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New approaches in phytopharmacological research*

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Abstract: The introduction of molecular biological models into phytopharmacology research is essential to get more detailed information on the underlying mechanisms of action of multivalent herbal plant preparations. By using new target directed pharmacological screening methods in combination with clinical trials, it will be possible to find a rational for synergistic effects of plant extract preparations. As shown on some examples, questions of the correct dosages, of dose dependent reversal effects of plant constituents and long-term therapeutic effects of phytopreparations at low doses are waiting to be answered. The elucidation of these phenomena can help to rationalize phytotherapy and to integrate it into an overall concept of modern medicine.

INTRODUCTION

Whereas the identification of the major active constituents and the standardization of a herbal drug or phytopreparation is nowadays in most cases possible with the help of the 'hightech' methods available since recently, the elucidation of the molecular biological mechanisms underlying the overall pharmacological profile of a herbal drug preparation is still in its very beginning.

It is, however, essential to get more detailed information on this part of phytomedicine, since the functional mechanisms hold the key of rational phytotherapy. Furthermore questions of the correct dosages and the adequate indication for phytopreparations are waiting to be answered. This goal can be achieved only by a thorough new approach in phytopharmacological research.

NEW SCREENING STRATEGIES IN PHYTOPHARMACOLOGY

In order to investigate complex systems—plant drugs and phytopreparation are complex mixture consisting of many bioactive entities—we have to follow the suggestion of the philosopher Decartes. He has proposed to investigate complex systems, which cannot be easily analyzed by simple means, by examining the individual parts of the system, hoping in this way to find a rational explanation for the whole. In other words we must carry out a more or less reductionistic research, aware, however, that this strategy will never quite explain the entire efficacy of the complete complex of active compounds, because the known sentence 'the whole is more than the sum of all its individual parts' can be suggested to be also true for phytopharmaceuticals.

To follow this approach means, to investigate extract fractions and single constituents of a herbal drug in various bioassays and compare the results obtained with those from the herbal raw drug or extract. In contrast to former pharmacological investigations, new targets such as enzymes, receptors, cell cultures and gene domains have to be included. The following examples from our research work should explain the new strategy.

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TARGET DIRECTED PHYTOPHARMACOLOGY RESEARCH

Example 1

In our approach into explaining the cardiotonic activity of Hawthorne (*Crataegus oxyacantha*) preparations, verified by numerous clinical studies [1], we have found that the procyanidines and flavon-C-glycosides appear to be the main constitutents responsible for the pharmacological activity of Crataegus phytopharmaceuticals. In our aorta model we found an endothelin dependent smooth muscle relaxing effect, which in turn seems to be caused by a phosphodiesterase inhibiting effect [2]. Additionally the procyanidins showed also an *in vitro* angiotensin converting enzyme (ACE) inhibiting effect, which accounts for a further blood vessel dilating effect and at the same time a redution of blood pressure [3]. By a systematic study with various typs of oligomeric procyanidins we found the highest inhibiting effect for the dimeric procyanidins. The flavon-C-glycosides vitexin, vitexin-rhamnoside, rutin and hyperoside act as anti-oxidants, inhibitors of cyclooxygenase and 5-lipoxygenase, so that additional anti-inflammatory and thrombocyte aggregation inhibitory effects can be expected. These results show that for increasing the efficacy of hawthorn medication, extracts have to be produced which contain both active classes of compounds in enriched form. Therefore, it seems to be reasonable to standardize these extracts on both classes of compounds. In this context it is of interest that the alcoholic extracts of two Southamerican *Cecropia* spezies (*Cecropia hololeuca* and *C. glaziovii*) and the African *Musanga cecropioides* plant, which are used in South American Traditional Medicine for the treatment of cardiovascular disorders, were found to have nearly the same composition of bioactive compounds and at the same time also the same pharmacological activity and therapeutic application [2].

Example 2

Water-alcohol extracts of the root of *Urtica dioica* (stingl nettle root) have long been used and are still in usage for the treatment of benigne prostata hyperplasia. Several observational and double blind studies have assessed the clinical efficacy [4]. The roots contain a mixture of isolectins (UDA) (Mol. weight ≈ 9500 d), which possess N-acetylglucosamine specifity [5]. By using a ^{125}J labeled epidermal growth factor receptor preparation, we were able to show that this UDA binds to the epidermal growth factor (EGF)-receptor of an epidermal cancer cell line CA 431 in a dose dependent manner [6]. This could result in a competitive inhibition of EGF induced proliferation. Additionally the polysaccharides isolated by us from the same root showed immunostimulating and anti-inflammatory activity [7]. Since the lectins are very stable against heat and acids and will be absorbed from the GUT after oral administration [8], it is likely that the well documented clinical efficacy of Urtica preparations is based on a synergism of anti-proliferative and anti-inflammatory effects caused by these two water soluble classes of compounds.

