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REVIEW ARTICLE

Metabolomics in the Context of Systems Biology: Bridging Traditional Chinese Medicine and Molecular Pharmacology

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The introduction of the concept of systems biology, enabling the study of living systems from a holistic perspective based on the profiling of a multitude of biochemical components, opens up a unique and novel opportunity to reinvestigate natural products. In the study of their bioactivity, the necessary reductionistic approach on single active components has been successful in the discovery of new medicines, but at the same time the synergetic effects of components were lost. Systems biology, and especially metabolomics, is the ultimate phenotyping. It opens up the possibility of studying the effect of complex mixtures, such as those used in Traditional Chinese Medicine, in complex biological systems; abridging it with molecular pharmacology. This approach is considered to have the potential to revolutionize natural product research and to advance the development of scientific based herbal medicine. Copyright © 2005 John Wiley & Sons, Ltd.

Keywords: metabolomics; systems biology; Traditional Chinese Medicine (TCM).

INTRODUCTION

Over the past thousands of years, several forms of traditional medicine have developed which are often based on fundamental principles that differ from those of so-called 'Western' medicine, thus making their scientific evaluation very difficult using existing conventional methods. One of the most prominent characteristics of many traditional forms of medicine, including that of Traditional Chinese Medicine (TCM), is a more holistic approach to examining the function and dysfunction of living organisms. In TCM, important starting points are the five element (phase) theory and the principle that every healthy organism is in a Yin-Yang balance (for review see Cheng, 2000). Balance is considered to be a complex interplay between body and mind, which is reflected at all levels, ranging from the biochemical component perspective to the energetic system control of the physical body. Internal imbalances can stem from a wide variety of factors and lead to a plethora of conditions ranging from short perturbations to chronic disease processes. The TCM approach recognizes the uniqueness of each human being and the necessity to develop a personalized medication to obtain optimal results based on multi-component treatment. Currently, Western medicine is showing an increased interest in traditional forms of medicine, including TCM and Ayurveda (Patwardhan *et al.*, 2004). The term 'alternative' is now often replaced by 'complementary' medicine and the newest term 'integrative' medicine implies that Western and alternative modalities are used together (Fontanarosa and Lundberg, 1998; Udani, 1998). At a first glance the present 'Western' medical approach may seem very different from holistic forms of traditional medicine. Western medicine relies on a detailed classification of diseases, empirical investigations and treatments targeting those disorders. However, the revolution in genomics that has taken place in life sciences during the past decade has provided considerable support for a more holistic view on diagnosis and treatment. Furthermore, the issue of personalized medicine is now receiving considerable attention due to the new insights in pharmacogenomics. Although the principle of homeostasis has been a cornerstone of Western physiology for more than a century, the enormous complexity of biological systems has often driven pharmaceutical research towards trying to identify and influence single targets that make the difference between health and disease. This approach has indeed provided many potent drugs, especially for the treatment

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of acute conditions such as infectious diseases, but also revealed major drawbacks. In fact, it involves trying to influence a system by interacting with a single protein that is often part of a complex pathway, and involved in a cascade of reactions and feed-back loops. The reality is that most diseases are multi-factorial which means that treating a single target provides a partial treatment and in the majority of cases no cure or in case of chronic diseases, serious side effects occur, particularly in the long term. Although this awareness is not new, it has been very difficult to find alternative routes given the mentioned complexity of the living system, which is almost impossible to reveal.

The last part of the previous century yielded many new technologies to study biology and many new insights have been generated since then. Of special interest has been the revolution in genomics that has yielded the complete DNA maps of several species including human and mouse. The enormous expectations of having the human genome data available and consequently the opportunity to identify many new 'drugable' targets based on transcriptomics has been tempered by new insights into the complex control mechanisms of biological systems. This has driven the scientific community to develop tools to study the organism at the level of proteins and protein–protein interactions; a field captured by the acronym proteomics. The wave of information from proteomics further stimulated the measurement of more and more elements to provide a systems approach, especially the level of metabolites and the field of metabolomics, appeared mandatory as well as it represents the chemical characterization of the phenotype. In this way the systems biology approach was born and although defined in several ways today, in our opinion it can be best described as 'the integrated approach to study biological systems—intracellular networks, cells, organs and any biological entity—by measuring and integrating genetic, proteomic and metabolic data' (Clish *et al.*, 2004; Lamers *et al.*, 2003a; 't Hart *et al.*, 2003).

