RAPID COMMUNICATION

EP1572: A novel peptido-mimetic GH secretagogue with potent and selective GH-releasing activity in man

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ABSTRACT. EP1572 (JMV1843 [Aib-DTrp-DgTrp-CHO]) is a new peptido-mimetic GH secretagogue (GHS) showing binding potency to the GHS-receptor in animal and human tissues similar to that of ghrelin and peptidyl GHS. EP1572 induces marked GH increase after sc administration in neonatal rats. Preliminary data in 2 normal young men show that: 1) acute iv EP1572 administration (1.0 µg/kg) induces strong and selective increase of GH levels; 2) single oral EP1572 administration strongly and reproducibly increases GH levels even after a dose as low as 0.06 mg/kg. Thus, EP1572 is a new peptido-mimetic GHS with potent and selective GH-releasing activity.

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INTRODUCTION

Ghrelin, a 28-amino acid acylated peptide mainly produced by the stomach, displays strong GH-releasing activity mediated by the hypothalamus-pituitary GH secretagogue (GHS) receptors (GHS-R) (1, 2). Ghrelin could be a provocative test for the diagnosis of GH deficiency while synthetic, orally active ghrelin analogues have been proposed as growth-promoting agents for the treatment of short stature and as anabolic drugs in somatopause and catabolic states (1, 2). To this goal peptidyl and non-peptidyl GHS have been generated and prolonged treatment with single oral administration of the spiroindoline MK-0677 has been shown to enhance the activity of the GH/IGF-I axis and to counteract food-restriction-induced catabolism (2, 3).

Ghrelin and GHS-R are also expressed in other central and peripheral tissues (3, 4) and ghrelin and GHS also possess orexigenic, gastro-entero-pancreatic, cardiovascular and antiproliferative effects (3). Thus, orally active GHS acting as agonists or antagonists would theoretically have perspectives for drug intervention in some non-GH-related clinical conditions such as eating disorders.

Herein we first report the pharmacological profile and the preliminary results about the GH-releasing activity of EP1572 [JMV1843 (Aib-DTrp-DgTrp-CHO)], a new peptido-mimetic GHS deriving from a chemical-pharmacological research program on down-sized analogues of peptidyl GHS (5).

SUBJECTS AND METHODS

Chemicals

^{125}I-Tyr^{4}-ghrelin (SA 2000 Ci/mmol) was radioiodinated using a lactoperoxidase method and purified by reverse-phase high performance liquid chromatography as previously described (6). This ghrelin analog has *in vivo* the same GH-releasing activity of the native molecule and represents reliable probe for labeling ghrelin receptors (7).

Binding studies

EP1572 was evaluated for its binding affinity to ghrelin receptors in a competitive binding assay with ^{125}I-labeled ghrelin as the radioligand. Ghrelin binding to human *post-mortem* pituitary gland or hypothalamus membranes was performed as previously described (7). Inhibition of ^{125}I-labeled ghrelin binding was obtained using 8 different concentrations of EP1572 (0.1 nmol/l to 1 µmol/l) and the IC_{50} was calculated by iterative nonlinear curve-fitting. Unlabeled ghrelin and hexarelin (HEX) were reference compounds.

Key-words: Synthetic GH secretagogues, GH, ghrelin receptors, oral administration, humans.

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Table 1 - *GH responses to oral EP1572 administration in 2 normal subjects.*

	GH peak (μg/l)		GH AUC$^{0\rightarrow300}$ (μg*min/l)		Time to peak (min)	
	Y1	Y2	Y1	Y2	Y1	Y2
EP1572 0.06 mg/kg	33.0	84.0	2539.5	5679.0	75	60
EP1572 0.125 mg/kg	53.5	58.0	5542.5	3523.5	90	60
EP1572 0.25 mg/kg	71.0	90.0	7717.5	6329.3	90	60
EP1572 0.5 mg/kg (on day 1)	90.0	111.5	6976.5	8000.3	60	60
EP1572 0.5 mg/kg (on day 2)	89.0	70.0	7305.0	6127.5	60	75

Animals

On post-natal day 10, male rat pups were given a fixed dose (300 μg/kg) of EP1572, HEX or isovolumetric amounts of saline sc and were sacrificed 15 min later by decapitation. Trunk blood was collected and plasma samples were stored at -20 C until GH assay (8).

