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OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.
Oral bioavailability of celastrol following administration of pure celastrol and its related tablets in rats

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General characteristics of TCM

- TCM characteristics:
  - multi-component mixture, holistic-regulating, the history of long-term use by human (experience-based);

- TCM resources:
  - 12807 species including 11146 herbs, 1581 animal-derived and 80 minerals; about 300-500 species commonly used in TCM clinical practice

- TCM Modernization:
  - a process of providing scientific evidence for the safety, quality and efficacy to develop evidence-based medicine.
Background

- Thunder God Vine,
  *Trypterygium wilfordii* Hook F.,
*Shennong Bencao Jing* (221BC- 200)
Chinese name: 雷公藤
- The xylem, TGV root (removed away the scarfskin)
- To treat rheumatoid arthritis, systemic lupus erythematosus, and cancer, etc.
Background

- **Pharmacological activities**: immunosuppression, anti-inflammatory, antioxidant, anti-bacterial, anti-cancer, and anti-fertility, etc.
- **Toxicity**: hepatotoxicity, nephrotoxicity, and reproduction toxicity, etc.
- More than 450 compounds isolated from TGV. About 90% of them were terpenoids, containing 118 diterpenes, 130 triterpenes, 77 sesquiterpene pyridine alkaloids, 78 sesquiterpene polyol esters and 53 other compounds.
- Triptolide and **Celastrol** have become the hot issue of research in recent years. They have been demonstrated to be not only effective, but also poisonous.
Celastrol, isolated from TGV in 1936.

Properties: anti-oxidant, anti-inflammation and anti-cancer.

Therapeutic usefulness: Alzheimer’s disease, arthritis rheumatoid, asthma, hypertension, systemic lupus erythematosus and different types of cancer.
Treatment of Obesity with Celastrol

- Diet-induced obese mice (Leptin resistance; Hyperleptinemia)
- db/db or ob/ob mice (Deficient leptin signaling)

Food intake, Body weight, Glucose homeostasis

Hypothalamus:
- ER stress
- Leptin sensitivity

Liu et al., 2015, Cell 161, 999-1011
Oral bioavailability of celastrol following administration of pure celastrol

Gender-related pharmacokinetics of celastrol following oral administration of TGV tablets
Animals and treatment:

- Two groups (female rats, n = 6, pure celastrol):
  - administered an oral dose: 1000μg·kg⁻¹ by gavage;
  - intravenous dose: 100μg·kg⁻¹ via the tail vein.

- Blood samples collection:
  - intravenous injection: 0, 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 36, 48 h;
  - oral administration: 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24, 36, 48 h.
Instrumentation and LC-MS conditions

Chromatographic condition:
Phenomenex Luna C8 column (2.0 × 50 mm, 3 μm); Mobile phase: methanol, acetonitrile, isopropanol, formic acid and water (15/27.5/27.5/0.03/29.97); Column temperature: 35 °C; Flow rate: 0.3mL/min

The optimized electrospray conditions:
ion spray voltage (ISV): 5500 V; turbo heater temperature (TEM): 500°C; collision activation dissociation (CAD): 10 psi; curtain gas (CUR): 20 psi. declustering potential (DP) and collision energy (CE) were optimized at 40 and 30 for celastrol and 25 and 15 for I.S.