Example 3

Since the pharmacological role of garlic (*Allium sativum*) in prevention and treatment of cancer and atherosclerosis has received increasing attention and thorough investigations into the molecular mechanisms of action of garlic compounds are lacking, allicin and ajoene have been investigated in two new *in vitro* models. The first used an apoptosis inducing model, whereas the second was done with the inducible nitric oxide synthase (iNOS) from human macrophages.

- In the first experiment it could be shown that ajoene induces apoptosis in human leucemic cells, but not in peripheral mononuclear blood cells of healthy donors. Ajoene increased the production of intracellular peroxide in dose and time dependent fashion, which could be partially blocked by preincubation of the human leucemic cells with the anti-oxidant N-acetyl-cysteine. This result suggests that ajoene might induce apoptosis via the stimulation of peroxide production and activation of the nuclear factor κB. [9]. This novel aspect is an important step in elucidating the underlying molecular mechanisms of its anti-tumor action.
- In the second experiment it has been found that allicin and ajoene inhibit the expression of iNOS in activated macrophages [10]. Since it is known that the inflammatory environment in human atherosclerotic lesions results in an expression of the inducible form of nitric oxide synthase and subsequently in the formation of peroxynitrite and by this aggravates the atherogenic process, these

results may provide an interesting basic contribution with regard to the beneficial effects claimed for garlic in atherosclerosis prophylaxis. Meanwhile it has been shown that also the mistletoe lectin I of *Viscum album* is able to induce the apoptosis of cancer cells, suggesting that besides the well known immunostimulating activity a second molecular biological mechanism for the described anti-tumoral activity of mistletoe preparation has been found.

Example 4

In a systematic screening of various constituents of plants with suggested diuretic, spasmolytic and anti-hypertensive activity for Ca-channel blocking activity using papillary muscles from the right ventricles of guinea pighearts and also the patch-clamp method we found active compounds among the class of phenylpropanoids (apiol, eugenol), naphthoquinones, garlic constituents, terpenoids (menthol), pyrano-coumarins (visnadin) and isochinolin alkaloids (sanguinarine) [11,12], The IC_{50}-values of some of them were in the range of 10–30 μm. Among the compounds listed in a review article written by Vuorela *et al.* [13] furanocumarines, coumarines and some mono- and sesquiterpenoids occupy a preferred place. In comparison to the activity of nifedipine or verapamil, the potential of most of these compounds are very low, however, if possible synergistic effects with other constituents of the same plant are taken into consideration a therapeutically relevant effect might result.

These examples, which could be augmented by many others (e.g. plant extracts and constituents with endothelin inhibiting-, COX 2-inhibiting, leucotrien-antagonistic-, immunomodulating, phosphokinase-C- or anti-fibrotic activity) may demonstrate the necessity and importance of introducing new molecular biological models for the screening of phytopreparations and herbal drug constituents.

MECHANISM OF ACTION OF PHYTOPHARMACEUTICALS

The experience based claim of phytotherapy that the therapeutic effects of plant extracts or constituents of herbal drugs are in many cases superior to isolated single compounds from the same plants or mixtures of them, has never been assessed in detailed pharmacological or clinical studies. There are, however, numerous practical experiences and clinical clues, which ascertain this claim. Previous findings of classical pharmacology with mixtures of bioactive compounds have shown that we have to differentiate between additive and synergistically acting overadditive or potentiating effects. If two substances of a mixture have the same pharmacological target an additive effect can be expected. If however, two or more substances of a mixture have different pharmacological targets, a pharmacologically synergistic effect may result, which can be greater that expected for the individual substances taken together [14]. Such dose–response investigations with mixtures of bioactive compounds can be carried out by using the isobol methods, as proposed by Berenbaum [15]. This method has been applied, e.g. by Hall & Duncan [16] for the rationalization of synergistic effects of mixtures of anti-viral compounds.

