MALDI/TOF-TOF Analysis of TCMs

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DAITEM #6

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TCMs—Traditional Chinese Medicines

— Rhinoceros horns (Xi Jiao)

- Rhino horns-black market
  - $65,000/kg
  - Asia
  - Yemen

- Rhinos in the wild
  - White Rhino - Near Threatened
  - Black Rhino - Critically Endangered
  - Javan - Critically Endangered
  - Indian - Vulnerable
  - Sumatran - Critically Endangered

- Poaching
  - Water Buffalo replacement (Shui Niu Jiao)
Rhino Location and Populations

- 500,000 at turn of 20th century
- 70,000 by 1970
- Less than 29,000 today

- Many countries have lost all rhinos despite the species surviving
Medical Uses

- From *The Classic of Herbal Medicine* ca. 200-250 AD

**Xi Jiao (Cornu Rhinocerotis)**

Xi Jiao (Cornu Rhinocerotis) is bitter and cold. It mainly treats the hundreds of toxins, gu influx, evil ghosts, and miasmic qi, kills Lip-hooking, zhen feather, and snake toxins, eliminates evils, and prevents confusion and oppressive ghost dreams. Protracted taking may make the body light. It is produced in rivers and valleys.

### Efficacy of rhino horn as medicine

<table>
<thead>
<tr>
<th>Prescribed use of rhino horn</th>
<th>Effective?</th>
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<tbody>
<tr>
<td>Antipyretic</td>
<td>Yes</td>
</tr>
<tr>
<td>Analgesic</td>
<td>Yes</td>
</tr>
<tr>
<td>Anti-inflammatory</td>
<td>Yes</td>
</tr>
<tr>
<td>Anti-spasmyotic</td>
<td>Yes</td>
</tr>
<tr>
<td>Diuretic</td>
<td>Yes</td>
</tr>
<tr>
<td>Bactericidal</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Table: Saving Rhinos LLC
Data: 1983. Yemen acts to halt rhino horn daggers; scientific tests fail to show rhino horn effective as medicine. *The Environmentalist* Vol. 3, No. 2
Goals of Analysis

- Analyze water buffalo replacement (Shui Niu Jiao)

- Determine if Rhino is in there
Overview of Analysis – “Proteomic” Approach

- Standards
  - Water Buffalo Horn
  - Shui niu jiao
  - Possible Rhino Horn
- Separate proteins
- Prepare proteins for analysis
- Collect MS
- Run MS/MS on peaks
- Compare fragmentation to database
Analysis -- MALDI/TOF-TOF

- Matrix Assisted Laser Desorption Ionization
- Nobel prize winning (Koichi Tanaka)
Sample Matrix and Preparation

- Collection of proteins
  - Water Buffalo
  - Rhino?
- Sample Prep
  - Purification
    - Chromatography
  - SDS Page

Fig. 1 Scheme for identification of new bioactive ingredients from TCMs by MALDI-TOF MS
Step 1: Separation of Proteins

- SDS Page
  - SDS (Sodium Dodecyl Sulfate)
  - Detergent
  - Anionic
  - PAGE (Polyacrylamide gel electrophoresis)

- SDS:
Step 2: Prep. For Analysis

- Open up proteins
  - Dithiothreitol (DTT)

- Alkylate to prevent refolding
  - Iodoacetamide
Step 2: Prep. For Analysis (Con’t)

- Digestion with Trypsin
- Mix with “Matrix”
- Crystallize for analysis
MALDI Mechanism of Action

α-Cyano-4-hydroxycinnamic acid, a common matrix molecule used

“Soft” Ionization = no fragmentation of analyte!
Step 3: MS Analysis

- Time-of-flight mass spectrometer (TOF)
- First Mass Spectrum
- Each peak = peptide
- Select peak for further analysis
Step 4: MS/MS Analysis

- Tandem mass spectrometry
- Specific peaks selected
- Fragmentation pattern
  - Ions of peptide
Step 5: Database Mining

- MASCOT
- Match Amino-Acid chains
- 3 peaks
Summary of Steps

- Separate proteins
- Prepare proteins for analysis
- Collect MS
- Run MS/MS on target peaks
- Compare fragmentation to database
Conclusions

- Possible use of LC/MS
- Use in other TCMs
  - Tiger
  - Elephant
  - Other endangered species
- Check purity of pharmaceuticals
- Use to assist law enforcement
  - Tracking illegal sellers
  - Poachers
- Don’t kill endangered animals, especially illegally


