Toxicological assessment of phytopharmaceutics

Olavi Pelkonen
Department of Pharmacology and Toxicology
University of Oulu, Finland
Co-opted member of HMPC, EMEA
olavi.pelkonen@oulu.fi

Topics of the talk

• What are phytopharmaceuticals?
• Safety and efficacy?
• Regulatory position in EU and elsewhere
• Guidelines for safety assessment
• Genotoxicity testing
• Other delayed toxicities
• Interactions
• Considerations and problems
Plant-derived preparations with health claims

- Folk Medicines
- Herbal Medicines
- Phytomedicines/pharmaceutics
- Chinese Herbal Medicines
- Ayurvedic medicines

- Food (botanical) supplements
- Functional foods
Characteristics of phytopharmaceuticals

- Complex plant-derived mixtures
- Variable and often unknown composition of products
  - Seasonal and geographical variations
  - Plant varieties
  - Unknown active ingredients
  - Identification may also be uncertain
  - Manufacturing and storage variations
- Variable formulations and dosage recommendations
Limited information from a purely chemical specification

Ginkgo-Extract

50 : 1

Herbal substance not present in the extract 98%

Ginkgolides 3%
Flavonoids 25%
Bilobalid 3%
Extract not specified 69%

Extract 2%
Complementary and Alternative Medicines (CAM)

- Traditional herbal medicines – one form of CAM implicating significant historical use
- Healthcare needs for a large proportion of the population in many developing countries rely on
  - traditional practitioners
  - their armamentarium of medicinal plants
- Myriads of traditions (Western, Chinese, Indian, African, Caribbean etc)
Efficacy and safety of herbal medicines - claims

- **Efficacy**: the whole preparation is ‘the active principle’
- **It is not possible to assign the therapeutic effect to any single constituent**
- **Safety**: because of a long experience, herbal medicines are of proven safety
- **Because herbal preparations are from natural sources, they are safe**
Efficacy of herbal medicines

- **Primary scientific literature:** scientifically rigorous evidence is often lacking
- **Meta-analyses:** very little evidence of efficacy
- **Preclinical research:** a large number of biological effects, but usually at very high concentrations and doses
- **Individual components:** voluminous literature, but how to translate it into the actual complex mixture
Safety of herbal medicines

• **Past:** herbs have been used as ‘clandestine’ poisons and abortifacients

• **Numerous botanicals have been developed into ‘ordinary’ medicines (digoxin, atropine, morphine etc)**

• **Present:** many examples of harmful and/or potent effects
Mechanismen toxischer Wirkungen/Angriffspunkte von Pflanzenstoffen

- Furocumarine → Phototoxizität
- Johanniskraut → Metabolisierung von Fremdstoffen
- Cumarin, Kavalaktone → Lebertoxizität
- Cyanogene Glykoside → Zellatmung

(Eisenbrand 2007)
Mechanismen toxischer Wirkungen/Angriffspunkte von Pflanzenstoffen

Ephedrin Alk.: adrenerge Rezeptoren

Solanin, Chaconin
Cholinesterasehemmung

α-Thujon
GABA – R-Antagonist

Morphin
ZNS

Neurotoxizität

(Eisenbrand 2007)
Mechanismen toxischer Wirkungen/Angriffspunkte von Pflanzenstoffen

Hormonsystem

Glycyrrhizin
mineralokortikoide Wirkung

Isoflavone
estrogene Wirkung, Signalling, DNA

Genistein

(Eisenbrand 2007)
Mechanismen toxischer Wirkungen/Angriffspunkte von Pflanzenstoffen

Aristolochiasäure

Genotoxizität/Kanzerogenität

Safrol

Pyrrolizidinalkaloide

Methyloegenol, Estragol

(Eisenbrand 2007)
Phytomedicines - what are the issues we should focus on?