We have carried out such an experiment using the thrombocyte aggregation assay with a mixture of Ginkgolide A and B, two major constituents of *Ginkgo biloba*, and found a typical synergistic effect as shown by the 'concave up' isobol curve in the diagram (Fig. 1) [17]. It is self-evident that this isobol method cannot be applied for mono-or multiherbal preparations. Therapeutic synergy have been described for the extracts of Kava-Kava [18,19], Valerian [20], Liquorice [21], Croton lechleri [22] or herbal plant mixtures of Traditional Chinese Medicine [23,24] In this context it may be mentioned that since recently in classical chemotherapy the application of mixtures of substances such as the three nucleotides containing cocktail for the therapy of AIDS has found a striking renaissance. The better overall effect of this triple therapy of AIDS can be explained by a polyvalent or multivalent action of these substances on three distinct target enzymes (reverse transcriptase, protease and glucosidase). In a similar way the synergistic effects within herbal extracts and multiherbal preparations has to be interpreted.

In discussing the possible molecular biological mechanisms of synergistic therapeutic effects, it has to be taken into consideration that a part of these effects can be also explained by an enhanced absorption or excretion rate and better bioavailability caused by nonbioactive constituents of the same herbal drug, such as tannins or saponins. On the other hand meanwhile many controlled clinical studies with standardized phytopreparations are available, which ascertain the hypothesis of synergistic effects of bioactive constituents within herbal extracts and drug combinations. The therapeutically used Ginkgo-and St. John's

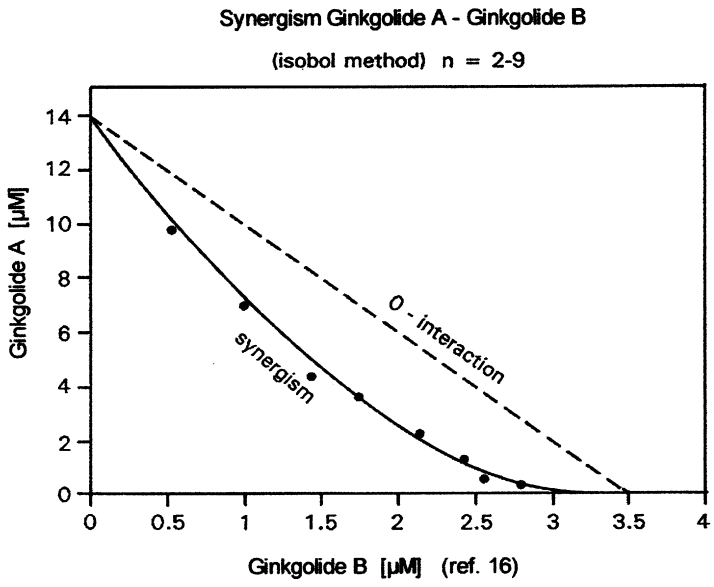

Fig. 1 Synergism ginkolide A–ginkolide B (isobol method $n = 2$–9).

Wort-phytopreparations may advocate this hypothesis. For the generation of a PAF-antagonizing effect in humans about 100–240 mg of a standardized Ginkgolide A, B, C mixture/day have to be administered. [25] The same effect can be achieved by a daily dose of 120 mg standardized Ginkgo extract containing altogether about 6–7 mg Ginkgolides, Bilobalide and flavonol-glycosides. Obviously, this low concentration of Ginkgo constituents in the Ginkgo extract has to be bioequivalent to 100–240 mg pure Ginkgolides. A similar calculation might be valid for St. John's Wort (*Hypericum perforatum*) extracts. One of the last clinical trials performed with standardized Hypericum extracts against the synthetic psychopharmacon Imipramin with the indication 'moderate' depression, resulted after a 6-weeks treatment in the same reduction of the depression score values on the HAMDA depression scale. [26]. It appears that 3×300 mg applied Hypericum extract/day containing altogether about 8–10 mg bioactive compounds (hypericines, hyperforins, flavonoids, procyanidins), can be regarded as bioequivalent to 75 mg synthetic Imipramin.

This comparison of bioequivalence let us conclude that the therapy with phytoprepartions with some exceptions is a 'low dose therapy' related to the amount of applied bioactive compounds of a plant extract. In terms of classical pharmacology, this low concentration of active compounds could be classified as 'underdosaged'. The successful results obtained with phytopreparations in controlled clinical trials, however, disprove this opinion. One of the recently performed investigations with mistletoe underlines the low dose hypothesis. Mistletoe lectin I, the presumed major active substance of *Viscum album* extracts, stimulates the cytokine production and phagocytosis activity of macrophages in mamma carcinoma patients to a maximum and by this the overall immune response at the surprisingly low concentration of 1.0 ng/kg (i.v. or i.m.) [27].