The ability to study biology using new analytical platforms enabling many elements to be measured in parallel created a great need for the informatics tools necessary to transform data into biological information and knowledge. Bioinformatics is a multidisciplinary area of research which has come to full bloom during the past years. Experts in the field of (computational) biology, statistics, mathematical modelling and informatics work closely together to get the best out of the vast amount of data obtained in a systems biology approach.

Obviously, interest in TCM is growing worldwide, also from the side of Western pharmaceutical industries. The latter category often tends to approach TCM from a non-holistic perspective, searching for single bioactive compounds. The idea is to isolate and characterize those and use them as a template for drug lead optimization studies. This approach is basically the same as that used in pharmaceutical companies for exploring natural products. This has been done over many years with great success, as an important part of drugs are derived from constituents from nature and not from the creation of chemical diversity within the constraints of the laboratory. However, this approach for bioactivity screening removes the important basis of multiple component intervention, inducing synergy being the basis of TCM's holistic approach. This leads to multi-target of multi-dimensional pharmacology based discoveries and strategies.

It is clear that systems biology can provide an important bridge function between the two complementary approaches used in TCM and Western medicine, because it can reveal the effects of simple perturbations such as a single drug and/or complex perturbations such as TCM or food. The use of systems biology to study the effect of TCM on humans is very promising but also one of the most complex challenges today in life science research. Moreover, within TCM an additional complicating factor is present, namely the quality control of the production.

The optimal bioactive fingerprint of the many components in a TCM preparation is not known. There is no direct control mechanism on the production and processing of plants and therefore it may cause a varying success factor in the evaluation process. In fact, differences in harvesting conditions that occur even during intervals as short as less than one day may already generate differences between batches. A more basic consideration in the evaluation of TCM comes from the fact that it is prescribed as a personalized preparation. Consequently, it cannot be evaluated in the more conventional way by applying clinical trials and generic treatments.

All the complicating factors mentioned above can be addressed in well-designed studies if technologies are used based on fingerprinting of not only the system but also the complex perturbation mixtures and linking both together using non-linear multivariate approaches. This paper describes the basic technology needed in systems biology and some first applications within the TCM field, as well as outlining potential approaches for future research studies.

SYSTEMS BIOLOGY: THE METABOLOMICS TECHNOLOGY PLATFORM

The technology platform used in systems biology comprises the elements as given in Fig. 1: transcriptomics, proteomics and metabolomics, at different levels: cell, tissue, organ, organism and system level. In addition, special attention is paid to profiling body fluids being an important source of information regarding the systems control functions or its biochemical 'body'-language. The extensive mapping of body fluids brings a new dimension into human physiology and is especially suited for the evaluation of the effects of treatments. Typical fluids are urine, blood, CSF, saliva, lymph, synovial fluid, etc. The ethical consideration favours urine and blood, which opens up especially the elements of proteomics and metabolomics measurements (Morel *et al.*, 2004; Lamers *et al.*, 2003b). In selected cases transcriptomics can be used in monitoring studies when blood cells are harvested or tissue biopsies are available (Van der Greef *et al.*, 2002). As for TCM, the medical preparation itself is also of a complex and changing character. This introduces a second set of variables. To make it even more challenging, not only the relative composition of a remedy is subjected to variation, but the quality of the starting materials may also differ.

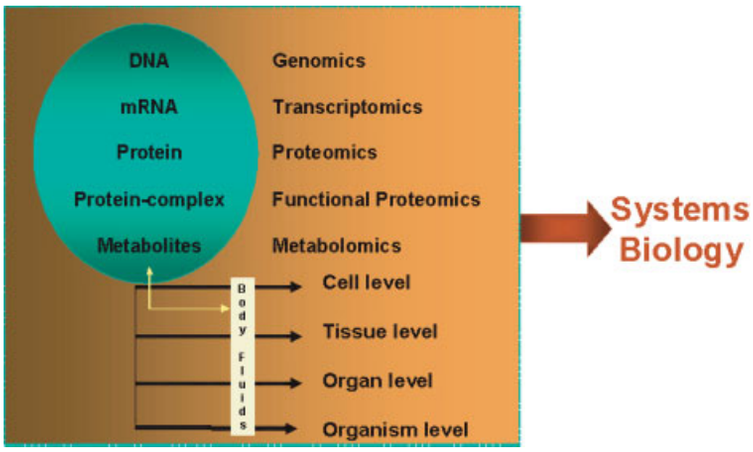

Figure 1. The different levels of measurement in a systems approach.

