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Natural products chemistry and phytomedicine in the 21st century: New developments and challenges*

H. Wagner

Centre of Pharma-Research, Pharmaceutical Biology, Butenandtstr. 5, University of Munich, D-81377 Munich, Germany

Abstract: The gradual transition from the long-standing use of monodrug therapy in classical medicine to the new concept of a multidrug and multitarget therapy has great implications for the research strategies of natural products chemistry and phytomedicine. The rationalization of the new strategies, however, requires great efforts in: standardization of mono- and multi-phytopreparations using all available high-tech methods; screening of extracts and their constituents by integration of modern molecular biological bioassays; and controlled, clinical studies, inclusive of pharmacokinetic and bioavailability investigations, aimed at evidence-based phytotherapy. The first results obtained in recent years are explained using several examples of phytopharmacological and clinical studies. These show the therapeutic superiority of many plant extracts over single isolated constituents, as well as the bioequivalence of many phytopharmaceuticals with synthetic chemotherapeutics.

INTRODUCTION

- The first change can be described as a gradual withdrawal from the dogma of mono-substance therapy and an increasing transition to the treatment of patients with drug combinations, as performed at present for the treatment of cancer, acquired immune deficiency syndrome (AIDS), malaria, or hypertension. We call this multidrug therapy.
- The second paradigm shift can be characterized as a transition to a new kind of multitarget therapy, which is directed primarily toward the activation of defense, protective, and repair mechanisms of the body rather than toward the direct killing of the damaging agents (e.g., the tumor cell or the pathogenic microorganism). Neither of these therapeutic strategies proposed at present by the new generation of clinicians and pharmacologists is absolutely new. Phytotherapy has long followed and developed these strategies by using mono-extracts or extract combinations containing mixtures of bioactive compounds and by activating primarily self-healing and protective processes of the human body, rather than attacking and directly destroying the damaging agents. These strategies are based on therapeutic experiences and the consideration that a complex pathophysiological process can be influenced more effectively and with fewer or no severe side-effects by a combination of several low-dosage compounds or the corresponding extracts than by a single highly dosaged isolated compound.

This task, however, can be mastered only in concerted cooperation among phytochemists, pharmacologists, molecular biologists, and clinicians. The efforts should focus primarily on three research areas:

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- application of all available modern, high-tech methods to standardize phytopreparations before conducting systematic pharmacological investigations and clinical studies;
- integration of new molecular biological assays into the screening of extracts and plant constituents to evaluate their exact pharmacological profiles, to elucidate the synergistic effects of the constituents of an extract, and thereby to gain a better understanding of the various mechanisms underlying these pharmacological effects; and
- controlled clinical studies paralleled or followed by pharmacokinetic and bioavailability studies.

Owing to the enormous progress of specific high-tech analytical methods, we are able today to analyze even complex composed extracts and phytopreparations and to quantify the major active compounds, which are supposed to be responsible for the efficacy of an extract. The successful three-dimensional high-performance liquid chromatography (HPLC) fingerprint analysis of a multiple extract combination of Kampo medicine prepared from eight herbal extracts and the quantification of their major constituents, illustrate the effectiveness of these modern tools [1]. At the same time, these methods meet the quality standards of drug authorities and the reproducibility of pharmacological studies, along with the performance of good clinical practice (GCP), conforms to clinical trials requirements.

MOLECULAR BIOLOGICAL APPROACH

The following examples will explain the molecular biological screening approach in more detail. Most of the models operate on a receptor, signal transduction, or genetic basis.

Crataegus oxyacantha

In an attempt to rationalize the cardiotonic activity of hawthorn (*Crataegus oxyacantha*) extract preparations, verified by numerous clinical studies, it was found that procyanidins and flavone C-glycosides appear to be the main constituents of the herb responsible for the therapeutically relevant cardiotonic activity [2]. Recently, we have reported that a hawthorn extract containing procyanidins and flavonoids, exhibits in vitro an angiotensin-converting enzyme (ACE)-inhibiting effect [3], which, together with the endothelin-dependent, smooth-muscle relaxing effect found recently in an aorta model [4], might account for the dilating effect on coronary vessels and a simultaneous reduction in blood pressure. Because the flavonoids also act as antioxidants, and cyclooxygenase and 5-lipoxygenase-inhibitors and have a thrombocytes aggregation reducing effect, it is necessary to produce special hawthorn extracts which contain both active classes of compounds in enriched form and standardized in the content of these procyanidins and flavone C-glycosides. In conclusion, we can state that the pharmacological multivalence of hawthorn constituents justifies the therapeutic application of hawthorn for the treatment of heart insufficiency grades I and II.

