PLANT EXTRACTS AS MODEL IN THE DEVELOPMENT OF METHODS FOR THE EVALUATION OF SYNERGISTIC EFFECTS

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Phytopharmaceuticals are complex mixtures and thus prototypes of multitarget interventions. The assessment of their mode of action is challenging. In combinations of different plant extracts, the challenge is even bigger. At the same time the rationale of combining different plant extracts is questioned. The common argument for the combinations is synergy, the elimination of adverse events by the chosen combination partners and a dose reduction of the single combination partners.

As an example we investigated for the elimination of adverse events the capability of STW5, a combination of 9 different plant extracts (*Iberis amara (L.), Menthae piperitae (L.) Chamomilla recutita (L.), Glycyrhiza glabra (L.), Angelica archangelica (L.), Carum Carvi (L.), Silybium marianum (L.) Gaertn. Melissa officinalis (L.) und Chelidonium majus (L.)*) to modulate interleukin 8 (IL-8). We demonstrated that the IL-8 releases which where induced by the different plant extracts were not simply additive.

We further could show in human skinfibroblasts treated with extracts of *Populus tremulus* L. (S1) [0.06%, 0.1%], *Solidago virgaurea* L. (S2) [0.02%, 0.1%], *Fraxinus excelsior* L. (S3) [0.02%, 0.1%], the multiextract mixture STW1 [0.05, 0.1%], acetyl salicyclic acid (ASA) [30 µg/ml] individually or in the presence of lipopolysaccharides (LPS)[5, 10µg/ml] that cytokine network responses induced by single extracts are not necessarily additive in combination.

"Synergy" is claimed to explain this type of results. However, how to define synergy has been disputed over decades followed by the question which method and which reference model should be used for its quantification.

It will be demonstrated how to further elucidate synergy in the above examples by using the "omic"-technologies (genomics, transcriptomics, proteomics and metabolomics and summarized under the heading "molecular system biology approach") will be shown.

These methods allow us to analyse complex modes of action and may thereby improve our understanding of combination therapies and synergistic effects and support us in finding suitable quantification and explanatory models.

ASSESSMENT OF ANTI-INFLAMMATORY EFFICACY OF A FIXED COMBINATION CONTAINING THYME AND IVY EXTRACTS IN THE MODEL OF ACUTE BRONCHOALVEOLITIS IN RATS

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Therapy of acute lung inflammation commonly includes symptomatic treatment using anti- or protussive agents, mucolytics, adrenergic beta-2 agonists, and in severe cases, antibiotics administration. Nowadays, herbal extracts are frequently used for treatment of acute bronchitis. Using an *in vivo* model of bronchoalveolitis we aimed to investigate whether the combination of thyme and ivy extracts suppresses bronchoalveolar inflammation and goblet cell hyperplasia in lipopolysaccharide (LPS)-instilled rats.

Acute bronchoalveolitis was modeled in male Wistar rats by intratracheal LPS instillation (100 mcg per animal). Test combination of thyme and ivy extracts was administered to animals intragastrically by oral gavage once daily at three doses — 1.7, 5.0 and 16.7 ml/kg for up to three days. Animals were sacrificed at 24 hour intervals up to 72 hours *post* LPS instillation to evaluate the inflammatory status (by granulocyte infiltration in bronchi, leukocyte counts in bronchoalveolar lavage fluid (BALF) and peripheral blood) and the number of mucus-comprising epithelial goblet cells presented in lungs.

Intratracheal LPS instillation induced rather intensive mucosal and sub-mucosal infiltration of granulocytes in bronchi that was maintained till 72 hours. Also, LPS-induced lung inflammation was characterized by signifi-

cant increases in leukocyte and granulocyte counts both in BALF and in peripheral blood. The number of bronchial mucosa goblet cells also significantly increased in response to LPS administration.

Combination of thyme and ivy extracts at all tested doses and almost all time points significantly inhibited the LPS-induced inflammatory process assessed by bronchial granulocyte infiltration, BALF and blood leukocyte counts and the number of bronchial mucosa goblet cells. For several investigated parameters the anti-inflammatory efficacy of the tested combination was similar to that of dexamethasone (5 mg/kg) used as a reference drug.

In conclusion, the combination of thyme and ivy extracts demonstrated significant anti-inflammatory effects and attenuated bronchial goblet cell hyperplasia/metaplasia in an *in vivo* model of acute LPS-induced bronchoalveolitis in rats.

A NEW APPROACH FOR DETERMINATION OF FLAVONOID CONTENT IN HERBAL EXTRACTS BY ^{1}H NMR SPECTROSCOPY

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According to different Pharmacopoeia the identification and quantification of flavonoids and their glycosides are held by HPLC and spectrophotometry methods [1], which need thorough sample preparation, rare and expensive standard samples, which are unavailable for many flavonol glycosides.

A new approach for determination of flavonol glycosides and their aglycones in herbal extracts by ^{1}H NMR spectroscopy method was worked out. This method is based on proton signals of hydroxyl group at position 5 in a ring A of flavonoids, which make a strong intermolecular hydrogen bond with oxygen of neighboring carbonyl group. These protons are least shielded and have chemical shifts from 11.5 to 13.2 ppm, depending on structure of aglycone, position and structure of glycoside parts of flavonol glycoside. The sample preparation is very simple and includes extraction using DMSO-d6. Quantification is held comparatively with residual proton signals of solvent as internal standard.

References:

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