Good Practice in Traditional Chinese Medicine Research in the Post-genomic Era

GP-TCM

223154

D2.7

Report on findings from workshop including minimum acceptable standards for extract preparation, purification and component analysis

Document description	
Name of document	Report on findings from workshop including minimum acceptable standards for extract preparation, purification and component analysis
Abstract	The 2nd Meeting of WP2 was hold as a joint meeting with WP1 on the 19th of April 2010 in Schloss Mickeln, Düsseldorf, Germany. The work shop's outcomes concerning techniques and standards employed for the extract preparation in TCM and a concept for the application of minimum acceptable standards are described.
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1 REPORT ON WORKSHOP

1.1 General

The Phase-II-Meeting of WP2 was planned as a joint meeting with WP1 on 18th and 19th of April 2010 in Schloss Mickeln, Düsseldorf. The meeting was hosted by Prof. Dr. Peter Proksch.

1.2 Workshop Program

Table 1 – Scheduled Workshop Program

Good Practice in Traditional Chinese Medicine Research in the Post-genomic Era

Workshop Düsseldorf/Germany

18th of April 2010 to 20th of April 2010

WP1 and WP2

Meeting location (Address for Taxi drivers (~40€ from Düsseldorf airport; ~20€ from Düsseldorf mainstation): Please don't forget to collect all your travel bills and receipts. Your travel costs will be refunded based on your collected bills

Schloss Mickeln
Alt Himmelgeist 25
40589 Düsseldorf

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Program

Sunday, 18th of April 2010
From 12.00 – check-in Schloss Mickeln
Up to 5.30 p.m. – Arrival in Schloss Mickeln
6.00 p.m. Dinner nearby Schloss Mickeln
(Haus Crevet, Steinkaul 3, ca. 50 m from Mickeln)
Topics:
First contact and session warm up

Monday 19th of April 2010

8.00 a.m. – 9.00 a.m.:
Breakfast

9.00 – 11.00 a.m. Session 1 (D2.7):
Salutory speech
Presentation of Prof. Dr. B. Kopp
Presentation of PD Dr. W. Knöß

11.00 a.m. – 11.15 a.m. coffee break

11.15 a.m. – 1.00 p.m. Session 2 (D2.5):
Presentation of Prof. Dr. I. Sutherland
Technology for the Extraction of Pharmaceuticals (STEP)
Discussion and Workshop:
Generation of a collection of TCM herb relevant extraction/analytical methods

1.00 p.m. – 2.00 p.m. lunch break

2.00 p.m. – 3.30 p.m. Session 3 (D1.8, D2.5, D2.10...):
Discussion and Workshop (Simmonds):
TCM Plant list
Selection criteria, availability of voucher collections

3.30 p.m. – 3.45 p.m. coffee break

3.45 p.m. – 5.00 p.m. Session 4 (D1.6, D2.7, D2.10):
Discussion and Workshop (Krenn):
Generation of a monograph draft considering minimum acceptable standards and data base design (What data need to be collected for later deliverables e.g. D.10?)

5.00 p.m. – 6.00 p.m. break

6.00 p.m. Dinner nearby Schloss Mickeln

Tuesday 20th of April 2010

8.00 a.m. – 9.00 a.m.:
Breakfast

9.00 – 11.00 a.m. Session 5:
Voting and further discussion of Session 3 and 4 topics

11.00 a.m. – 11.15 a.m. coffee break

11.15 a.m. – 1.00 p.m. Session 6:
Voting and further discussion minimum exactable standards for D2.7

1.00 p.m. End and lunch break

2.30 p.m. check-out Schloss Mickeln

1.3 List of expected Participants

1.3.1 Participants involved in WP1

Youping Zhu (Netherlands)
Rob Verpoorte (Netherlands)
Pierre Duez (Belgium)
Ian Sutherland (UK)
Svetlana Ignatova (UK)
Michael Heinrich (UK)