QUALITY AND VARIATION OF THE STARTING MATERIAL

In TCM more than 80% of the constituents of the preparations are derived from plants. Like any other organism, plants are constantly interacting with their changing, and often harsh, environment during the several phases of their life cycle. Plant secondary metabolites provide chemical protection against invading pathogens and predators to attract, for example, pollinators, and physical stress but may also act to give a typical smell or colour. Plants can make several thousands of these secondary metabolites. This has resulted in a natural treasure house with highly diverse and often very potent compounds with a wide diversity of application in human health (Verpoorte *et al.*, 1999). A complicating factor when using plants is the variability of the material. This can be caused by differences occurring during growth, but also after harvesting the plant, due to decomposition during post-harvest processing, extraction and preparation. Quality control and standardization are therefore highly relevant to assure proper preparation and standardization of a medication. The importance of metabolomics for quality control for phytomedicine has been described elegantly in a recent article (Wang *et al.*, 2004). However, standardization is still a matter of debate. Even the latest (2002) EMEA (European Agency for the Evaluation of Medicinal Products) guidelines for Good Agricultural Practice (GAP) for starting materials of herbal origin do not provide rules for standardization (Scholten, 2003). This is illustrated by two examples from our own laboratory, showing the cultivar dependent variation and the effects of time of harvesting on the chemical composition of plants. Figure 2 shows the effect of harvesting time on some groups of the metabolites of *Ginkgo biloba* leaves. Leaves were harvested from trees grown under identical conditions (soil, stress and weather conditions) in early morning and late evening. Methanol extracts of the leaves were analysed for the presence of secondary metabolites, e.g. ginkgolides, bilobalides and flavonoids by means of TLC. Harvesting the leaves after a light period dramatically increased both the ginkgolide and bilobalide content. Not only the quantity but also the quantitative composition of the secondary metabolites was affected

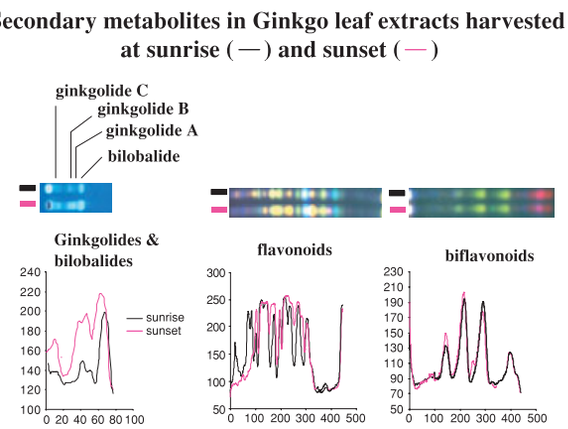

Figure 2. Effects of harvesting time (sunrise and sunset) on metabolites in Ginkgo leaves. Ginkgolide, bilobalide, biflavonoid and flavonoid content in *Ginkgo biloba* leaves at sunrise (red line) and at sunset (black line); results of thin layer chromatogram (TLC, above) and relative density as a function of the R_f-value (arbitrary units). Each spot in the TLC represents at least one substance in the extract, its density corresponding to its concentration. It can be seen that many substances are present in higher concentrations at sunset than at sunrise.

by the time of harvesting. This effect was most pronounced for the flavonoid content. Figure 3 shows a similar phenomenon, namely the cultivar dependent variation in metabolites in *Cannabis sativa*. Flower tops or leaves were harvested from cannabis plants grown under identical conditions (soil, stress and growth chamber). Chloroform or methanol/water extracts of the flower tops or leaves were analysed for the presence of secondary metabolites. These results demonstrated that the metabolites in the flowers and leaves were quite different. The metabolomic profile of the flower extract from cultivar CS18 was different from cultivars CS4 and CS12, whereas the metabolic profiles of cultivar CS4 and CS12 were very similar. Moreover, light and darkness have also significant effect on cannabinoid concentrations and total metabolite profile as measured by HPLC and NMR (data not shown)