- Are they therapeutic agents?
- Are they foods or food supplements?
- Should they be classified as drugs?
- If so what issues should be considered?
  - Safety, efficacy, interaction potential etc
  - Tradition, experience, cultural factors etc
- Should they be regulated?
- How are they being regulated?
Regulatory positions

- Western “modern” positions
  - USA: dietary supplements
  - EU: medicinal products
  - Australia: risk-based 2-tiered approach

- Chinese Traditional Medicine

- Ayurvedic medicine
Are herbal medicinal products drugs in EU?

• According to Community legislation: Yes, they are legally, but..
• They are in a special status in terms of testing requirements
• Test and trial results can partially be replaced by appropriate scientific literature
• Which kind of scientific literature?
Marketing Authorisation

Pharmacovigilance

Consumer information; labeling; advertising

Efficacy
- new trials
- bibliographic

Safety
- new tests
- bibliographic

Quality Control

Good Manufacturing Practices

Good Agricultural and Collection Practices

Registration

Applies to registered and to authorized HMP

May be replaced by a monograph or the list from the HMPC in registrations

Identical for marketing authorizations and registrations

new
well-established
traditional
Marketing Authorization (bibliographic)

- **2001/83/EC as amended by CD 2004/27/EC**
- **Article 10a**
- ...
- By way of derogation from Article 8(3)(i), … the applicant shall not be required to provide the results of pre-clinical tests or clinical trials if he can demonstrate that the active substances of the medicinal product have been in well-established medicinal use within the Community for at least ten years, with recognized efficacy and an acceptable level of safety in terms of the conditions set out in the Annex.

- In that event, the test and trial results shall be replaced by appropriate scientific literature.
Bibliographical Sources for Assessment reports

• ESCOP Monographs (European Scientific Cooperative on Phytotherapy)
• WHO Monographs
• European Pharmacopoeia
• Other Pharmacopoeias
• Any Published Literature
• other information available to national competent authorities. e.g. pharmacovigilance data, studies etc.
Monographs for Herbal Medicinal Products

Community herbal monograph on Valerian root:
*Well-established use (marketing authorisation)*

**Preparations:**
Extract prepared with water, ethanol/water (ethanol max. 70 % V/V); Tinctures (1:5, ethanol max. 70 % V/V)

**Indication:**
Herbal medicinal product for the relief of mild nervous tension and difficulty in falling asleep (non-organic insomnia according to ICD-10, F51.0).

**Posology:**
Adolescents over 12 years of age, adults, elderly. Single dose: Tincture (1:5, ethanol max. 70 % V/V) or extracts with water, ethanol/water (max. 70 % V/V) equivalent to 2 to 3 g of the drug.
EU List for Traditional Herbal Substances

List entry for Valerian root:

*Traditional use registration*

Preparations covered:
- Dried Valerian root, Fresh plant juice, Valerian root oil

Indication:
- Traditional herbal medicinal product for support of mental relaxation and normal sleep exclusively based on long-standing use.

Posology:
- Single dose: 0.3 to 1 g dried Valerian root, 15 ml of fresh plant juice, 15 mg of Valerian root oil. …
EMEA/HMPC (Committee for Herbal Medicinal Products)

- First Meeting on September 23, 2004
- Chair: Konstantin Keller (Germany)
- Vice chair: Ioanna Chinou (Greece)
- Meetings at EMEA 6 per year for 3 to 4 days
- 25 delegates and 25 alternates
- Members from Norway and Iceland; observers from e.g. Turkey, Croatia etc
- + up to 5 additional co-opted members
- Working Group of Monographs and Lists, Drafting Groups for Organizational matters and Quality
HMPC Guidelines concerning Safety & Efficacy

- Guideline on non-clinical documentation for well-established and traditional herbal medicinal products
- Guideline on assessment of clinical safety and efficacy for well-established and traditional herbal medicinal products
- Guideline on clinical assessment of fixed combinations of herbal substances / herbal preparations
Non-clinical documentation of well-established herbal medicinal preparations

• Comprehensiveness and quality of evidence
• Tests not required, if **sufficient experience in humans is available**:
  • single dose toxicity,
  • repeated dose toxicity,
  • immunotoxicity
  • local tolerance testing
  • pharmacological tests including safety pharmacology,
  • pharmacokinetic studies.
Safety concerns of "old" substances