1.3.2 Participants involved in WP2

Proksch, Peter (Germany)
Bayer, Mirko (Germany)
Sendker, Jandirk (Germany)
Werner Knöss (Germany)
Friederike Stolte (Germany)
Kirsten Knapp (Germany)
Liselotte Krenn (Austria)
Helen Sheridan (Ireland)

Annika Orland Germany (Germany)

1.3.3 Participants involved in WP1 and WP2

Brigitte Kopp (Austria)
Monique Simmonds (UK)
Dr. Dong (China)
Hewei Li (China)

Figure 1: Work shop participants in front of Mickeln Manor

2 PROGRESS

2.1 Session 1

The session began with a welcome speech of the host Prof. Dr. Peter Proksch followed by a brief report about the status and recent activities of WP2 given by Dr. Jandirk Sendker. Lectures by Prof. Dr. Brigitte Kopp and PD Dr. Werner Knoess were given successively about the processing and extraction of Traditional Chinese Medicine (TCM) herbal drugs and the existing European regulatory framework concerning herbal medicinal products, respectively. Both lectures were followed by discussions which were kept very brief at this point but were resumed in the forthcoming sessions which were scheduled as round table discussions. The subjects of the brief discussions of Session 1 are reported together with the subjects of the later sessions.

2.2 Session 2

Session 2 started with a lecture of Prof. Dr. Ian Sutherland and Dr. Svetlana Ignatova about the principles and application of Counter Current Chromatography (CCC). The discussion afterwards issued the potential of CCC for solving WP1 and WP2 related problems, e.g. for the yield of reference substances for quality control or for quality control itself.

After this last lecture, the round table discussion was opened. The first scheduled objective was to discuss extraction methods used in TCM and TCM-specific post-harvest-treatments and drug processing methods (*pao zhi*).
The discussion yielded the conclusions stated below:

- Besides aqueous decoctions and medicinal wines which represent the most common extraction methods in TCM, more lipophilic extracts of herbal drugs are used by minorities which employ oil, wax, butter or milk. As these methods are seldom used they will not be focused by WP2.
- Drug processing methods (*pao zhi* e.g. frying, steaming, roasting) which are applied to raw drugs in order to produce medicinal drugs are most likely expected to influence the chemical composition. The decomposition of the ester alkaloids of Chinese Aconite drugs had been given as an example by Prof. Dr. Kopp.
- The question about a defined end-point of drug processing methods has been raised. Dr. Dong reported that “masters” would control the processes due to personal experience by sense e.g. by evaluating viscosity of continuous color changes occurring during the process.
- Another TCM-specific procedure is the remoistening of raw drugs before cutting and further procession. The remoistening of once dried plant material is known to rapidly boost pathological reactions that may again influence the chemistry (e.g. reactivation of hydrolytic or ROS generating enzymes).
- European legislative would require a complete process definition from the recent plant to the ready-to-use medicinal product including quality control procedures of relevant biomarkers for every process step (e.g. harvest, drying, remoistening, *pao zhi*, extraction, formulation, see Ref. 1 for further information).
- Accordingly, the preparation of CHM includes numerous steps that have the potential to influence a product’s quality. Critical points of post-harvest-treatments and drug processing (endpoint?) should be identified. This issue apparently represents a gap of knowledge for many CHM that can be addressed by modern techniques of hyphenated analytical and statistical technologies. However, the new Chinese Pharmacopeia is going to address a number of these issues. Its English translation is oncoming.

2.3 Session 3

The scheduled objective of session 3 was to identify criteria for the selection of a small number of priority plant species from a comprehensive species list prepared by WP1 that engulfs any species of particular interest within the consortium. A detailed literature research will exemplarily be undertaken on the priority list species in order to identify typical gaps of knowledge from a European point of view.