The importance of the use of plant metabolomics for characterizing medicinal plants can be further illustrated with the results of the analysis of the total metabolite profiles of *Ginkgo* preparations available on the Dutch market. ^{1}H-NMR analysis of the extracted metabolites

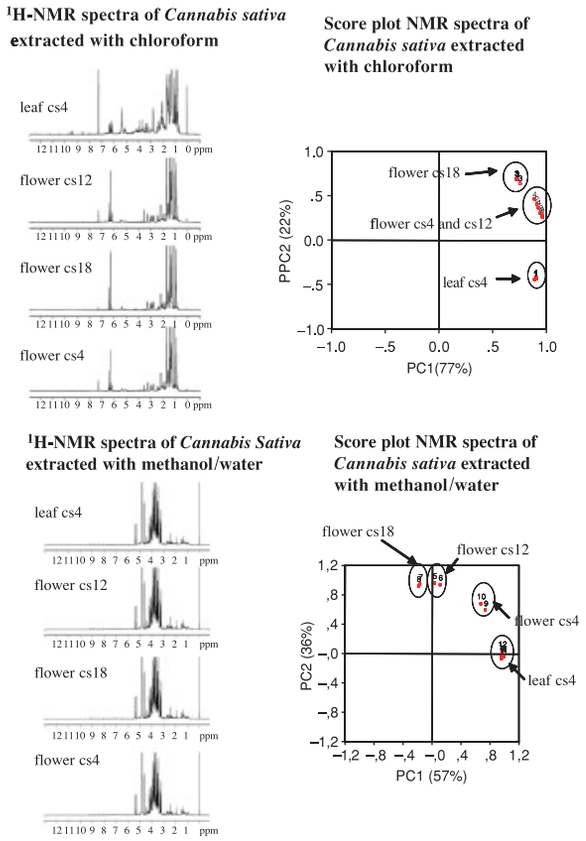

Figure 3. Cultivar dependent variation on metabolites in *Cannabis sativa.* Metabolites in the various chloroform and methanol/water extracts were profiled by means of ^{1}H-NMR-spectrometry and spectra analysed by multivariate data analysis. 400 MHz ^{1}H-NMR spectra of $CHCl_3$ extract of *Cannabis sativa* flower tops in $CDCl_3$. Score plot of principal component analysis of the $CHCl_3$ extracts of *Cannabis sativa* flower tops (upper panels). 400 MHz ^{1}H-NMR spectra of MeOH/water extracts of *Cannabis sativa* flower tops in KH_2PO_4 buffer (pH 6.0) Score plot of principal component analysis of the MeOH/water extracts of *Cannabis sativa* flower tops (lower panels).

showed that only one out of six different preparations contained the ginkgolides thought to be responsible for the activity of this medicinal plant (Table 1). In fact in many studies on the activity of medicinal plants, and in particular in clinical studies, the plant material was not properly defined, making the results very doubtful. At the same time with all the possible variables it is almost impossible to measure each separately to determine which one is the more active. Again by using the multivariate analysis of the data from either the treated animals or patients and of the metabolomes of the different preparations tested, the optimal composition can probably be calculated.

More recently, novel techniques such as Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-MS) represent a quantum leap forward in the capabilities of mass spectrometers for metabolite analysis. Due to the exceptionally high resolution of these instruments, metabolites with mass differences of less than 2 ppm can be separated on a chromatographic time scale (Van der Greef *et al.*, 2004). The accurate masses obtained give elemental compositions, which enable unequivocal metabolite identification. The power of such a set up is illustrated by the analysis of a *Ginseng* extract by nano-LC-FT-MS. A capillary C_{18} column with an internal diameter of 50 μm was used, the tapered tip

Table 1. Analysis of ginkgolic acid in six commercial preparations

Code	GC method	NMR method
KG1	20.79 μM/g	18.35 μM
KG2	4.77 μM/g	9.05 μM
KG3	0.00 μM/g	0.00 μM
KG4	17.82 μM/g	38.31 μM
KG5	61.74 μM/g	112.83 μM
KG6	8.64 μM/g	55.54 μM