• Old substances are widely used, but there are few formal toxicological studies
• Very little incentives to make such studies; no protection of data and intellectual property
• Animal experiments are difficult to justify, if only the "completeness of dossier" is the goal
• What is the value of past experience?
Possible solutions to safety concerns of "old" substances

• Require formal studies (as pharmaceuticals; check-box approach)

• Accept common experience and/or past ‘pharmacovigilance’ data for safety assessment (if no serious concerns)

• Mixed approach depending on a substance/preparation and specific toxicity

• What creates "cause for concern"

Herbal drugs with serious risks without any accepted benefit
(Not acceptable for revision)

Aconitum all species
parts: all parts
reason: contains aconitine and other toxic alkaloids, benefit not proven.

Angelica archangelica L.
parts: fruit, herb
reason: contains phototoxic furanocumarins, benefit not proven.

Aristolochia all species
parts: all parts
reason: contains aristolochic acids, strong carcinogen, genotoxicity, benefit not proven.

Artemisia cinerea (BERG.) WILKCOMM.
parts: Flower-bud
reason: contains the toxic lactone santonin, benefit/risk negative.

Berberis vulgaris L.
parts: bark, root bark, root
reason: contains the alkaloid berberine.

Borago officinalis
parts: herb, flowers
reason: contains pyrrolizidine-alkaloids with genotoxic, carcinogenic and hepatotoxic properties.

Bryonia all species
parts: root
reason: cytotoxic cucurbitacines, drastic laxative and emetic.

Chenopodium ambrosioides L. var. anthelminticum (L.) A.GRAY
parts: essential oil
reason: contains the toxic principle ascaridole, benefit/risk negative.

Chrysanthemum vulgare (L.) BERNH.
parts: flower, herb
reason: may contain essential oil with the neurotoxic thujone.

Citrus colocynthis (L.) SCHRADER.
parts: fruit
reason: contains cytotoxic cucurbitacines, drastic laxative.

Claviceps purpurea (FR.) TULASNE
parts: Secale cornutum (Sclerotium)
reason: contains toxic ergot-alkaloids, benefit/risk negative.

Convolvulus scammonia L.
parts: resin
reason: drastic laxative with irritant properties.
Pharmacovigilance/Safety - examples

- soya or peanut protein
- Capsicum / capsaicin
- Chamomilla
- Aristolochia species
- estragole
- methyleugenol
- pulegone and menthofuran
- asarone
- black cohosh

http://www.emea.eu.int/htms/human/hmpc/hmpcguide.htm
Problem issues in safety assessment of "old" substances

• Long-term use = Safety (to a certain extent)
• Certain forms of toxicity not easy (not possible) to recognize in daily use or via pharmacovigilance
  – Genotoxicity/mutagenicity
  – Carcinogenicity
  – Reproductive toxicity
• Potential interactions with pharmaceuticals
Safety problems in genotoxicity and mutagenicity

• A long lag period to detect the outcome (if any and if at all)
  – Germ cells - generations
  – Somatic cells – decades (carcinogenicity)
  – Embryo cells – next generation

• Exposure to multiple chemicals
• Hazard detection by preclinical tests
• Risk assessment?
## Mutagenicity/Genotoxicity