The below-stated criteria were raised for discussion:

- Known Chemistry
- Plants which are used in activity studies in other WPs
- Plants for which processing appears to be an important factor
- Not endangered species
- Known for high chemical variability
- Different plant parts involved
- Toxic plants of which the active constituent is not known
- Plants already used in Europe
- High variability of preparations on the European market
- Active plants with unknown mode of action
- Many possible substituents known
- A plant selection that embraces a wide chemical variety

Finally, the participants agreed to use three priority criteria as follows:

1. Plants which are used in activity studies in other WPs
2. Plants for which processing appears to be an important factor and may produce variability in the final products
3. A plant selection that embraces a wide chemical variety

The above mentioned WP2 specific criteria were selected because *(i)* it was agreed that the most important criteria for a consortium-wide species list would be clinical and toxicological relevance which is dealt with by WP3 and WP6 (criterion 1), *(ii)* the postharvest processing and *pao zhi* methods are a major difference to European phytopharmacy and significant changes in the herbal metabolite profile's can be expected due to this procedures (criterion 2). *(iii)* it is reasonable to look at different classes of compounds as they will variably be affected by postharvest treatments and *pao zhi* (e.g. glycosidic compounds, alkaloids, essential oils…).

2.4 Session 4

This session was used to draft a comprehensive standardized from that should *(i)* aid the data collection for the priority species to be selected and *(ii)* help to identify issues that require the application of Minimum Acceptable Standards. Prof. Dr. Krenn had provided a preliminary version which was presented for discussion. The result of this Session is reported as part of 3 Minimum acceptable standards.

2.5 Problems

Due to the eruption of the volcano Eyjafjallajökull on Iceland any air transport was impossible around the time of the meeting. Also train and Eurotunnel transport was problematic because of short capacities. Though amazingly 17 of 19 expected attendees managed to come, the organisators suggested skipping the program of the second day so that the participants who were partly uncertain about their return trip would be able to return as previously planned. The subjects of Sessions 5 and 6 which were meant to state decisions were successfully included in the Sessions 1 to 4.

3 MINIMUM ACCEPTABLE STANDARDS

3.1 General

The "Minimum Acceptable Standards" to be defined by WP2 are meant to work as an internal tool that will help to identify gaps of knowledge that can later be recommended for future research projects. "Minimum acceptable standards" can be applied to processes (in the sense of e.g. ICH guidelines for the validation of analytical methods) which is of minor interest for this project, or to knowledge that should be present for certain aspects of the production chain (e.g.: How are relevant secondary metabolites affected by postharvest treatment or *pao zhi*?). Existing standards and requirements will be consulted whenever possible. Important documents in this context would be e.g. guidelines about quality control of herbal medicinal products or the ICH guidelines for the validation of analytical procedures. An overview about existing relevant documents of the European regulatory framework is given in 3.2 European regulatory framework for Herbal Medicinal Products.
As some TCM-specific procedures are not specifically covered by existing European standards, the participants drafted a monograph layout (3.3 Monograph draft) that should *(i)* aid the data collection for the priority species to be selected and *(ii)* help to identify issues that require the application of Minimum Acceptable Standards. This draft will in its final version be used for D2.10 and related WP2 deliverables that will be used for the evaluation of D2.10. The draft will successively be refined.

3.2 European regulatory framework for Herbal Medicinal Products

In the European Union there is a legal regulatory framework which is addressing the requirements for marketing authorisations or registrations for herbal medicinal products and traditional herbal medicinal products. In article 1 No. 31 of Directive 2001/83 EC of the European Parliament and of the Council of 6 November 2001 amended by Directive

2004/24/EC of the European Parliament and of the Council of 31 March 2004 there are definitions for herbal medicinal products, herbal substances and herbal preparations. The criteria to define traditional herbal medicinal products are laid down in article 16 of this directive. These criteria have been established primarily to harmonise herbal products in medicinal use in the different member states. Consequently, the particular characteristics of traditional Chinese medicines are not fully covered by this framework.