Analysis of six different commercial registered ginkgo preparations in Dutch pharmacies for the present of ginkgolic acid by means of gas chromatography and ^{1}H-NMR spectrometry. The NMR method gives the total of all ginkgolic acids; GC result represents the sum of the acids that could be identified by means of comparison with reference compounds.

of the column served as nanospray ion source. Figure 4 presented an overview of all positively charged ions detected during a single analysis. Several hundreds of compounds were detected. Saponins, typical constituents of *Ginseng*, have a molecular mass between 600 and 1300 Da. For a dammarene-type of saponin the mass trace at *m/z* 801.49948, with a tolerance of only 10 ppm, is shown in Fig. 5A. The accurate mass indicated a molecular composition of $[C_{42}H_{72}O_{14}]$ H^+ (Fig. 5B). These types of data are essential in metabolite identification. Normally, the question of identity arises very quickly after comparison of metabolite profiles.

THE POTENTIAL OF PLANT METABOLOMICS FOR TCM

Plant metabolomics provides one of the pillars for studying the relation between the composition of complex and variable mixtures of plant-derived remedies and their—also complex—biological effects. Plant metabolomics starts with the analysis of as many as possible detectable individual components that are present in the material. Extracts made from individual herbs/plants, total mixtures or combinations of individual herbs/plants and extraction/mixing/preparation-methods as used in TCM can be analysed by means of different techniques (LC-MS, GC-MS, NMR, etc.), resulting in total metabolite profiles.

Next, extracts (individual, total, combinations) are investigated for bioactivity (effects in cell lines, animal models, human volunteers) studying their effects at various biological levels using the above mentioned body fluid studies. The databases from plant metabolic profiles, bioactivity and animal studies can be linked and analysed by means of multivariate data analysis (MVDA). MVDA is a powerful technique for the analysis of data sets with a large number of variables (e.g. Sumner *et al.*, 2003). It enables, for example, the visualization and interpretation of patterns in NMR data that correlate with a target variable such as bioactivity. In this way, a plant metabolomic database can be constructed which will be extended with single compounds and metabolic profiles of all types of commercial extracts available from vegetables/crops/herbs that were grown, harvested and stored under different conditions.

In the past a major tool in identifying new activities was testing unknown compounds or extracts on whole

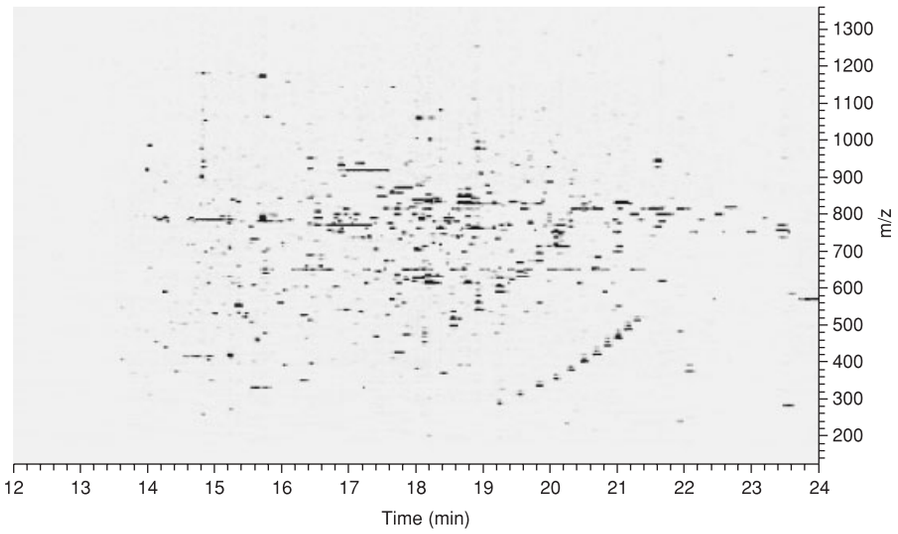

Figure 4. An ion map obtained from a nano-LC-MS analysis of a Ginseng extract indicating the large number of metabolites which can be profiled in a single analysis. The ion map shows the retention time on a nano-C_{18} reversed phase system versus the *m/z* value of the individual ions (metabolites). The MS was a LTQ-FTMS (Thermo), which is an ion trap based Fourier-Transform Ion Cyclotron Resonance mass spectrometer.