Table 3.7.1: Methods currently available for genotoxicity/mutagenicity testing

<table>
<thead>
<tr>
<th>Annex V EC</th>
<th>OECD TG</th>
<th>Name of test</th>
<th>Endpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vitro</strong></td>
<td></td>
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<td></td>
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<tr>
<td>B.13–14</td>
<td>471</td>
<td>Bacterial reverse mutation test (Ames test)</td>
<td>Gene mutations in bacteria</td>
</tr>
<tr>
<td>B.10</td>
<td>473</td>
<td>Mammalian chromosome aberration test</td>
<td>Chromosome aberrations</td>
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<tr>
<td>B.17</td>
<td>476</td>
<td>Mammalian cell gene mutation test (mouse lymphoma test)</td>
<td>Gene mutations</td>
</tr>
<tr>
<td>B.19</td>
<td>479</td>
<td>Sister chromatid exchange assay in mammalian cells (SCE)</td>
<td>Mammalian DNA damage</td>
</tr>
<tr>
<td>B.15</td>
<td>480</td>
<td><em>Saccharomyces cerevisiae</em> gene mutation assay</td>
<td>Gene mutations in yeast</td>
</tr>
<tr>
<td>B.16</td>
<td>481</td>
<td><em>Saccharomyces cerevisiae</em> mitotic recombination assay</td>
<td>Recombination in yeast</td>
</tr>
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<td>B.18</td>
<td>482</td>
<td>Unscheduled DNA synthesis (UDS) in mammalian cells</td>
<td>Mammalian DNA damage in liver cells</td>
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<tr>
<td><strong>In vivo</strong></td>
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<tr>
<td>B.12</td>
<td>474</td>
<td>Mammalian erythrocyte micronucleus test</td>
<td>Structural and numerical chromosome aberrations in somatic cells</td>
</tr>
<tr>
<td>B.11</td>
<td>475</td>
<td>Mammalian bone-marrow chromosome aberration test</td>
<td>Chromosome aberrations</td>
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<tr>
<td>B.20</td>
<td>477</td>
<td>Sex-linked recessive lethal test in <em>Drosophila melanogaster</em></td>
<td>Gene mutations in germ line</td>
</tr>
<tr>
<td>B.22</td>
<td>478</td>
<td>Rodent dominant lethal test</td>
<td>Chromosome aberrations and/or gene mutations in germinal tissue</td>
</tr>
<tr>
<td>B.23</td>
<td>483</td>
<td>Mammalian spermatogonial chromosome aberration test</td>
<td>Inheritable chromosome aberrations</td>
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<tr>
<td>B.24</td>
<td>484</td>
<td>Mouse spot test</td>
<td>Mutagenicity in fetal cells</td>
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<tr>
<td>B.25</td>
<td>485</td>
<td>Mouse heritable translocation assay</td>
<td>Heritable chromosome aberrations</td>
</tr>
<tr>
<td>B.39</td>
<td>486</td>
<td>Unscheduled DNA synthesis (UDS) test with mammalian liver cells</td>
<td>Mammalian DNA damage in liver cells</td>
</tr>
</tbody>
</table>
Genotoxicity testing of herbal medicinal products

• The "full set" of ICH/OECD genotoxicity tests versus a reduced set of selected tests?

• What is the absolute minimum for acceptance of a registration/marketing authorisation of old herbal substances

• In current practice, no agency is asking for a full genotoxicity testing programme
Guideline on non-clinical documentation for well-established and traditional herbal medicinal products (adopted 2006)

- In this guidance a step-wise procedure for assessing genotoxicity of herbal medicinal products was established. The basic requirement is to assess genotoxicity initially in a bacterial reverse mutation test using a test battery of different bacterial strains and metabolic activation. If positive results cannot be clearly attributed to specific constituents with a well-established safety-profile for example quercetin, additional in vitro, e.g. mouse lymphoma cell assay, and, if necessary, in vivo studies should be performed.
Guideline on the assessment of genotoxicity of herbal substances/preparations

- Adoption 2008
- A step-wise procedure
  - a bacterial reverse mutation test (Ames)
  - mouse lymphoma cell assay
  - \textit{in vivo} studies (mouse micronucleus test)
- Risk assessment problematic
  - ALARA, MOE, Uncertainty factors, TTC
Problems in genotoxicity testing of herbal medicinal preparations

- Complex and variable mixtures
  - Current tests not necessarily suitable
  - Matrix effects, interactions, etc
  - Unspecific effects on bacteria or cells
- Trace mutagenic compounds (known or unknown)
  - quercetin
- Differences between preparation and internal exposure
- Extrapolation from one formulation to another
Angelica archangelica

- resistance to bacterial infection (trad.)
- efficacy – no evidence
- safety – furocoumarins
- Interactions – no data
Problems with risk assessment of Angelica archangelica