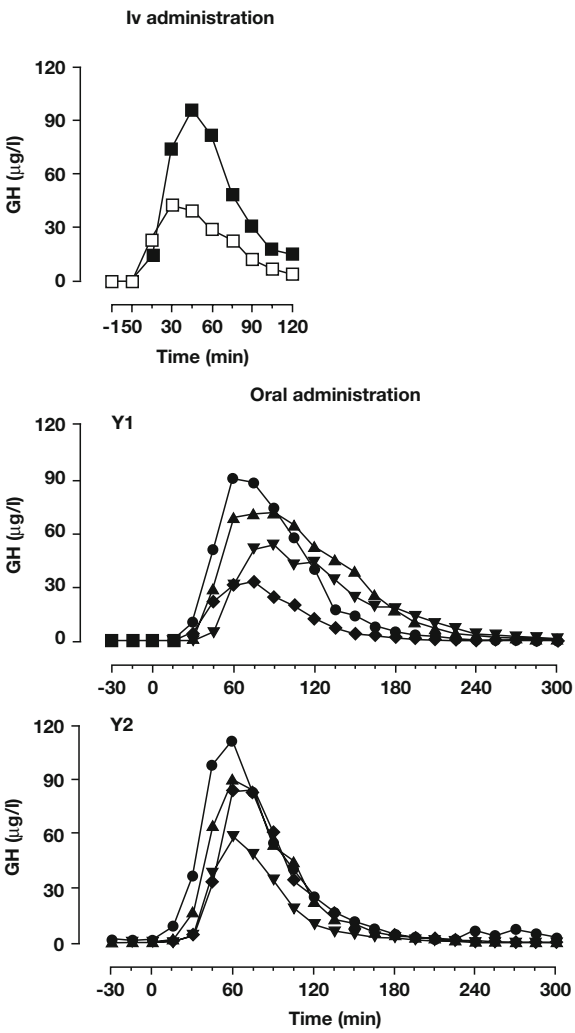

Fig. 1 - *GH responses to 1.0 μg/kg iv (Y1, □; Y2, ■) and to 0.06 (◆), 0.125(▼), 0.25 (▲) and 0.5 (●) mg/kg po EP1572 administration in 2 normal subjects.*

Humans

Two healthy male young volunteers (Y) (Y1: age: 27 yr, BMI: 23.1 kg/m^2; Y2: 29 yr, 19.5 kg/m^2) underwent the following tests, in the morning (08:30-09:00 h) and at least 10 days apart:

- session A: acute iv EP1572, ghrelin (both at 1.0 μg/kg as a bolus at 0 min) or placebo administration. Blood samples were taken every 15 min up to +120 min to assay GH, PRL, ACTH and F levels (9);
- session B: oral EP1572 administration of 0.06, 0.125, 0.25 and 0.5 mg/kg doses. The test with 0.5 mg/kg was repeated on 2 consecutive days. Blood samples were taken every 15 min up to +300 min for GH assay.

The responses are expressed as absolute values or as AUC calculated by trapezoidal integration.

Statistical analysis

GH levels in rats were evaluated with Dunnet's *t* test for multiple comparisons, preceded by an analysis of variance (ANOVA).

The study was approved by an independent Ethics Committee.

