Synthèse et évaluation biologique de ligands de la Ghréline

Du laboratoire à la Clinique
Ghrelin, the natural ligand of the GHS receptor

• Ghrelin was characterized in 1999 as the endogenous ligand of the GHS-R1a
• Ghrelin is produced by the stomach as a 28 amino-acid peptide with a lipidic group on Ser-3
• GHS are compounds that stimulate GH secretion by a pathway independent from GHRH
• GHS work on GH secretion through a G-protein-coupled receptor, the GHS receptor GHS-R1a

Biological Activities of Ghrelin

- Influence on food intake, sleep and behaviour
- Stimulation of GH, PRL, ACTH and AVP secretion; Inhibition of gonadotropin secretion
- Influence on gastric acid secretion and motility
- Influence of exocrine and endocrine pancreatic function
- Modulation of cell proliferation and survival
- Influence on cardiac performances and vascular resistances
- Influence on energy metabolism
- Influence on glucose metabolism
Therapeutic Interest of GH

ESSENTIAL ROLE FOR:

STRUCTURAL GROWTH in children

INTEGRITY MAINTENANCE in adults

Growth Hormone interacts:

★ Directly on the LIPIDIC METABOLISM

- by increasing the fatty acid liberation in adipose tissue and using them as energy

★ Undirectly, on the stimulation of the synthesis and secretion of IGF-I, by promoting

- bone formation
- muscle growth, cellular proliferation, ...
THERAPEUTIC APPLICATIONS FOR GH

Currently, only used in promoting growth in children who present a GH secretion deficiency

2 major disadvantages
- High cost of the treatment (10 000 to 15 000 $ / year)
- Daily painful injection

Future Applications?
A large number of studies (hrGH) suggest benefits of using GH:

- In elderly patients with osteoporosis, wasting conditions...
- In recovery after major surgery, burn victims....
- Cachexia in cancer or AIDS patients

Interest of an alternative to GH therapy (GHSs)
Development of GHS Agonists

ENKEPHALIN ANALOG

\[ \text{Tyr-(D)Trp-Gly-Gly-Phe-Met-NH}_2 \]

*Bowers et al. 1977*

\[ \text{His-D-Trp-Ala-Trp-D-Phe-Lys-NH}_2 \]

\[ \text{EC}_{50} = 10 \text{ nM} \]

*Bowers et al. 1984*

\[ \text{His-D-Mrp-Ala-Trp-D-Phe-Lys-NH}_2 \]

*in vitro \text{EC}_{50} = 3 \text{ nM}*

**Hexarelin/Hexamorelin**

*Deghenghi et al., et al. 1994*
Development of GHS Analogs

EP-51389

More potent than Hexarelin
sub-cutaneous injection in rat
Dehgenghi et al., et al. 1999

JMV 1843 (EP-1572)

gem-diamino structure
An orally Active Analog
Guerlavais et al., et al. 2003
JMV 1843: *in vitro* Pharmacological Characterization
Cloned GHS-1a Receptor (CHO Cells)

**Binding affinity by displacement**
- IC$_{50}$ = 0.7 nM
- IC$_{50}$ = 40 nM

**Scatchard representation**
- $K_d$ = 0.1 nM
- $K_d$ = 120 nM

**IPs Production**
- EC$_{50}$ = 0.8 nM
- EC$_{50}$ = 0.5 nM

**Calcium Mobilisation**
- EC$_{50}$ = 0.8 nM
- EC$_{50}$ = 0.8 nM

### JMV 1843: *in vitro* Pharmacological Characterization

<table>
<thead>
<tr>
<th>Affinity IC$_{50}$ nM</th>
<th>h-Hypothalamus Membranes</th>
<th>h-Pituitary Membranes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghrelin</td>
<td>7.9 ± 1.3</td>
<td>9.4 ± 0.8</td>
</tr>
<tr>
<td>Hexarelin</td>
<td>19.3 ± 2.5</td>
<td>11.4 ± 0.5</td>
</tr>
<tr>
<td>MK-0677</td>
<td>32.0 ± 1.7</td>
<td>26.2 ± 0.4</td>
</tr>
<tr>
<td>JMV1843</td>
<td>23.8 ± 1.9</td>
<td>14.6 ± 0.2</td>
</tr>
</tbody>
</table>

Binding affinity. Displacement experiments were performed using $^{125}$I-Tyr4-Ghrelin.