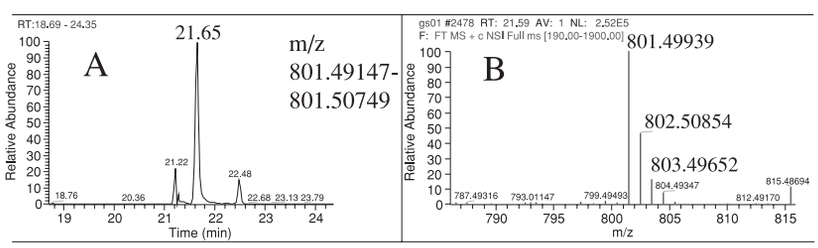

Figure 5. Nano-LC-MS analysis of a Ginseng extract (see also Fig. 1). (A) Mass trace of a known dammarene-type of saponin at *m/z* 801.49948 with a tolerance of 10 ppm. (B) Mass spectrum (FT-MS) at 21.6 min. The difference between measured mass and theoretical mass was 0.12 ppm.

animals and making a whole set of observations at regular time intervals (Hippocratic screening) (e.g. Malone and Robichaud, 1962). By validating this method with various known compounds, it was very useful for the identification of novel activities. However, for determining which were the active compounds this approach had the disadvantage that it was elaborate and quite large samples were needed, something which is difficult to realize with bioassay guided fractionation. By administering plant extracts of different composition and using MVDA, it will be possible to calculate which compounds or groups of compounds are associated with the highest bio-activity.

THERAPEUTIC SYNERGY IN HERBAL MEDICINE

Synergy is an aspect that will be lost in a target driven single lead discovery programme with TCM. For example, the National Cancer Institute of the USA and the USD department of agriculture have screened 35 000 samples from different tissues from 12 000 plant species and only three new drugs were discovered (Yuan and Lin, 2000). Nevertheless, the ancient Oriental pharmacopoeias contain thousands of therapeutic formulations, indicating that the biological activity of these preparations might result from synergy of active compounds rather than from a single chemical entity. Such synergistic activities have, for example, been reported for the antimicrobial activity of the alkaloid berberine that is 100 times enhanced by 5′ methoxyhydnocarpin (5′ MHC). 5′ MHC is a compound found in the same plant as the alkaloid but it has no antimicrobial effect (Stermitz *et al.*, 2000). Synergistic effects were also found to occur in the activity of *Ginkgo* extracts, for example for their anticlastogenic, antioxidant, vasoregulatory, cognition-enhancing, stress alleviating and gene-regulatory effects (Roy *et al.*, 1998; Alaoui-Youssefi *et al.*, 1999; Curtis *et al.*, 1999). For cannabis, tetrahydrocannabinol (THC) has often been considered as the major active component, but synergy with other compounds present in the cannabis extract had already been reported by the early 1970s (Mechoulam, 1972; Carlini and Karniol, 1974) Recently, Williamson and Evans (2000) suggested that THC in the presence of cannabidiol (CBD) and possibly some other compounds, may be more beneficial as a medicine than the single compound. The synergetic effect of THC and CBD in cannabis was recently proven (Williamson and Whalley, 2002). The antiproliferative activity of pomegranate juice extract is enhanced by both peel and seed extracts (Lansky *et al.*, 2003, personal communication). Moreover, the synergism between solanine and chaconine in relation to destabilization of synthetic phosphatidylcholine-sterol liposomes (Roddick and Rijnenberg, 1987) was shown to hold also for membranes of living cells from taxonomically diverse groups and are responsible for the antifungal activity

(Roddick *et al.*, 1988). The synergism between solanine and chaconine on the antifungal activity extended over a wide range of glycoalkaloid ratios (Roddick *et al.*, 1990). Obviously, the above described synergism between different compounds originated from one plant species. However, it would be impossible to use a single bioassay to demonstrate the efficacy of a multi-herb mixture, with the polyvalent components plus synergism aiming multi-targets in a living organism.