RESULTS

In vitro EP1572 displaced in dose-dependent manner ^{125}I-labeled ghrelin in human pituitary gland with a binding potency very similar to that of ghrelin or HEX. The IC_{50} values (mean±SE of 3 experiments) were: ghrelin, 10.2±1.1; HEX, 12.3±0.7; EP1572, 15.6±0.4 nmol/l. Binding values in human hypothalamus were overlapping.

In rats *in vivo*, EP1572 increased GH levels 15 min after sc administration. Peak GH levels (mean±SE of 9 rats) after EP1572 (158.8±39.4 μg/l) were higher (p<0.05) than in control rats (11.3±3.9 μg/l) and similar to those after HEX (222.8±26.2 μg/l).

In 2 men, marked and prompt increase of GH levels was observed after iv EP1572 (Y1: peak: 42.4 μg/l at 30 min, AUC$^{0\rightarrow120'}$: 2656.5 μg*min/l; Y2: 96.0 μg/l at 45 min, 5549.3 μg*min/l) as well as ghrelin (Y1: peak: 79.5 μg/l at 30 min, AUC$^{0\rightarrow120'}$: 5317.5 μg*min/l; Y2: 178.5 μg/l at 30 min, 10642.5 μg*min/l).

Differently from ghrelin, iv EP1572 did not modify PRL, ACTH and F levels (data not shown).
In both subjects, all oral EP1572 doses elicited strong increase in circulating GH levels (Table 1) (Fig. 1). A marked GH response to the highest oral dose of EP1572 on the following day was also observed. Specifically, Y1 displayed similar responses in both sessions, whereas in Y2 in whom the GH response on day 1 was higher than in Y1 was about 30% lower on day 2 (Table 1).
No side-effects were recorded though subjects referred appetite after ghrelin or EP1572 administration.

DISCUSSION

This study shows that EP1572, a new peptido-mimetic GHS, binds with potency the GHS-R and is able to increase GH secretion after sc administration in neonatal rats, elicits strong and selective increase in GH levels in humans either after acute iv or single oral administration. Even an oral EP1572 dose as low as 0.06 mg/kg shows remarkable GH-releasing effect.
EP1572 derives from a chemical-pharmacological research program on down-sized analogues of peptidyl GHS (5). Following previous orally active GHS, it was generated taking into account the potential clinical perspectives of ghrelin analogues: 1) diagnostic potential; 2) therapeutic potential in GH-related disorders for treatment of short stature and as anabolic intervention in somatopause and catabolic states; 3) theoretical therapeutic potential in non-GH-related disorders taking into account the wide spectrum of ghrelin biological activities (3, 9).
Binding studies showed that EP1572 displaces ^{125}I-labeled ghrelin in dose-dependent manner in human pituitary and hypothalamus with binding potency similar to that of ghrelin and HEX. Studies in rats *in vivo* demonstrated its GH-releasing activity after sc administration. Thus, the endocrine effects of EP1572 were preliminarily tested in men. Our results firstly show that iv EP1572 administration elicits prompt and specific increase of GH to very high levels, at variance with ghrelin that also stimulates PRL, ACTH and F levels (3, 9). This selectivity could be related to the possibility that this synthetic GHS could bind different pockets of the GHS-R 1a or activate GHS-R subtypes selectively devoted to the GH-releasing activity (2, 3).
The stimulatory effect of EP1572 after oral administration of a wide range of doses is very remarkable; even a dose as low as 0.06 mg/kg elicited very high GH increase lasting for approximately 180 min. Oral EP1572 administration at the highest dose induced a marked GH response even when given on the following day indicating reproducible effect and absorption.
Within our experimental conditions, EP1572 does not induce any relevant side effect. Subjects referred to be hungry after EP1572 administration further indicating its activity, as was shown in animals and humans after ghrelin administration (3, 9).
In all, EP1572 is a new peptido-mimetic GHS with potent and selective GH-releasing activity. It is very active even after oral administration of very low doses and likely undergoes reproducible adsorption. Based on these preliminary results EP1572 has been selected for further clinical investigation.

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