*Guerlavais et al., J. Med. Chem. (2003)*
*Broglio et al., J. Endocrinol. Invest., 2002*
### GH Secretion in Rodents (s.c. injection) Induced by JMV 1843

<table>
<thead>
<tr>
<th></th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>11.3 ± 3.9</td>
<td>6.54 ± 0.96</td>
</tr>
<tr>
<td>Hexarelin</td>
<td>222.8 ± 26.2</td>
<td>177.1 ± 18.7</td>
</tr>
<tr>
<td>JMV1843</td>
<td>258.8 ± 39.4</td>
<td>232.3 ± 30.2</td>
</tr>
</tbody>
</table>

10 days old Rat model, s.c. injection of 160 µg/kg each compound. GH dosed after 15 min. Values are means ± SE of 9 rats.

Broglio et al., J. Endocrinol. Invest., 2002
In vivo GH Secretion in Dogs Induced by JMV 1843 (i.v. Administration)

(125μg/kg i.v. Administration)

Unpublished Results
### In vivo GH Secretion in Dogs Stimulated by JMV 1843 (1 mg/Kg Oral Route)

<table>
<thead>
<tr>
<th>Time (min) After Administration</th>
<th>GH Concentration (ng/ml)</th>
<th>AUC (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.8 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>5.3 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>3.5 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>180</td>
<td>3.0 ± 0.4</td>
<td>676 ± 94</td>
</tr>
</tbody>
</table>

Saline 1.5 ml/kg (180 min)  
1.5 ± 0.6  
119 ± 36

Values are means of experiments carried out on 5 Beagle Dogs.  
Unpublished Results
Phase 1 Clinical Study of **JMV 1843** Administered by Oral Route (Healthy Males)

**OBJECTIVES**

To demonstrate the effect of **JMV 1843** on GH secretion
To investigate the effect on ACTH; Cortisol; Ghrelin; Prolactin; Insulin and Glucose
To delineate the pharmacokinetic profile of **JMV 1843**
To assess safety/tolerability

**METHODS**

36 healthy, male subjects were included in this study
A dose escalation study investigating 5 different doses (0.005; 0.05; 0.125; 0.25 and 0.5 mg/kg) of **JMV 1843** as a single oral administration of an aqueous solution in comparison to a placebo
Effect of Different Doses of Oral JMV1843 on GH Secretion Over Baseline (Healthy Males)

Plasma Concentration of JMV1843 After Oral Administration of Different Doses of JMV1843 (Healthy Males)

Phase 1 clinical study of JMV 1843 administered by oral route

- **JMV 1843** was well tolerated and no adverse events were reported

- Maximal GH release was achieved following 0.5 mg/kg orally

- Maximal GH release is achieved when plasma levels of **JMV 1843** are between 5 – 6 ng/ml

- Stimulation of GH appears to be selective as no other hormones measured were affected by administration of **JMV 1843** (No effects on ACTH; cortisol; ghrelin; prolactin; insulin and glucose)

- Next development phase is Phase 2 clinical study in cachectic cancer patients who can benefit from the anabolic effect of GH stimulated by **JMV 1843**
SolorelTM (AEZS-130)
Test diagnostique de la déficience en hormone de croissance chez les adultes (endocrinologie)

**Champ thérapeutique** : Thérapie endocrinienne

**Indication principale** : Test diagnostique de la déficience en hormone de croissance

**Stade de développement** : Phase 3

SolorelTM (AEZS-130), un secrétagogue de l'hormone de croissance, est une petite molécule synthétique novatrice oralement active qui agit comme un mimétique de la ghréline en stimulant la sécrétion de l'hormone de croissance (GH).

En plus de l'utilisation comme diagnostic, SolorelTM (AEZS-130), pourrait être potentiellement utilisé comme traitement de la cachexie, une condition souvent associée à des maladies chroniques sévères comme le cancer, les maladies pulmonaires obstructives chroniques et le SIDA.
Ghrelin and Energy Homeostasis

- Ghrelin is the only known peripheral mediator which acts on the CNS to increase food intake and energy storage
- Ghrelin increases adiposity in rodents
- Ghrelin increases lipogenesis in peripheral adipocytes
- Central and peripheral effects of Ghrelin are probably mediated through different types of receptors
Potentially Novel Treatment of Obesity with Ghrelin Antagonists?

