New Drug Development for Hepatic Insulin Resistance

Innovative Drug Research Center for Metabolic and Inflammatory Disease
College of Pharmacy

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AMPK as an energy sensor, (A novel drug target for metabolic disease)
Upstream signals of AMPK activation

- **CaMKK**
- **LKB1**
- **PKCζ**
- **TAK1**
- **SIRT1**
- **Ca**
- **CaMKK**
- **AMP**
- **[AMP]:[ATP]**
- Stress (ROS)
- **AMPK**
- **Activation**

(AMPK activation is indicated by an increased ratio of AMP to ATP.)
LKB1–independent AMPK activation
LKB1-independent AMPK activation

• Oltipraz activates AMPK through a distinct pathway independent of LKB1 or CaMKK.
• Not a direct AMPK activator.

Bae et al., Hepatology, 2007
AMPK activation by oltipraz leads to S6K1 inhibition.

(A) Oltipraz time course showing the upregulation of p-ACC, p-AMPKα, and AMPKα, and downregulation of p-S6 and S6.

(B) Western blot images comparing pCDNA and DN-AMPK conditions with and without Oltipraz treatment. S6K1 and S6 levels are reduced in DN-AMPK compared to pCDNA.

(C) Western blot images showing the effect of Oltipraz on Akt, p-Akt, GSK3β, and p-GSK3β under pCDNA and DN-AMPK conditions. Insulin treatment increases Akt activation, and TNFα decreases p-Akt and GSK3β levels.

(Bae et al., Hepatology, 2006)
Inhibition of S6K1 by Oltipraz

(Bae et al., Hepatology, 2006)
Inhibition of insulin signaling by S6K1

(Um et al., Nature, 2004)
S6K1 inhibition by oltipraz enhances insulin signaling.

(Bae et al., Hepatology, 2006)
Inhibition of IRS Ser-307 phosphorylation by oltipraz

(Bae et al., Hepatology, 2006)
Inhibition of LXRα-SREBP-1c Pathway by AMPK-activating Candidates?

Bae et al., *Hepatology* (2007)

**Hepatic lipogenesis through LXRα-SREBP-1c pathway**

Oltipraz

DTTs

AMPK → S6K1 → Insulin Resistance

closely linked

FATTY LIVER
LXR-dependent Lipogenic Gene Induction

- **LXR** and **RXR** form a complex that binds to the LXRE in the nucleus, leading to transcriptional activation of target genes (e.g., SREBP-1c).
- **Endogenous LXR ligand** mediates the interaction of LXR with RXR.
- **Insulin** and **T0901317** are ligands that can modulate the activity of LXR.

**Cytosol**
- **T0901317** (T0901317)
- **Insulin**

**Nucleus**
- **Endogenous LXR ligand**
- **LXRE**
- **Target gene transactivation** (e.g., SREBP-1c)

**Lipogenic gene transactivation** (FAS, ACC, SCD-1)
- Nuclear translocation

**SREBP-1c expression** (tethered in ER membrane)
Opposite Regulation of LXRα Activity by AMPK and S6K1
Inhibition of SREBP-1c Expression and Activation by Oltipraz

(Hwahng et al., *Hepatology*, 2009)
Attenuation of SREBP-1c Target Gene Expression by Oltipraz

SRE-containing FAS promoter

SREBP-1c target gene FAS, ACC, SCD-1

(Hwahng et al., Hepatology, 2009)
Oltipraz

AMPK
S6K1
SREBP-1c

Lipogenic Enzymes

FAS  ACC  SCD-1
Repression of SREBP-1c by S6K1 Inhibition

(Hwahng et al., Hepatology, 2009)
Role of AMPK in Inhibition of T090-stimulated SREBP-1c Induction

(Hwhanng et al., Hepatology, 2009)
Oltipraz

? ?