The potential of the systems biology approach in understanding the activity of phytomedicine can be demonstrated with some examples. Probably the best example is one of the most successful medicines ever, acetylsalicylate. This compound was synthesized more than 100 years ago. The expected activity was based on the use of the bark of *Salix* to treat pain and headaches. But interestingly in the bark no salicylate is found, instead salicin is present. This compound is a glucoside, after hydrolysis it gives saligenin (salicylic alcohol) which in the gut is oxidized to yield salicylic acid. In other words this medicinal plant does not contain an active compound, but a pro-drug (e.g. Jack, 1997). Systems biology would easily register the effect and link this with compounds present in the plant material which need to be present to obtain activity. Even in the case of the presence of salicylate itself in a medicinal plant, it is doubtful if by conventional screening salicylic acid (SA) would have been selected for development.

Plants have provided a multitude of life-saving drugs. Fourteen of the drugs in current use for cancer chemotherapy occur naturally, and five of these originate from plants (Talalay and Talalay, 2001). The re-discovery of natural compounds with its synergism in the concept of systems biology will open a new era for drug discovery.

LINKING A MULTI-COMPONENT PREPARATION WITH ITS COMPLEX PATTERN OF ACTIVITY

The next step is to correlate complex pharmacological or toxicological effects that are elicited by a highly complex medical preparation. This requires input from bioinformatics and computational biology. This multidisciplinary combination is extremely powerful and the outcome will provide support for efficacy claims and also produce numerous opportunities for new discoveries, including new bioactives, molecular targets etc. In our institute we apply different models to study this. A relatively simple approach is depicted in Fig. 6. In our cannabis research programme we compare different cultivars and preparations using *in vitro* models to measure effects. In this example, the effects on a cell are analysed in a holistic way, using the proteome or the transcriptome. One of the goals is to link specific cannabinoids or combinations of cannabinoids with cellular effects. This will enable us to unravel new mechanisms and find new bioactives.

One level of integration higher is the analysis of *in vivo* effects. Effects of medication are studied in animal models or humans using urine, blood, CSF, saliva, lymph or synovial fluid as matrices to study all metabolites and proteins. In selected cases, transcriptomics can also be used in monitoring studies when blood cells are harvested or tissue biopsies are available. An example of this approach from our own research is the studies done with *Ginkgo* in rats and human volunteers. Here a metabolomics approach was used taking urine as a matrix that can be collected noninvasively. For this, a combination of ^{1}H-nuclear magnetic resonance spectroscopy (^{1}H-NMR) and MVDA was applied. ^{1}H-NMR is a very suitable technique to analyse biofluids because it provides both quantitative as well as qualitative information without sample preparation. Nevertheless, due to the complexity of the mixture of metabolites, MVDA is needed to find significant differences in metabolic NMR profiles that can not be recognized visually.

Rat studies

Urinary ^{1}H-NMR spectra were classified in three groups as control versus *Ginkgo* treatment of mildly stressed rats versus *Ginkgo* treatment of unstressed rats. Supervised MVDA was performed on the urinary spectra. The resulting components were plotted to visualize clustering based on urinary NMR spectra. The resulting score plot shows (Fig. 7, left) that the three groups can be discriminated based on small differences in urinary ^{1}H-NMR spectra. Compared with control rats,

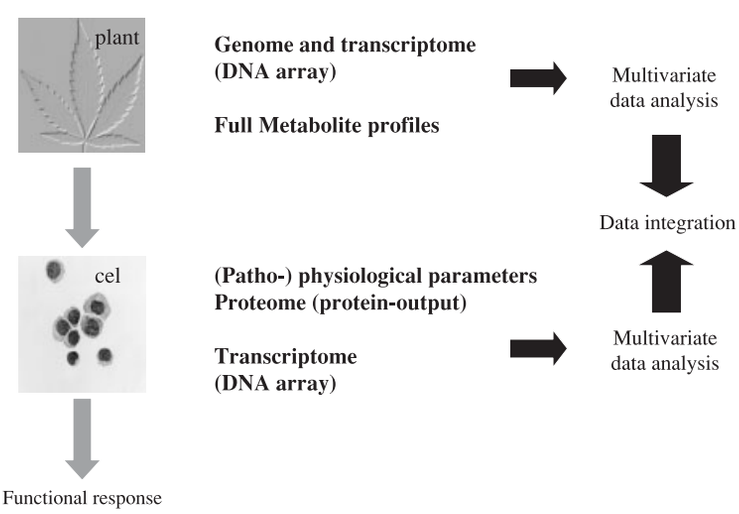

Figure 6. Schematic representation of the *in vitro* analysis of effects of Cannabis preparations on cultured cells.