- Acting on meal initiation and energy balance to decrease body weight
- But so far no ghrelin receptor antagonist has been administered to humans
- Oral absorption and blood-brain barrier permeability?
- Selectivity of the ghrelin antagonistic effect?
- Still unknown physiological effects of ghrelin?

![Weight Control Diagram](image)
Synthesis of Substituted 1,2,4-Triazoles

JMV 1843
Novel Triazole Derivatives as Ghrelin Ligands

- Based on the triazole scaffold more than 350 derivatives were synthesized
- 5 derivatives showed binding affinity to GHS-R1a < 1 nM
  - 1 in vitro competitive antagonists
  - 4 in vitro agonists
- 5 derivatives showed binding affinity to GHS-R1a 1-10 nM
  - 4 in vitro competitive antagonists
  - 5 in vitro agonists

Martinez et al., US Patent Application 05017732.8 Filed on August 2005;
Martinez et al., European patent Application 05017732.8 Filed on August 16, 2005
Novel Triazole Derivatives as Ghrelin Ligands

SAR Conclusions (GHS1-a Receptor, *in vitro*)

- $R_1$ Indole provided the best results. Configuration is not significant although $(R)$ configuration is preferred.
- $R_2$ Aromatic moiety is requested. Crucial for Agonist/Antagonist Character (p-Methoxybenzyl, 2,4-Methoxybenzyl yielded Antagonist compounds)
- $R_3$ Methyl, Ethyl-Indole is preferred. $(R)$ configuration provided the best results.
- $R_4$ Can modulate Agonist/Antagonist Character in connection with $R_2$. Aib, 2-Pyridyl, Piperidine carboxylic acid are preferred.
Novel Triazole Derivatives as Ghrelin Ligands

**JMV 2810**

\[ R_1 = \begin{array}{c} \text{[structure]} \end{array} \quad R_2 = \begin{array}{c} \text{[structure]} \end{array} \quad R_3 = \begin{array}{c} \text{[structure]} \end{array} \quad R_4 = \text{Aib} \]

**JMV 3002**

\[ R_1 = \begin{array}{c} \text{[structure]} \end{array} \quad R_2 = \begin{array}{c} \text{[structure]} \end{array} \quad R_3 = \begin{array}{c} \text{[structure]} \end{array} \quad R_4 = \begin{array}{c} \text{[structure]} \end{array} \]

# Pharmacological Characterization of Triazole Compounds

<table>
<thead>
<tr>
<th></th>
<th>GHS1a Receptor (1)</th>
<th>GH Release (2)</th>
<th>Feeding (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Binding IC&lt;sub&gt;50&lt;/sub&gt; nM</td>
<td>[Ca&lt;sup&gt;++&lt;/sup&gt;]&lt;sub&gt;i&lt;/sub&gt; Release EC&lt;sub&gt;50&lt;/sub&gt; nM</td>
<td>GH Release</td>
</tr>
<tr>
<td>JMV2810</td>
<td>27 ± 4</td>
<td>39 ± 5</td>
<td>Partial Agonist (22%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No Antagonist</td>
</tr>
<tr>
<td>JMV3002</td>
<td>1.1 ± 0.3</td>
<td>No Effect</td>
<td>Antagonist Kb 24 ± 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No Antagonist</td>
</tr>
</tbody>
</table>

1) Cloned GHS1a Receptor in CHO or LLC-PK1 cells.
2) 10 days old Rat model, s.c. injection of 160 µg/kg each compound. GH dosed after 15 min. Values are means ± SE of 9 rats. 3.
3) Cumulative food intake (6 h) in Rats (s.c. injection).

*Unpublished Results*
JMV 2810: *in vivo* Pharmacological Characterization

GH Secretion in Rodents (s.c. injection)

JMV 2810 at 160 µg/kg, s.c. does not stimulate GH secretion, nor inhibits GH secretion induced by hexarelin in Rats.
**JMV 2810: *in vivo* Dose-Effect Curves on Food Intake in Rats (s.c. injection)**

JMV 2810 at 20, 80, 160 µg/kg, and 320 µg/kg s.c. inhibits food intake induced by hexarelin
Pharmacological Characterization of JMV 3002 on h-GHSR-1a Receptor

