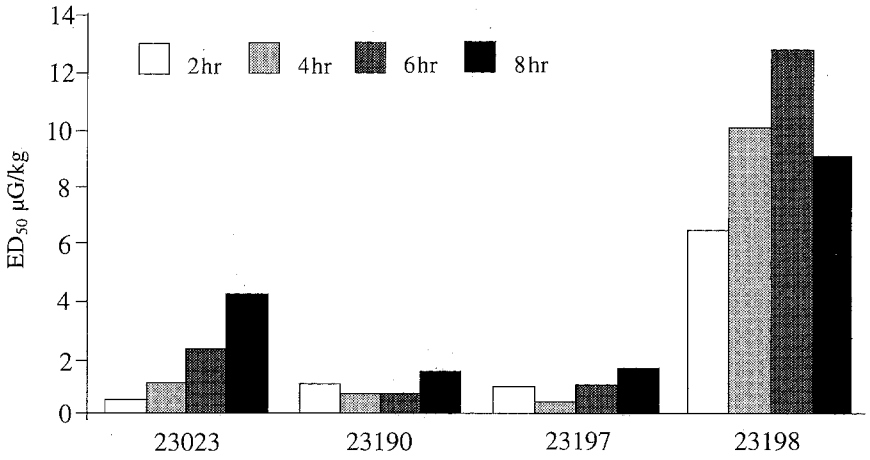

Fig 1. Inhibition of D-Ala^2-GRF–induced GH release in the rat at various times following subcutaneous administration of test peptide.

CONCLUSIONS

Over the next 5 years, there is little doubt that more efficient, and therefore more cost-effective, SRIF analogs will become available to clinical medicine. We anticipate that these new analogs may be combined with improved, novel delivery methodologies, such as PPIC, to yield more effective formulation strategies. The properties of these new agents will make it possible to consider them for first-line treatment in acromegaly, as well as for novel treatment opportunities where the dosage regimen is currently impractical.

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