For these synergistic and dosages phenomenon a scientific explanation is lacking. We are also unable to rationalize the frequently observed reverse effects of bioactive compounds, if they are used once in a high and then in a very low concentration. Particularly impressive are experiments, we have carried out with classical cytostatic agents of natural and synthetic origin such as vincristine, taxol, podophyllotoxin, naphthoquinones, Methotrexate or Fluoruracil. These substances exhibited in subtoxic amounts (pg–fg-range) in *in vitro* granulocyte- or T-lymphocyte-assays immunostimulatory effects and in high dose (µg–ng) the known cytostatic, cytotoxic or immunosuppressive activity [28]. This dose dependent reverse effect is shown in Fig. 2 for vincristine, which in a pg-and fg-concentration range shows *in vitro* immunstimulating effect against human T-lymphocytes. When lymphocytes were exposed to a cold shock (4 °C for 1 h), before incubation with vincristin, the cells were found to be more sensitive and

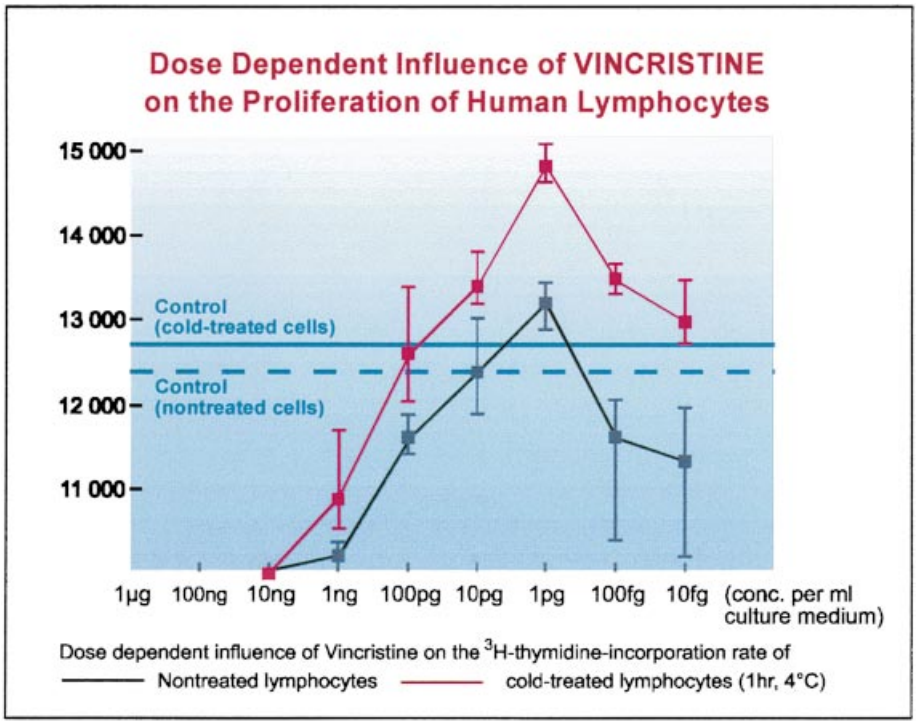

Fig. 2 Dose dependent influence of vincristine on the ^{3}H-thymidine-incorporation rate of nonpretreated lymphocytes and cold-pretreated lymphocytes (1 h, 4 °C).

showed a higher stimulating rate than nonpretreated cells. The same effect was observed when the lymphocytes were exposed to a heat shock (40 °C for 30 min).

Another, as yet, unresearched area of phytotherapy is the phenomenon of the increasing effects of phytopreparations in the course of a long-term therapy with the smallest of active substance doses. Measurements of the myocardial blood perfusion in dogs using a thermo-probe led, in the course of a three week administration of very low doses of Crataegus extracts, to a maximum of blood supply (three to four times higher than on day 0 of administration) not earlier than after a three weeks treatment. This result agrees with the subjective results noted by patients in clinical trials [29].

Similar observations that the summation of very low doses results in an effect after reaching a threshold concentration, until now are known from plant physiology only.

REFERENCES

1 M. Tauchert, M. Bloch, W. D. Hübner. *Münch. Med. Wschr.* **136**(Suppl. 1), 27 (1994).

2 U. Franck, B. Günther, W. Vierling, H. Wagner. *Phytomedicine Supplement* **1**, 93 (1996).

3 H. Wagner, G. Elbl, H. Lotter, M. Guinea. *Pharm. Pharmacol. Lett.* **1**, 15–18 (1991).

4 H. Schilcher. *Herbal Drugs in the Treatment of Benign Prostatic Hyperplasia in Phytomedicines of Europe* (L. D. Lawson, R. Bauer, eds), ACS Symposium Series 691, American Chemical Society, Washington, DC (1998).