JMV 3002 is a competitive antagonist of h-GHS-R1a

IC$_{50}$: $1.23 \times 10^{-9}$ M
• JMV 3002 (160 µg/kg, s.c.) does not inhibit GH secretion induced by hexarelin in Rats.
JMV 3002: *in vivo* Dose-Effect Curves on Food Intake in Rats (s.c. injection)

JMV 3002 at 20, 80 and 160 µg/kg, s.c. inhibits food intake induced by hexarelin. The higher dose seems to be less effective.
CONCLUSIONS

• We have identified *in vitro* potent ligands for the Ghrelin Receptor GHS-1a, Agonists, Antagonists and Partial Agonists

• Most of the compounds were tested *in vivo* for their activity on GH Release and Feeding Behavior

• We have identified several compounds (including **JMV 3002** and **JMV 2810**) with no activity on GH secretion and able to stimulate or inhibit Hexarelin-stimulated Food Intake

• There was no clear correlation between *in vitro* and *in vivo* results; there was no clear correlation between the agonist/antagonist *in vitro* character and *in vivo* antagonist/agonist character.

• There was no clear correlation between *in vivo* results on GH secretion and control of food intake suggesting the presence either of receptor subtypes or a specific mechanism of action of the GHS-1a receptor for GH and Food intake responses.
Contributors

Institut des Biomolécules Max Mousseron IBMM
UMMR5247
Département des Aminoacides, Peptides et
Protéines, Universités de Montpellier 1 et 2, France

J.A. Fehrentz
V. Guerlavais
L. Demange
A. Moulin
D. Boeglin
J. Ryan
J. C. Galleyrand
D. Gagne
G. Bergé

Department of Experimental & Environmental
Medicine and Biotechnologies
University of Milano-Bicocca, Italy

A. Torsello
V. Locatelli
E. Ghigo
F. Broglio

Department of Internal Medicine
University of Torino, Italy

G. Muccioli
C. Ghé
F. Catapano

Department of Anatomy, Pharmacology & Forensic Medicine
University of Torino, Italy

R. Deghenghi

Europeptides
Argenteuil, France

Zentaris
Frankfurt, Germany

D. Perrissoud
Development of GHS Agonists

Novonordisk 1999, 2001

NN 703 or Tabimorelin
Ki (liaison) = 50 nM (hGHS-R1a)
EC<sub>50</sub> (GH) = 2.7 nM
Oral Bioav. (Dog) = 35%
t<sub>1/2</sub> > 3 h (Dog)

NCC 26 1167
EC<sub>50</sub> (GH) = 9 nM
Oral bioav. (Dog) = 35%
t<sub>1/2</sub> = 1.9 h (Dog)

Merck Sharp Dohme 1996

MK 0677 or Ibutamoren
Ki (binding) = 0.3 nM (hGHS-R1a)
EC<sub>50</sub> (GH) = 1.3 nM
Oral Bioav. (Dog) > 60%
t<sub>1/2</sub> = 5 h (Dog)

CP 424 391-18 or Capromorélin
IC<sub>50</sub> (biinding) = 7 nM (hGHS-R1a)
EC<sub>50</sub> (GH) = 3 nM
Oral Bioav. (Rat) = 65%
t<sub>1/2</sub> = 0.8 h (Rat)

Pfizer 2001
Development of GHS Partial Agonists

Sumimoto Pharmaceuticals 2000

Tullins et al, 2000

SM 130686

IC$_{50}$ (binding) = 1.2 nM (hGHS-R1a)
E$_{50}$ (GH) = 6.3 nM
Oral bioav. (Rat) = 28%
$\text{t}_{1/2} = 1.5 \text{ h (Rat)}$

(R)-PIA

EC$_{50}$ (Ca$^{2+}$) = 0.5 nM
Development of Peptide GHS Antagonists

**Zentaris 2002**

IC₅₀ 300mg/kg (Inhibit GH release Rat)

**Ipsen Beaufour 2004-5  BIM 28 163**

Ki = 8nM
Inhibit Ghrelin-stimulated GH secretion, i.v. (Rat)
Enhances Food Intake (Rat)

**Tranzyme Pharma 2005**

Ki (binding) 1 - 10 nM
Development of Non-Peptide GHS Antagonists

**Abbott 2005**

IC$_{50}$ (binding) = 9 nM (hGHS-R1a)
IC$_{50}$ (Ca$^{2+}$) = 10 nM

**Abbott 2005**

IC$_{50}$ (binding) = 16 nM (hGHS-R1a)
IC$_{50}$ (Ca$^{2+}$) = 29 nM
Oral bioav. (Rat) = 19%