Allium sativum

Because the pharmacological role of garlic (*Allium sativum*) in the prevention and treatment of atherosclerosis has received increasing attention, we decided to investigate ajoene and allicin, two major constituents of fermented garlic juice, in a suitable molecular biological assay. It could be shown that both compounds inhibit the expression of inducible nitric oxide (iNO) synthase in activated macrophages. Because it is known that the inflammatory environment in human atherosclerotic lesions results in an expression of the inducible form of nitric oxide synthase [5] and, subsequently, in the formation of peroxynitrite, thereby aggravating the atherogenic process, these results may provide an interesting basic contribution with regard to the beneficial effects claimed for garlic in atherosclerosis prophylaxis. If we consider all the other known pharmacological effects caused by garlic preparations, including thrombocyte aggregation-inhibiting, anti-inflammatory, antioxidant, triglyceride-decreasing, and cholesterol-

decreasing effects, garlic can be designated as one of the most notable examples of a multivalent herbal drug, in which the major bioactive constituents reveal synergistic, cardiovascular-directed effects.

Fixed herbal combination

A fixed herbal combination product*, consisting of extracts from nine plants**, clinically proven to have an effect comparable to metoclopramide and cisapride against functional dyspepsia and irritable bowel syndrome (IBS), was investigated pharmacologically and in several molecular biological assays using binding affinity receptor models with isolated guinea pig ileum [6]. The results obtained with the extract combination were compared with those of the individual plant extracts. A given table shows the magnitude of different pharmacological effects of each plant extract on the multiple pathomechanisms of the disease: for example, *Iberis* selectively inhibited binding to M3 receptors, while celadine herb and chamomile flowers were selective to 5-HT4-, and licorice roots to 5-HT3 receptors. In conclusion, the multiherbal drug combination exerts a synergistic, multiple effect on various parameters, including hypersensitivity, atonia, spasms, acid secretion, inflammation, and radical production, which explains the therapeutic bioequivalence of Iberogast with some synthetic drugs as well as its superiority over monoextract preparations.

Multi-extract traditional Chinese medicince preparation

This example describes the attempt to evaluate and rationalize the pharmacological profile and therapeutic potential of a multi-extract preparation from traditional Chinese medicine (TCM). The research project, headed by Prof. Sucher of the University of Hong Kong***, is the chemical and pharmacological investigation of a fixed herbal drug combination which has a great reputation in TCM for the prevention and therapy of stroke [7]. The combination investigated consists of eight herbal drugs: root of *Salvia miltiorhiza*, rhizome of *Ligusticum sinense*, root of *Paeonia rubra*, root of *Angelica pubescens*, root of *Stephania tetrandra*, Ramulus c. Uncis of *Uncaria rynchophylla*, rhizome of *Gastrodia elata*, and root of *Panax ginseng*. As a result of the detailed chemical and molecular-biological investigations, the researchers were able to assign defined pharmacological effects and mechanisms of action to the individual herbal drugs and their major bioactive constituents. The individual TCM drugs were classified into four categories—channel collateral-stroke affecting, anti-inflammatory, antithrombotic, and neuroprotective—and described as improving blood circulation, increasing cerebral blood flow, and protecting the brain from ischemic and reperfusion injuries [7].

Meanwhile, many other herbal drug extracts have been screened using new molecular-biological in vitro assays. These include extract preparations of *Hypericum*, *Ginkgo*, *Silybum*, *Harpagophytum*, or *Vitex agnus-castus*.

From all these results we can conclude:

- all herbal drug extracts investigated with these new methods were found to have much greater pharmacological potential and broader profiles than suggested previously;
- most of the additional pharmacological effects were found to be synergistically additive to the earlier evaluated pharmacological activities (this provides a more comprehensive and a better understanding of the mechanism of action of the plant extracts); and
- all investigations have shown that most of the extracts and individual constituents thereof exert multivalent or pleiotropic pharmacological effects (this multivalence of pharmacological activities can generate additive or overadditive, potentiated synergistic effects).