Agilent 1200 HPLC coupled with a API4000 mass spectrometer, Analyst software version 1.4.1.
ESI+, with acquisition in MRM mode.
m/z 451.3→201.1 for celastrol, m/z 471.4→317.4 for I.S.
Fig. 2 Typical MRM chromatograms of celastrol (I) and glycyrrhetinic acid (I.S., II)
(a) blank plasma, (b) blank plasma spiked with celastrol (4.34 ng·mL⁻¹) and I.S. (960 ng·mL⁻¹),
(c) rat plasma sample 5 h after oral administration of pure celastrol,
(d) rat plasma sample 7 h after intravenous administration of pure celastrol.
Fig. 3 Plasma concentration–time profiles of celastrol following (a) intravenous injection and (b) oral administration of pure celastrol in female rats (n=6)
Table 1 Pk parameters of celastrol following single intravenous and oral administration of pure celastrol in female rats (n=6, mean values ± SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Oral group (1000 μg·kg⁻¹)</th>
<th>intravenous group (100 μg·kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{max}$ (h)</td>
<td>3.00 ± 0.89</td>
<td>0.083</td>
</tr>
<tr>
<td>$C_{max}$ (μg·L⁻¹)</td>
<td>13.75 ± 7.94</td>
<td>38.83 ± 12.83</td>
</tr>
<tr>
<td>$AUC_{(0-tn)}$ (μg·h·L⁻¹)</td>
<td>130.90 ± 79.39</td>
<td>76.74 ± 19.03</td>
</tr>
<tr>
<td>$AUC_{(0-∞)}$ (μg·h·L⁻¹)</td>
<td>135.50 ± 79.76</td>
<td>79.35 ± 19.85</td>
</tr>
<tr>
<td>$T_{1/2β}$ (h)</td>
<td>10.20 ± 2.17</td>
<td>8.33 ± 0.84</td>
</tr>
<tr>
<td>$CL/F$ (L·h⁻¹)</td>
<td>11.29 ± 6.16</td>
<td>0.45 ± 0.16</td>
</tr>
<tr>
<td>$MRT_{(0-tn)}$ (h)</td>
<td>12.04 ± 1.20</td>
<td>7.63 ± 0.75</td>
</tr>
<tr>
<td>$MRT_{(0-∞)}$ (h)</td>
<td>14.11 ± 1.60</td>
<td>9.46 ± 1.43</td>
</tr>
</tbody>
</table>

The oral absolute bioavailability: 17.06%
Which?

There are 16 drug approval numbers.
Determination of celastrol in TGV tablets

Chromatographic condition:
Zorbax Eclipse XDB-C8 column (5μm, 4.6mm × 150mm) Mobie phase:
methanol:1% glacial acetic acid solution (83:17 )
Column temperature: 30 °C; injection volume: 5μL
Detection wavelength: 425nm
Flow rate: 1.0 mL/min.

Fig 1  HPLC profiles of reference standards (A, containing 50.65μg · mL⁻¹ celastrol) and Tripterygium Preparation (B, from one pharmaceutical company in Zhejiang province)
The contents of celastol of Tripterygium Preparations in 6 different batches (n=5)

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Manufacturer</th>
<th>Batches</th>
<th>Contents of celastol (μg per tablet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tripterygium Tablets</td>
<td>Sanjiu</td>
<td>090604</td>
<td>356.03</td>
</tr>
<tr>
<td>Tripterygium Glycosides</td>
<td>Zhejiang-1</td>
<td>090602</td>
<td>37.84</td>
</tr>
<tr>
<td>Tripterygium Glycosides</td>
<td>Hunan</td>
<td>090601</td>
<td>400.62</td>
</tr>
<tr>
<td>Tripterygium Glycosides</td>
<td>Shanghai-1</td>
<td>091010</td>
<td>17.66</td>
</tr>
<tr>
<td>Tripterygium Glycosides</td>
<td>Shanghai-2</td>
<td>090603</td>
<td>26.77</td>
</tr>
<tr>
<td>Tripterygium Glycosides</td>
<td>Zhejiang-2</td>
<td>0910107</td>
<td>26.77</td>
</tr>
</tbody>
</table>
Gender-related pharmacokinetics of celastrol

Animals and treatment:

- Group 1: seven male rats, Group 2: seven female rats.
- Dose: 1.5 tablets·kg⁻¹ by gavage.