**Abbott 2006**

IC$_{50}$ (binding) = 7 nM (hGHS-R1a)
IC$_{50}$ (Ca$^{2+}$) = 19 nM
Oral bioav. (Rat) = 55%
Non selective (DHFR)

**Sumitomo Pharmaceutical 2005**

Active *in vivo* on Food Intake (*db/db* mice)

**Amgen Inc. 2006**

IC$_{50}$ (Ca$^{2+}$) = 1 - 10 nM
Development of GHS Inverse Agonists

Merck Sharp Dohme 2004

His - (D)Trp - (D)Lys - Trp - (D)Phe - Lys - NH₂

(D)Lys (3) - GHRP - 6
IC₅₀ (liaison) = 0.9 µM (hGHS-R1a)

(D)Arg - Pro - Lys - Pro - (D)Phe - Gln - (D)Trp - Phe - (D)Trp - Leu - Leu - NH₂

L 756 867 ou ((D)Arg¹, (D)Phe⁵, (D)Trp⁷,⁹, Leu¹¹)substance P
IC₅₀ (IP₃) = 5.2 µM

7TM Pharma A/S 2004

TM 27 810
IC₅₀ (IP₃) = 6.5 µM
Screening of Ghrelin Receptor Ligands
Targeting the Treatment of Obesity

In vitro binding and functional test
In cells expressing hGHS-R1a

In vivo reduction of food intake acutely stimulated by hexarelin or fasting in Rats

In vivo modification of GH release stimulated by hexarelin in Rats

Pharmacokinetics / oral bioavailability

Body weight reduction in diet induced obesity in mice
Synthesis of Substituted 1,2,4-Triazoles
Synthesis of Substituted 1,2,4-Triazoles
SYNTHESIS OF COMPOUND JMV1843

Martinez et al., PCT WO 01/96300 A1 (2001)
JMV 1843: a Ghrelin Receptor Agonist Candidate for Clinical Development

- **JMV 1843 pharmacological profile**
  - Binding affinity to GHS-R1a: \( IC_{50} = 40 \text{ nM} \)
  - Binding affinity to h-Pituitary Glands: \( IC_{50} = 23 \text{ nM} \)
  - Binding affinity to h-Hypothalamus: \( IC_{50} = 15 \text{ nM} \)
  - Activation of GHS-R1a *in vitro* (calcium release) \( ED_{50} = 0.8 \text{ nM} \)
  - GH secretagogue effect by i.v., s.c. injection in rats
  - GH secretagogue effect by s.c. and oral route at 1 mg/kg in dogs

- **Requirements for the performance of a first trial in humans**
  - Preparation of amount of substance necessary for the preclinical studies at a GMP (Good Manufacturing Procedure) quality
  - Acute toxicity studies in males and females of 2 animal species
    - No toxic dose of **JMV 1843** by i.v. route: 30 mg/kg
  - Toxicity after repeated administration (2 to 4 weeks) in 2 animal species
    - Maximum tolerated dose of **JMV 1843**: 3 mg/kg/day by i.v. route
  - Safety pharmacology tests including central nervous system, cardio-vascular system, gastro-intestinal and metabolic assays
  - Evaluation of mutagenic potential
  - Preparation of a pharmaceutical formulation suitable for i.v. and oral administration in humans
  - Clinical trial authorization after approval by an Ethic Committee
Synthesis of Substituted 1,2,4-Triazoles

(i) BOP, $\text{H}_2\text{N-}R_1$, NMM, DCM, (ii) Lawesson’s reagent, DME, 85°C, (iii) $\text{H}_2\text{N-}\text{HN-COR}_2$, $\text{Hg(OAc)}_2$, RT, THF, (iv) HCl, AcOEt, (v) Boc-Aib-OH, BOP, DIPEA, DCM, (vi) HCl, AcOEt
Novel Triazole Derivatives as Ghrelin Ligands
(GHS1-a Receptor, in vitro)

JMV 2952

IC$_{50}$ 0.3 ± 0.2 nM
Full Agonist
EC$_{50}$($\text{Ca}^{2+}$) 3 ± 1 nM

JMV 3018

IC$_{50}$ 0.7 ± 0.2 nM
Antagonist
$\text{Kb(S)}$ 12 ± 1 nM