Metabonomics in the study of Traditional Chinese Medicine

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Traditional Chinese medicine seeks to adjust the circulation of qi (metabolic energy flow) in the body using a variety of therapeutic techniques.

Some of these techniques include

- Special diets
- Physical training regimens (qigong, tai chi chuan, and other martial arts training)
- Massage
- Acupuncture
- Moxibustion
- Herbal medicines

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The Five elements:

Tree, traditionally Wood, (木, pinyin: mù)
Fire (火, pinyin: huǒ)
Earth (土, pinyin: tǔ)
Metal (金, pinyin: jīn)
Water (水, pinyin: shuǐ)

An increasing number of trials:

Effectiveness of a TCM preparation in cough treatment in uncomplicated upper respiratory tract infection

- Randomised, double blind, placebo-controlled, between groups study of 80 patients
- Average 4 days history of cough
- 5 days treatment, followed up for 6 days
- Evaluated by cough score

Ledebouriella root
Fritillaria cirrhosa bulb
Citrus reticulata peel
Effectiveness of a TCM preparation in cough treatment in uncomplicated upper respiratory tract infection

- Randomised, double blind, placebo-controlled, between groups study of 80 patients
- Average 4 days history of cough
- 5 days treatment, followed up for 6 days
- Evaluated by cough score
- No significant overall difference between groups

Problems with conducting trials on TCM

The test material itself

Plant extracts (phytomedicines) are mixtures of many compounds

Verification of synergy

Bioactivity-guided fractionation of a plant with reputed antimicrobial activity

Berberis species

Anti-infective modulators

Antimicrobial activity of a Berberis sp

Stermitz FR, Lorenz P, Tawara JN, Zarewicz JA and Lewis K; PNAS, 2000; 97(4), 1433-1437
Distinguishing feature of phytomedicines

Extracts (mixtures of compounds) *not single compounds*

Accepted conventional pharmaceutical methods not really applicable to plant extracts

Nonetheless, standardisation often uses single actives or markers

This produces significant difficulties

DNP-22 whole chromatogram

TLC products are mixtures of mixtures
**Metabolite profiling**

**Metabonomics**

**Metabolomics**

What is metabolite profiling?

- Investigating and describing genetic expression by non-targeted measurements of all metabolites in a biological sample.
- Profiling complex matrices such as *biofluids (plasma and urine)*.
- Biomarkers for metabolic studies and diagnosis.
- Environmental samples.
- Plant matrices (identification).

Tools available for metabolite profiling are several and can be hyphenated:

**Chromatographic**
- HPLC or GC
- GC-MS; HPLC-MS; HPLC-MS-MS
- Detection: selectivity
- Derivatization
- LC-UV-SPE-NMR-MS (cryogenic flow probe)

**Spectroscopic**
- ESI-MS
- Ionisation and fragmentation variability
- IR
- Data information limited
- Peak deconvolution algorithms necessary

High field $^1$H nuclear magnetic resonance spectroscopy

- Data rich fingerprint; requires data reduction/simplification.
- Statistical analysis:
PCA – Intraspecific variation: target (field samples) and non-target accessions

PCA – Intraspecific variation: target and non-target accessions + Year 1 Harvest

PCA – Intraspecific variation: target and non-target accessions + Year 2 Harvest

PCA – Intraspecific variation: target and non-target accessions + Year 3 Harvest

Conclusion

‘Fingerprinting’ approaches already recognised by
WHO
Chinese State Food and Drug Authority

Only HPLC profiles considered
Reductive (not all compounds taken into account)
Subjective in operation (impossible to set criteria for more than one peak)

NMR and PCA gives an approach to fuzzy fingerprints
Application to metabolic studies

Application of metabonomics to detect metabolic effects of plant products

High field nmr spectroscopy to analyse urine to investigate effects of chamomile tea ingestion

Experimental design

Human volunteers

Matricaria chamomilla tea prepared from Roman chamomile flowers

Matricaria camomilla
Experimental design

Human volunteers
Matricaria chamomilla tea
  – 5g dried flowers infused in 200ml hot water (10 minutes)
  – Resultant tea drunk
3 phases
  – Pretreatment or control
  – Treatment
  – Post-treatment or washout
Urine collected 2 hours after dosing
Urine examined by 600MHz nmr spectroscopy
Data analysed by partial least squares analysis
Bioinformatics and Traditional Chinese Medicine

Traditional Chinese Medicine

<table>
<thead>
<tr>
<th>TCM category</th>
<th>Western Equivalent</th>
<th>Signs and Symptoms</th>
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</thead>
<tbody>
<tr>
<td>Tonify Yin</td>
<td>Endocrine agent, Antihypertensive, Anticholesterolmic</td>
<td>Dizziness, tinnitus, weak lower back and knees, low-grade fever, menopausal symptoms, scanty dark urine, red dry tongue, thin pulse</td>
</tr>
<tr>
<td>Shen</td>
<td>Tranquilizer, Sedative, Nerve Tonic</td>
<td>Palpitations, anxiety, insomnia</td>
</tr>
<tr>
<td>Wind Cold</td>
<td>Diaphoretic, Antiviral, Antibacterial</td>
<td>Chills, headache, body &amp; neck pain, no fever/mild fever</td>
</tr>
<tr>
<td>Heat (Qi)</td>
<td>Refrigerant, Antipyretic, Anti-inflammatory, Antimicrobial</td>
<td>High fever, irritability, thirst, delirium, certain skin diseases</td>
</tr>
</tbody>
</table>

Drug discovery from TCM plants

Construction of Two Novel Databases:
Chinese Herbal Constituents Database (CHCD)
Bioactive Plant Compounds Database (BPCD)
Phytochemical Informatics of TCM

- Random forest models used to create TCM profiles
- Combined with Random forest models for target activities
  - Predict activity for drug discovery
- Analysed using Michael Eigen’s CLUSTER program
- Visualized using Eigen’s TREEVIEW program

Random ordering of compounds

Herbal constituents • Targets

Compounds ordered by chemical class

Compounds ordered by plant family
Compounds ordered by plant family

Compounds ordered by TCM profile/usage

Constituents of Herbs in the Invigorate Blood TCM Category

For treating severe pain and ulcer

Voltage sensitive Na+ channel blockers - effective in treating inflammatory pain

5-lipoxygenase & cyclooxygenase-2 inhibitors - anti-inflammatory & analgesic

Protein kinase A & calmodulin dependent protein kinase - implicated in H. pylori potentiation of peptic ulcer

Drug Discovery prediction verified by laboratory study

Application to database screening

Understand nature of drug-receptor interaction
Use mathematics to predict interactions (hydrophobic, van der Waals, electrostatic and hydrogen bonding) between substrate and new molecules (not drugs) and calculate affinities
Obviously need precise 3D structures of compounds
Search for molecules with high binding affinity
Effectively screening of many thousands of compounds against target without physically testing them

Screening in silico
Dihydrofolate reductase with methotrexate at active site

Perform correlation analyses of compound data against target, eg
- Receptor binding characteristics
- Enzyme inhibition
Using molecular modelling techniques
Hopefully find a class or classes over represented compared with chance
Screen those in detail

Aromatase inhibition

The current situation
A few TCM-derived products in trial
One or two approved in the US
But these are principally fractions
- not really the whole medicine
Summary

We have improved tools now

We have better tools now

We just have to do the work properly!!

Thank you for your attention

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