*Iberogast (STW)

***Iberis amara, Angelica archangelica, Carum carvi, Silybum marianum, Chelidonium majus, Glycyrrhiza glabra, Chamomilla recutita, Melissa off., Mentha pip.*

***Now Burnham Institute, La Jolla, CA 92037.

It is a rule in classical pharmacology that if two bioactive 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 that can be greater than expected for the individual substances taken together (provided that none of the substances in the mixture exerts an antagonizing effect) [8]. Those dose–response investigations with two-component containing mixtures of bioactive pure compounds can be carried out using the isobol method as proposed by Berenbaum [9]. We have carried out such an in vitro experiment using the thrombocyte aggregation assay with various mixtures of ginkgolide A and B, two major constituents of *Ginkgo biloba*. From the concave-up isobol curve we have obtained, a typical potentiated synergistic effect could be deduced [10]. It is plausible that the isobol method cannot be applied to herbal extract mixtures. Here, detailed in vitro or in vivo comparative investigations with single constituents or mixtures and extract fractions or whole extracts must be performed. Evidence supporting the occurrence of synergy within phytomedicines is accumulating and has been reviewed recently by Williamson [11].

The synergistic effects that have been measured exceed the effects of single compounds, or mixtures of them at equivalent concentrations, by a factor of two to four, or more. These synergistic additive or potentiated effects are of interest only if they can be verified by clinical studies. The next example may be instructive.

Cannabis sativa

The *Cannabis sativa* herb has received much attention recently because of the detection of an endogen cannabinoid system in some parts of the human brain and the immune system, represented by the two cannabinoid receptors CB1 and CB2. New molecular biological investigations resulted in the identification of arachidonylethanol-amide (anandamide) and 2-arachidonyl-glyzerol (ether) as endogen CB-ligands. According to current knowledge, this endogenic cannabinoid system plays an important role in the development of memory, in pain transduction and inhibition, in control of appetite, in lactation, in generation of emesis, and as an immunomodulator. Among the most conspicuous pharmacological effects of (–) *trans*-Δ 9-tetrahydrocannabinol (THC), which came onto the drug market under the drug name Dronabinol* [12], the muscle-relaxant, appetite-stimulating, and analgesic effects are the most interesting.

Meanwhile, the idea of using THC as a pure compound for the treatment of multiple sclerosis (MS) patients has been given up after a comparative study with *Cannabis* has shown that the extract exhibited a much better antispastic activity than the THC substance alone, as measured in an immunogenic model of MS [13]. At present, over 1 % of MS patients take *Cannabis* illegally for amelioration of spasticity and the pain associated with this condition [14]. The reason for this better effect is probably due to the content of cannabidiol (CBD), in the extract, which amplifies the antispastic effect markedly and simultaneously reduces the undesirable psychotropic side-effects of THC. It is not yet clear how this amplifying antispastic effect can be explained on a molecular basis, but there are some indications that CBD increases the permeation of THC into the muscle cells.

CLINICAL STUDIES

The best clinical evidence for existing therapeutic synergism can be demonstrated by results obtained in a clinical trial performed with a standardized *Hypericum* extract in comparison with the synthetic psychopharmacon Imipramine in the treatment of patients with moderately severe depression [14].

The *Hypericum* extract was administered over 6 weeks in a dose of 500 mg extract per day, which corresponds to about 8–10 mg bioactive compounds together (hypericins, hyperforin, xanthons, flavonoids, procyanidine). Imipramine was given in a concentration of 150 mg/day. The efficacy was

*Marinol® in USA.

measured by the procentual reduction of the HAMD-score values after 6 weeks' treatment. The results of the comparative study show that 8–10 mg bioactive constituents of the *Hypericum* extract together must be therapeutically equivalent to 150 mg imipramine. Interestingly, the *Hypericum* treatment has far fewer side-effects than the synthetic drug [8]. The reason for this therapeutic equivalence of the medications has to be interpreted as a potentiated synergistic effect caused by the combination of *Hypericum* constituents, which are suggested to have different molecular targets in the brain. The possibility that the increased efficacy could be explained only due to an enhanced resorption rate of the constituents and thereby improved bioavailability, because of additional non-bioactive byproducts of the *Hypericum* extract, is not very likely and can be ruled out.