5 W. J. Peumans, M. De-Ley, W. F. Broehaert. *F. E. B. S. Letters* **177**, 99–103 (1984).

6 H. Wagner, W. N. Geiger, G. Boos, R. Samtleben. *Phytomedicine* **4**, 287–290 (1995).

7 H. Wagner, F. Willer, R. Samtleben, G. Boos. *Phytomedicine* **1**, 213–214 (1994).

8 W. N. Geiger. *Studien zur, p.o. Bioverfügbarkeit von Urtica dioica—agglutinin (UDA) und Untersuchungen von UDA und anderen Lektinen zur Wirkung auf Wachstumsfaktoren und Tyrosinphosphorylierung*. Thesis, München (1998).

9 V. M. Dirsch, A. L. Gerbes, A. M. Vollmar. *Molecular Pharmacology* **53**, 402–407 (1998).

10 V. M. Dirsch, A. K. Kiemer, H. Wagner, A. M. Vollmar. *Atherosklerosis* **139**, 333–339 (1998).

11 W. Vierling, O. Sensch, K. Neuhaus-Carlisle, H. Wagner. *Naun-Schmiedeberg Arch. Pharmacol.* **351**, R96 (1995).

12 K. Neuhaus-Carlisle, W. Vierling, H. Wagner. *Pharm. Pharmacol.* **3**, 77–79 (1993).

13 H. Vuorela, P. Vuorela, K. Törnquist, S. Alaranta. *Phytomedicine* **4**(2), 167–180 (1997).

14 Provided none of the substances in the mixture exerts an antagonizing effect.

15 M. C. Berenbaum. *Pharmacol. Rev.* **41**, 93 (1989).

16 M. J. Hall, I. B. Duncan. In *Antiviral Agents: the Development and Assessment of Antiviral Chemotherapy*, Vol. II (H. J. Field, ed.), pp. 29–84. CRC Press, Boca Raton, FL (1988).

17 B. Steinke. *Chemisch-analytische und pharmakologische Untersuchungen von pflanzlichen PAF-Antagonisten und Inhibitoren der Thrombozytenaggregation (Allium-Arten, Ginkgo biloba, Pinellia ternata)*. Thesis, Munich (1993).

18 M. W. Klohs, F. Keller, R. E. Williams, M. I. Toekes, E. G. Cronheim. *J. Med. Pharm. Chem.* **1**, 95–99 (1959).

19 Y. N. Singh, M. Blumenthal. *Herbalgram* **39**, 33–56 (1997).

20 J. Hölzl. In: *Medicinal and Aromatic Plants- Industrial Profiles*, Vol. 1 *Valerian. The Genus Valeriana* (P. J. Houghton, ed.), pp. 55–75, (series ed. R. Hardman). Academic Publishers. the Netherlands (1997).

21 G. Cantelli-Forti, F. Maffei, P. Hrelia, F. Bugamelli, M. Bernadi, P. D'Intino, M. Maranesi, M. A. Raggi. *Environ. Health Perspect.* **102**(Suppl. 9), 65–68 (1994).

22 Z. P. Chen, Y. Cai, J. D. Phillipson. *Planta Med.* **60**, 541–545 (1994).

23 J. D. Phillipson. *Eur. Phytotelegram* **6**, 33–40 (1994).

24 S. J. Sheu, Y. J. Han. *Chinese Pharm. J.* **46**, 565–573 (1994).

25 P. Chung, C. Brambilla, J. Duchier, A. Taytard, C. Summerhayes. In *Ginkgolides—Chemistry, Pharmacology and Clinical Perspectives* (P. Braquet, ed.), pp. 345–354. JR Prous Science Publishers, Barcelona (1988).

26 E. H. Vorbach, W. D. Hübner, K. H. Arnoldt. *Nervenheilkunde* **12**, 290–296 (1993).

27 T. Hajto, K. Hostanka, H. J. Gabius. *Cancer Res.* **49**, 4803–4808 (1989).

28 H. Wagner, B. Kreher, K. Jurcic. *Arzneim. Forsch. (Drug Res.)* **3**, 273–275 (1988).

29 W. H. Mävers, H. Hensel. *Arzneim. Forsch. (Drug-Res.)* **24**, 783 (1974).