Combination screening of synthetic drugs and plant derived natural products
Potential and challenges for drug development

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Comparison of Treatment Approaches of traditional phytomedicine and conventional medicine

Phytopharmaceuticals

- Multi Component Mixtures
- Multitargeting
- Synergy

Synthetic Drugs

- Monosubstances (chronic Diseases, Autoimmunity, Cancer, HIV)
- Poly-Pharmacology

„Polypill“: Prevention of Cardiovascular Disorders
(Eur HeartJ 2011, Science 2011)
Multimodal Therapy Concept for the Treatment of acquired Hemophilia

Rare Autoimmune Disease (Incidence: 1-4*10^6)
Development of Antibodies against Clotting Factor VIII
Life-threatening Bleedings, Mortality up to 22%

Modified Bonn-Malmö Protocol

Inhibitor and Factor VIII Concentrations in the Course of Therapy

Result: lasting Remission in 92% of all Cases

Zeitler, Ulrich-Merzenich et al. Blood 2005
Zeitler, Ulrich-Merzenich et al, Atherosclerosis Suppl 2009
Zeitler, Ulrich-Merzenich et al. Atherosclerosis Suppl 2013
Why are we looking for Synergistic Effects?

- Address multiple Targets
- Dose Reduction
- Reduction of Adverse Events
- Sensitisation of drug resistant cells
- Assessment of Synergy

How does it work?

- Gene expression profiles?
- Cytokine modulations?
- Signal Cascade modulation?
Assessments of Synergy

1- Isobologram analysis (Dose-oriented)

2- Combination index (Effect-oriented)
antagonism = negative interaction
synergism = positive interaction or potentiation
zero-interaction = effects-addition of individual components

IC<sub>50</sub> – values for various dose-combinations of PAF-induced thrombocyte aggregation*

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Wagner et al. 2001
Wagner, Ulrich-Merzenich et al. Phytomedicine 2009
Challenges in Synergy Screening

According to Chou T.C 2013

Phytopharm, 23rd July 2015, Bonn, Germany
The Dose-Effect Curve:

\[ m = 1, 3, 5; D_m = 1 \]

\[ m = 1; D_m = 0.5, 1, 2, 4, 6, 8, 16 \]

The Median-Effect Plot: (Chou Plot)

Computer simulation: CompuSyn generated dose-effect curves with their corresponding median-effect plots, based on the median-effect equation. CompuSyn software has been used.

Source: Chou TC, 2013
Assessments of Synergy

3 – Sensitisation factor
Measures the increase in the sensitivity of cancer cells to a certain drug after combination.

4 – Dose reduction index (DRI)
Measures how much the dose of each drug in a combination may be reduced at a given effect level compared with the doses of each drug alone.
Example:
Cannabis extract is a better antispastic agent then THC

Fig. 4. Cannabis extract is a better antispastic agent than tetrahydrocannabinol at an equivalent dose (Baker et al. 2000; Williamson E. (2001) with permission).
SD-Rats
Treatment: 14 days p.o.

- **Group A (n=12):** Total extract (TS) (willow) (30 mg/kg)
- **Group B (n=12):** Ethylacetat fraction (30, 11.3, 9 mg/kg)
- **Group C (n=12):** n-Butanol fraction (30, 16, 7.5 mg/kg)
- **Group D (n=12):** Ethanol fraction (30, 15, 9 mg/kg)
- **Group E (n=12):** Water fraction (30, 6.8, 1.5 mg/kg)
- **Group F (n=12):** Imipramin (20 mg/kg)
- **Group G (n=12):** Control (0 mg/kg)

**Gene Expression Profiling:**
1. Collection of blood in PAX-Gene-tubes
2. Isolation of RNA from whole blood (Qiagen)
3. Use of Agilent whole Genome Rat Array

**Ulrich-Merzenich et al. Phytomedicine 2012**
Gene expression in responders: Data analysis

<table>
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<tr>
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<th>Willow bark</th>
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<td>Rec. IPA**</td>
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Non-linear relationship between the number of constituents of a drug preparation and the number of genetic targets modulated in a biological system

*Filter Criteria: > 2-fold / \( p \leq 0.01 \)

**IPA: Human, Mammal, Cell Culture, Organ, Organism
Core Analysis

Uploaded Data Set is compared to Data Base (IPA)

Relevant Functions, canonical Pathways, Networks associated with the Data Set are „returned“

Tox / Clinical Pathology Endpoint Analysis

Search in the Gene Expression Profile for

well studied Groups of Genes / Molecules which are known to participate in clinical Pathology and lead to toxicological Events or Processes with specific Tissues / Organs

Reduction of Adverse Events
**Gene expression profiling: „Adverse event“ gene clusters**

Combination K activates less gene clusters than its component B or the mono-substance imipramine.

Ulrich-Merzenich et al. Phytomedicine 2012
The Adverse Events Potential for Willow Bark is lower than the one for Imipramine at comparable Efficacy.

Can Multi Component Mixtures have a specific therapeutic Effect at a lower Adverse Events Profile?

Which Role does Synergy play?
Gene involvement

Gene expression profiling: numeric analysis

**Example:** a) 3 herbal extracts  b) their combination  c) 3 chem. mono-substances

- three combination partners

- neuroglia cells (TG98G) in vitro, gene expression analysis

- Uncovering of synergisms/antagonisms

- Study conducted at Institute of Pharmaceutical Biology, University of Mainz, Germany, Prof. Thomas Efferth

Panossian et al. 2013, Frontiers in Neuroscience 7, 1-17

From O. Kelber
1056 – 735 = 321 genes are influenced only by the combination of A, B and C → synergistic interactions

2188 – 735 = 1453 genes are not influenced by the combination of A, B and C → antagonistic interactions

1056 – 735 = 321 genes are influenced only by the combination of A, B and C → synergistic interactions
Gene expression profiling proposes:

- A combination is a new active substance (chemical combination or herbal combination).

- Benefit / Risk profile results from the synergistic and antagonistic effects of the components.

→ Chance for development of combinations with higher selectivity and better AE profile than mono substances

Panossian, A et al. Frontier Neuroscience 2013;7:1-17
Inflammation

Phytochemical:

Willow bark (Salix spec.)
Natural source and forerunner of aspirin (ASS)

Pharmacokinetics: 240 mg “Salicin” ≈ 87 mg ASS (Schmid et al. 2001)

Inhibition of Inflammation through the combination
Salicylates + Polyphenols + Flavonoids

(Kayyal et al., 2005, Nahrstedt et al. 2007, Bonnaterra et al. 2010, Freischmidt et al. 2012)
Agonism of salicylic acid and the polyphenol quercetin through cytokine networks (IL4-signaling)
P53 Signaling by the combination of salicylic acid and quercetin

Prepared with ToxWiz, Cambridge Cell Network
Network responses are not additive

Dosage dependent interaction of redundant, convergent and divergent signaling pathways

▶ System Biology

Antiinflammatory mode of action by salicylate containing Plant extracts:

Agonism of salicin and polyphenols through cytokine networks

Final goal: Network centric therapeutic approaches

combination of targets and modulators acting on different therapeutic areas to produce synergistic effects
Why are we looking for Synergistic Effects?

- Improved Efficacy
- Lower Drug Dosages
- Less Drug Toxicity
- Less Drug resistance

Definition of Synergy

How does it work? Search for non-additive

- gene expression profiles
- Cytokine modulations
- Signal Cascade modulations
From Decoctions with Genomics and Metabolomics to a better understanding of Synergy for a Rational Co-medication
Colleagues and Cooperation partners

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Thank You for Your Attention!

Bird’s Eye View

Institutes