- Blood samples collection:
  0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24, 36, 48 h.
Fig. 3 Plasma concentration–time profiles of celastrol following oral administration of TGV tablets in (c) female and (d) male rats
### Table 2 PK parameters of celastrol following single oral administration of TGV tablets (1.5 tablets·kg⁻¹) in female and male rats (n=7)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Female group</th>
<th>Male group</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>6.71 ± 4.57</td>
<td>5.14 ± 3.58</td>
</tr>
<tr>
<td><strong>$C_{\text{max}}$ (μg·L⁻¹)</strong></td>
<td>32.03 ± 8.41↑</td>
<td>14.31 ± 7.33</td>
</tr>
<tr>
<td><strong>$AUC_{(0-\infty)}$ (μg·h·L⁻¹)</strong></td>
<td>443.52 ± 138.95↑</td>
<td>221.87 ± 135.44</td>
</tr>
<tr>
<td>$T_{1/2\beta}$ (h)</td>
<td>10.02 ± 3.36</td>
<td>8.38 ± 1.98</td>
</tr>
<tr>
<td><strong>$CL/F$ (L·h⁻¹)</strong></td>
<td>0.96 ± 0.53↓</td>
<td>2.58 ± 0.66</td>
</tr>
<tr>
<td>$MRT_{(0-\infty)}$ (h)</td>
<td>16.72 ± 1.43</td>
<td>16.96 ± 2.56</td>
</tr>
</tbody>
</table>
Comparison of major PK parameters

<table>
<thead>
<tr>
<th>species</th>
<th>drug</th>
<th>Targeted compound</th>
<th>Dose (μg·kg⁻¹)</th>
<th>Tmax (h)</th>
<th>Cmax (ug/L)</th>
<th>AUC₀-ₜ</th>
<th>AUC₀-∞</th>
<th>t₁/₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>celastrol</td>
<td>celastrol</td>
<td>100 (i.v.)</td>
<td>0.083</td>
<td>38.83 ± 12.8</td>
<td>76.74 ± 19.03</td>
<td>79.35 ± 19.85</td>
<td>8.33 ± 0.84</td>
</tr>
<tr>
<td>Rat</td>
<td>celastrol</td>
<td>celastrol</td>
<td>1000 (i.g.)</td>
<td>3.00 ± 0.89</td>
<td>13.75 ± 7.94</td>
<td>130.90 ± 79.39</td>
<td>135.50 ± 79.76</td>
<td>10.20 ± 2.17</td>
</tr>
<tr>
<td>Rat (♀)</td>
<td>TGV tablets</td>
<td>celastrol</td>
<td>534 (i.g.)</td>
<td>6.71 ± 4.57</td>
<td>32.03 ± 8.41</td>
<td>379.49 ± 118.1</td>
<td>443.52 ± 138.95</td>
<td>10.02 ± 3.36</td>
</tr>
<tr>
<td>Rat (♂)</td>
<td>TGV tablets</td>
<td>celastrol</td>
<td>534 (i.g.)</td>
<td>5.14 ± 3.58</td>
<td>14.31 ± 7.33</td>
<td>188.17 ± 92.33</td>
<td>221.87 ± 135.44</td>
<td>8.38 ± 1.98</td>
</tr>
<tr>
<td>Dog</td>
<td>TGV tablets</td>
<td>Triptolide</td>
<td>356 (i.g.)</td>
<td>1.75 ± 0.76</td>
<td>2.78 ± 0.39</td>
<td>11.54 ± 1.49</td>
<td>13.18 ± 1.69</td>
<td>2.59 ± 0.6</td>
</tr>
</tbody>
</table>

The oral absolute bioavailability of celastrol significantly increased from 17.06% for pure celastrol to 94.19% for TGV tablets containing equivalent celastrol.
Challenges

- Large content variance in different TGV tablets.
- Choose which ingredient for the quality control of TGV
- The low concentration of compounds in blood makes it detect difficultly.
- When TGV was administrated with other drugs, is there potential drug–drug interaction?
Resolution

- The plant of TGV should be cultivated according to Good Agricultural Practices.
- Multi-marker quantification plus fingerprint analysis is the future direction for the comprehensive quality control of TGV.
- Investigate the effects of TGV extract on CYP activity to predict the potential clinical drug–drug interaction.
Publications


Acknowledgements

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2 Leading Talents of scientific research in TCM of Jiangsu Province, LJ200906

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Our team
Thank you for your attention!

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Let us meet again..

We welcome you all to our future conferences of OMICS International 7th World Congress on Bioavailability & Bioequivalence: BA/BE Studies Summit On

August 29 - 31, 2016 at Atlanta, USA

http://bioavailability-bioequivalence.pharmaceuticalconferences.com/