At present, about 500 controlled, mono- and double-blind, placebo-controlled clinical trials have been performed, which meet all international requirements of performance and efficacy. About 80 % of the existing studies have been made with standardized versions of long-used mono-extracts, including *Ginkgo*, *Hypericum*, *Harpagophytum*, *Valeriana*, *Sabal*, *Urtica*, *Aesculus hippoc.*, *Crataegus*, *Silybum*, or *Echinacea*. About 20 % were tested against hemosynthetic drugs, showing therapeutic equivalence of both medications at the same indication. Clinical evidence is available for the therapeutic superiority of Salix root extract over an isolated single constituent thereof [16] and for the herbal drug combinations *Valeriana/Piper methysticum* [17] and *Ginkgo/Ginseng* [18] over only one of the two herbal drug combinations.

It can be concluded that the therapeutic superiority of an extract over a single isolated constituent thereof, and of an extract combination over one extract of the extract combination, is caused by additive or potentiated effects of the mixtures of constituents in the extract. It must be emphasized that in the comparative studies with synthetic drugs, the concentrations of all bioactive constitutents of an extract together, applied per day, were much lower than the applied dosages of the synthetic drugs. This accounts for synergistic effects and simultaneously for the absent or minimal side-effects reported for phytopreparations.

MULTITARGET THERAPY

The idea of developing new drugs for a rational multitarget therapy, in both classical medicine and phytomedicine, is still in its infancy. The new strategy can be explained using the following examples of possible new therapies for cancer and hepatitis B/C.

- The standard drug therapy for cancer in classical medicine uses cytostatic chemotherapeutics, which, in ideal conditions, arrest or destroy tumor cells. Most of the cytostatic drugs possess severe side-effects and reduce the quality of life. Apart from some exceptions, these drugs are not able to heal cancer patients or to extend their life span by more than 5 or 10 years. The new strategy follows a quite different concept. It aims at the stimulation of defense and repair mechanisms of the healthy tissues, e.g., induction of apoptosis, inhibition of angiogenesis, stimulation of heat-shock proteins and oncogen-suppressor genes. This multitarget therapeutic concept requires a cocktail consisting of several individual or multivalent drugs, which, in a concerted and synergistic way, might be able to arrest the tumor growth. Many laboratories worldwide work on the development of such a multidrug and multitarget concept.
- The standard therapy to treat hepatitis B+C uses pegylated interferon-β and/or some chemotherapeutic drugs such as ribaverine or lamivudine. The disadvantages are that the response rate for interferon is only 50–60 % and the resistance rate for AZT and analogs is very high. The healing chances are limited. More success is expected with the new strategy of stimulating the immune system and repair mechanisms concertedly, to protect the healthy liver cell with antioxidants and inhibit apoptosis, as well as inflammatory and fibrotic processes.

SUMMARY

Two new paradigm shifts in medicine characterize the beginning of the 21st century: the gradual renunciation of the long-standing reliance on monosubstance therapy in favor of a multidrug therapy, and the transition to a new kind of multitarget therapy, through which the interference of drugs with protective-, repair-, and immunostimulatory mechanisms of the human body, rather than with single disease-causing agents, gains more and more importance. Phytomedicine research has a good chance of contributing to these new strategies through the development of new and better drugs for an evidence-based and rational therapy. Great efforts in three research areas, however, are compulsory: (1) the increase in efforts to develop suitable standardization methods for phytopreparations; (2) the integration of molecular biological assays into the screening of plant extracts, single isolated compounds thereof and phytopreparations; and (3) the performance of further placebo-controlled, mono- or double-blind, clinical trials, paralleled or followed by pharmacokinetic and bioavailability studies. One major concern will be to investigate the multivalent and multitarget actions of plant constituents and standardized extracts, with the aim of rationalizing the therapeutic superiority of many plant extracts over single isolated constituents.

Phytomedicine and chemosynthetic pharmaceutical research find themselves in a race to develop new medicines, with fewer or no side-effects, for therapeutic and preventive application in illnesses for which causality-based treatments are nonexistent or imperfect.

Although the dominant opinion has been that the use of phytotherapy should be restricted to the treatment of moderate and moderately severe diseases, or used for prevention of diseases only, recent clinical trials have shown clearly that some phytopharmaceuticals possesss therapeutic equivalence with synthetic drugs and can also be applied to the treatment of severe diseases. Therefore, a rational, evidence-based phytotherapy has a good chance of gaining a new legitimation in contemporary medical use.

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