AMPK
S6K1

LXRα

SREBP-1c

Lipogenic Enzymes

FAS ACC SCD-1
Suppression of LXRα Activity by Oltipraz

(Hwahng et al., Hepatology, 2009)
Direct Phosphorylations of LXRα by S6K1 and AMPK

(Hwahng et al., Hepatology, 2009)
The Opposing Action of S6K1 and AMPK on LXRα Activity

(Hwahng et al., Hepatology, 2009)
In case of LXR activation

- AMPK (Inactive) with Thr (p)
- LXR activity
- S6K1 (Active) with Ser (p)
- TSC2
In case of LXR inhibition
A summary on the LXRα–SREBP-1c regulation

- Oltipraz
- DTTs

AMPK

- Inactive
  - Thr (phosphorylated)

TSC2

S6K1

- Active
  - Ser (phosphorylated)

LXR activation

Binding to LXRE

SREBP-1c

- FAS
- ACC
- SCD-1

Hepatic lipogenesis
In vivo Anti-steatotic Effect

C57BL/6 mice

(Hwahng et al., Hepatology, 2009)
Dithiolethiones

Degradation

RSK

C/EBP

β

C/EBP

β

HIF-1α

Drug Targets

Drug Targets

Drug Targets

Drug Targets

Description

Insulin

IR

IRS1

PI3K

Akt

TSC1/2

mTOR

RAPTOR

AMPK

p70S6K1

Activating Phosphorylation

Inhibitory Phosphorylation

Nucleus

LXR

RXR

SREBP-1c

LXRE

S6K1: insulin resistance, lipogenesis

AMPK: insulin resistance, lipogenesis

FAS, ACC, SCD-1

↓

↑
IX. OVERALL SCHEME OF OLIPRAZ DEVELOPMENT

- Process development for large-scale production
- Set up specification & test method (raw material)
- Stability study (raw material)

Preliminary Clinical
- Clinical marker setup
- Clinical trial protocol preparation

Prof. S.G. Kim
Seoul National Univ.

Synthesis

Toxicity Study

Formulation

Efficacy

PK

IND

Ph. Phase I Phas e II Phas e III

NDA

Launch

Prof. M.G. Lee
National Cancer Center (J.W. Park, M.D.)
Samsung Medical Center (G.C. Koh, M.D.)
Korea Cancer Center Hospital (C.J. Han, M.D.)

Preformulation study

Formulation study

Set up specification & test method (finished product)

Stability study (finished product)

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<th>1st Year ('02)</th>
<th>2nd Year ('03)</th>
<th>3rd Year ('04)</th>
<th>4th Year ('05)</th>
<th>Launching ('06)</th>
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</table>
Pharmacokinetic profiles of oltipraz in cirrhotic patients
(Patients safety verified)

LKB1–dependent AMPK activation
Sauchinone (Sau)

- A lignan in *Saururus chinensis*
  - frequently used medicinal plants
  - treat fever, jaundice, and inflammation.

- **Anti-inflammation:**
  Inhibits iNOS, COX-2 and TNF-α induction in macrophages. *Br J Pharmacol, Lee et al., 2003*

- **Liver protection:**
  Attenuates liver toxicity in primary hepatocytes. *Biol Pharm Bull, Sung et al., 2000*
AMPK activation by Sau

Kim YW et al., *FRBM* (2009)
LKB1-dependent AMPK activation by Sau

Kim YW et al., FRBM (2009)
Improvement of insulin sensitivity

Kim YW et al.,
*FRBM* (2010)
Inhibition of LXRα activity by Sau

Kim YW et al., *FRBM* (2010)
SREBP-1c repression by Sau

A) Western blot analysis of SREBP-1c and Actin levels in HepG2, H4IE, and AML12 cells treated with different concentrations of Sau and T090. Controls are shown for comparison.

B) Bar graph showing the relative mRNA levels of SREBP-1c in HepG2 cells treated with different concentrations of Sau and T090. Significant changes are indicated by statistical symbols.

C) Western blot showing the protein levels of SREBP-1c and Actin in cells treated with Sau and the presence of 22(R)-HC or GW3905.