- Furocoumarins – phototoxic, photogenotoxic, photocarcinogenic
  - carcinogenicity 8-MOP class 1 (IARC)
  - PUVA therapy (30 mg/dose ~200 doses)
- Dietary exposure (celery etc)
  - average 1.45 mg/day, up to 14-15 mg/day
- Exposure via diet and herbal preparation
  - matrix effects
  - difference in exposure pattern
Safety problems in carcinogenicity

• A long lag period to detect the outcome (if any)
  – Usually 20-40 years
  – Several mechanisms of action (genotoxicity is only one albeit an important one)
  – Requires multiple “hits”

• Exposure to multiple chemicals

• Testing problematic
Carcinogenicity testing

• In vivo carcinogenicity tests (24-month rat study, 18-month mouse study)
• TG or KO strains (short-term tests)
• Colony forming assays (transformation assays)
• Gap junction inhibition assays
• Justified for herbal medicines?
Carcinogenicity – some considerations in non-clinical guideline (4.4.)

- Is the suspicion based on results of genotoxicity studies and can it be clarified in further genotoxicity studies, mainly *in vivo*?
- Is the suspicion based on a possible epi-genetic mechanism?
- Are the extent and the quality of the available scientific data (non-clinical, clinical, epidemiological, post-marketing etc.) sufficient to refute the suspicion taking into account the intended use?
- Are the extent and the quality of the available scientific data (non-clinical, clinical, epidemiological, post-marketing etc.) sufficient to come to a positive benefit-risk assessment taken into account the expected benefit from the herbal medicinal product?
Safety problems in reproductive toxicity

- Teratogenicity - the outcome manifests after birth or later
  - Different sensitivity of the target
  - Constantly changing target
  - Damage often irreversible
- Fertility – infertility difficult to detect
- Testing
Reproductive toxicity testing

- Teratogenicity tests (rodent, non-rodent)
- 3-generation reproductive toxicity test
- Perinatal toxicity test
- Developmental neurotoxicity test
- (Behavioural teratogenicity test)
Toxicity to Reproduction

• 4.2. …Reproductive toxicological tests in animals are not necessary if one of the following criteria is fulfilled:

  – Results from post-marketing studies or epidemiological data of adequate power or post-marketing safety studies are available.
  – The assessment of the results of a systematic and comprehensive scientific literature search and post-marketing experience does not identify a positive signal of reproductive toxicity and the herbal medicinal product is not intended to be used during pregnancy and lactation.
  – Results from investigations in pregnant women and neonates are present.
  – The medicinal product is not intended to be used in women of childbearing potential.
St. John’s wort (hypericum perforatum)

- Antidepressant (mild to moderate)
- e.g. hyperforin, hypericin, flavanols
- Efficacy – up to SSRIs?
- Safety – problems of interactions with drugs
StJW: Structures of Biological Interest

Rutin

Hypericin

Hyperforin
St John’s wort

- Continuous intake increases the expression of intestinal (and hepatic)
  - P-glycoprotein (PGP)
  - CYP3A4
- The combined up-regulation of PGP and intestinal/hepatic CYP3A4
  - Impairs absorption of cyclosporin
  - Stimulates metabolism of cyclosporin
- Subtherapeutic plasma levels of cyclosporin
Safety assessment of herbals – problem topics

- Long experience vs formal toxicity studies
- What creates cause for concern (signals)
- Retroactive vs proactive testing
- Hazard detection vs risk assessment
- Genotoxicity: testing of complex mixtures
- Carcinogenicity: threshold for toxicological concern (TTC) vs other types of risk assessment
- Interactions: in vitro testing of complex mixtures
Phytopharmaceutics –
take-home messages

- Drugs – and still not ‘real’ drugs
- Benefit (efficacy) - risk (safety) considerations
- Tradition, experience vs science
- Comprehensive bibliographical evidence – question of quality
- Other traditions
- Scylla vs Kharybdis: Boundaries between foods, herbals and pharmaceuticals
- Problems in testing complex mixtures
Botanical Health Products

- Food
- Food Supplement
- Cosmetics
- Medical Devices
- Medicinal Products