Concerning quality the current requirements in the European Union are described by some guidance documents issued by the Committee on Herbal Medicinal Products (HMPC) at the European Medicines Agency (EMA) in London. The most important guidelines are:

- Guideline on Quality of Herbal Medicinal Products/ Traditional Herbal Medicinal Products (CPMP/QWP/2819/00 Rev. 1)
- Guideline on Specifications: Test procedures and Acceptance Criteria for Herbal Drugs, Herbal Drug Preparations and Herbal Medicinal Products/Traditional Herbal Medicinal Products (CPMP/QWP/2820/00 Rev. 1)
- Guideline on Quality of Combination Herbal Medicinal Products/Traditional Herbal Medicinal Products (EMEA/HMPC/CHMP/CVMP/214869/2006)
- Guideline on Good Agricultural and Collection Practice (GACP) for Starting Materials of Herbal Origin (EMEA/HMPC/246816/2005)
- Guideline on Declaration of Herbal Substances and Herbal Preparations in Herbal Medicinal Products/ Traditional Herbal Medicinal Products in the SPC" (EMEA/HMPC/287539/2005)

Further quality standards are defined by the validated methods described in the European Pharmacopeia and the monographs on herbal drugs and herbal drug preparations. The methods include analytical techniques like TLC, MS, GC, HPLC and assays on impurities, heavy metals, pesticides, aflatoxins etc.. A process of introducing herbal substances from Traditional Chinese Medicine into the European Pharmacopeia has been started. This development will contribute to further harmonisation of standards in future.

Based on the existing and accepted techniques to characterise herbal substances and herbal preparations it is challenging to use new and complementary techniques. Molecular fingerprinting, metabolomics, mathematical including multi-variate analysis, new analytical techniques and hyphenated techniques (LC-MS, LC-NMR…) will improve the knowledge on medicinal plants and give new insights in their therapeutical benefit. For the characterisation of Chinese herbal medicines well established methods as well as more sophisticated techniques that provide complex data suited for multi-variate data analysis (LC-MS, NMR) should be used.

3.3 Monograph draft

- General
  - Plant species
    - grading system for quality
    - Voucher?
  - Pinyin drug name, way of processing
  - Latin drug name
  - DNA-Identification
    - Adulterations?
  - NIR-Identification
- Reference
  - Bibliographic data
  - Language
  - Fulltext/Translation available?
- Pretreatment, Processing
  - Purpose
    - Chemical rationale
    - Etc.

- Ingredients
- Methods
- Parameters of methods
  - Critical points
- [Batch size]
- Result
- Grading of information

- Extraction
  - Herbal substance
    - postharvest treatment, remoisturing (effects?)
    - particle size of drug
  - Solvent
    - Type
    - Composition, quality
  - Drug-extract-ratio
  - Parameters of extraction
    - Batch size
    - method of grinding
    - Drug-Solvent-Ratio
    - Mode (ASE, sonification, reflux etc.)
      - Temperature, Pressure, Duration, pH, ionization
      - Conditions (steady-state, exhaustive, flow rate etc.)
    - Filtration etc.
- Preparation of processed material
  - Purpose
  - Method
  - Parameters of method
  - [Batch size]
  - Result
- Preparation of dry/soft/fluid extracts
  - Method of drying/concentration:
  - Mode (lyophilization, evaporation, spray drying etc.)
  - Excipients
  - Parameters of drying/concentration:
    - Batch size
    - Temperature
    - Pressure
    - Duration
- Purification of extracts
  - Enrichment of compounds with biological activity:
    - Purpose
    - Class of compounds (CAS no.)
    - Method
    - Parameters of method
    - Result
  - Removal of undesirable accompanying compounds:
    - Purpose
    - Class of compounds (CAS no.)
    - Method
    - Parameters of method
    - Result
- Component Analysis
  - Purpose (fingerprinting, quantification)
  - Kind of compounds
    - categorization of compounds (CAS, concentration)
    - undesired compounds due to adulteration etc.
  - Sample preparation

- Analytical method
- Parameters of method
  - Details
- Validation
- Results
- “Quality of method” ICH guideline

- Other Aspects

4 REFERENCES

[1] Groot MJ; van der Roest J (2006) Quality control in the production chain of herbal products. In: Bogers RJ; Craker LE; Lange D (eds.) Medicinal and Aromatic Plants, 253-260.