Kim YW et al., FRBM (2010)
Inhibition of SREBP-1c target gene

Kim YW et al.,
FRBM (2010)
Inhibition of lipogenesis in mice fed HFD

Kim YW et al., FRBM (2010)
Sau treats LXR$\alpha$-dependent lipogenesis by activating AMPK.
LKB1-dependent AMPK activation by resveratrol

Pharmacological modulations of AMPK activity

Kim SG et al., *Handbook of Type I DM* (2009)
Cytoprotective, antioxidant, & mitochondrial protective effects of AMPK-activators
Inhibition of HFD-induced liver injury

Kim YW et al., FRBM (2010)
Inhibition of HFD-induced oxidative stress

Kim YW et al., *FRBM* (2010)
Mitochondrial permeability transition pore

- Non-selective large conductance channel in the mitochondrial inner membrane

- ANT, CypD and VDAC → regulatory factors

- Opening of mPTP causes MMP transition and cytochrome c release → apoptosis.

(Miura and Miki, Circ J., 2009)
1) Mitochondrial protective; 2) $H_2O_2$ scavenging effect
Mitochondrial protective and H$_2$O$_2$ scavenging effects of Sau
Mitochondrial protective and H$_2$O$_2$ scavenging effects of Res
Mitochondrial protective and $H_2O_2$ scavenging effects of ILQ
AMPK and GSK3β

AMP-activated Protein Kinase Activation Increases Phosphorylation of Glycogen Synthase Kinase 3β and Thereby Reduces cAMP-responsive Element Transcriptional Activity and Phosphoenolpyruvate Carboxykinase C Gene Expression in the Liver

Resveratrol Protects Mitochondria against Oxidative Stress through AMP-Activated Protein Kinase-Mediated Glycogen Synthase Kinase-3β Inhibition Downstream of Poly(ADP-ribose)polymerase-LKB1 Pathway

Sang Mi Shin, Il Je Cho, and Sang Geon Kim
Increase in GSK3β phosphorylation by ILQ

A) 

B)
Effect of GSK3β inhibition on mitochondrial protection

A) Relative cell viability (%)

B) Relative rhodamine-negative cell population (%)

C) Relative rhodamine-negative cell population (%)

Mitochondrial Membrane Permeability
AMPK activation alone by metformin is not sufficient to protect cells from severe oxidative stress!!!

Kim YW et al., FRBM (2010) (Unpublished data)

B) DCFH-DA

No H$_2$O$_2$ scavenging effect

(Unpublished data)
Down-stream functions of AMPK

PARP, SIRT, NO, NQO1
PKA, PKCζ, RSK

AMP/ATP↑ TAK1 LKB1 CaMKK

ACC phosphorylation:
Lipogenesis↓

eNOS phosphorylation:
Anti-inflammation↑
Mitochondrial protection↑

Antioxidant capacity:
MnSOD, catalase, NQO1↑
Glutathione biosynthesis↑

S6K inactivation:
IRS Ser-phosphorylation ↓
Insulin sensitivity↑
LXR Ser-phosphorylation ↓
Lipogenesis↓

Mitochondria biogenesis:
PGC1α induction ↑

GSK3β inactive phosphorylation:
Glycogen synthesis↑
Mitochondrial protection↑

LXR Thr-phosphorylation:
Lipogenesis↓

Kim SG et al., Handbook of Type 1 DM (2009)
Clinical & Investigational AMPK activators

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<thead>
<tr>
<th>1. Biguanides</th>
<th>5. Phytochemicals</th>
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<tr>
<td>Metformin</td>
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<th>2. Thiazolidinediones (TZDs)</th>
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<tr>
<td>Pioglitazone</td>
<td>EGCG</td>
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<tr>
<td>Oltipraz</td>
<td>Sauchinonc</td>
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<th>4. α-lipoic acid</th>
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<td>![α-lipoic acid Structure]</td>
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Kim SG et al., *Handbook of Type I DM* (2009)
Clinical & Investigational AMPK activators (continued)

6. Others

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<tr>
<th>Metadoxine</th>
<th>β-lapachone</th>
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Kim SG et al., *Handbook of Type I DM* (2009)
Collaborations
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