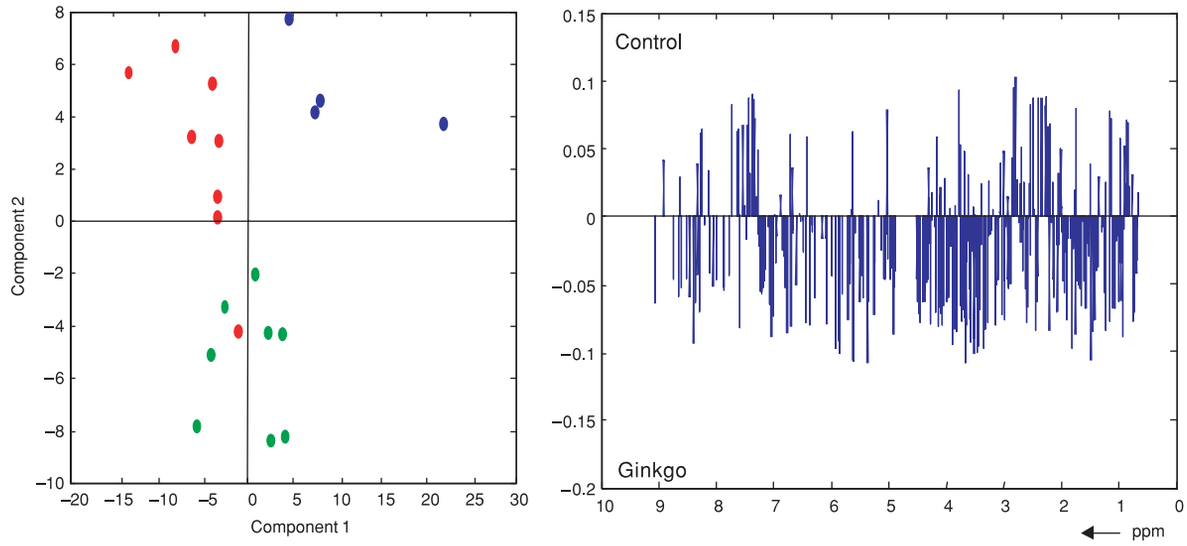

Figure 7. Effects of Ginkgo leaf extracts on rat. Score plot (blue, controls; red, Ginkgo treated; green, Ginkgo treated, mildly stressed) of urinary NMR spectra of rats treated with Ginkgo versus control rats (left, each point represents a complete NMR spectrum). The shift of the Ginkgo treated groups in horizontal direction away from the controls can be ascribed to an effect of Ginkgo. The shift in vertical direction is supposed to reflect stress. The factor spectrum (right) represents NMR signals, and thus metabolites, that are lower in control than treated animals (negative direction) and larger in control than in treated animals (positive direction) in urine of rats treated with Ginkgo.

the *Ginkgo* treated groups were shifted in a horizontal direction. *Ginkgo* is thought to be responsible for this effect. The shift in vertical direction is supposed to reflect stress. NMR signals, and thus metabolites, that are responsible for the separation in the horizontal direction, the *Ginkgo* effect, are visualized in a so-called metabolic fingerprint (Fig. 7, right).

Human volunteer studies

One of the aims of this study was to investigate whether *Ginkgo* has an effect on blood flow. The peripheral blood flow of volunteers was measured in the foot before and after *Ginkgo* treatment. Subsequently, supervised MVDA was performed on the urinary NMR spectra of the subjects. Spectra were classified in three groups as subjects showing no effect on blood flow upon *Ginkgo* treatment *versus* subjects with increased blood flow *versus* subjects with decreased blood flow upon *Ginkgo* treatment. The resulting components were plotted to visualize the grouping of the urinary NMR spectra in relation to the blood flow and *Ginkgo* treatment. The score plot (Fig. 8) shows that a separation between the groups can be made based on the urinary NMR spectra. The separation between groups reflects the effect of *Ginkgo* on the blood flow. Subjects with an increasing blood flow upon *Ginkgo* treatment have a different urinary metabolic composition to those subjects with a decreasing blood flow or subjects showing no effect (Boelsma *et al.*, 2004).

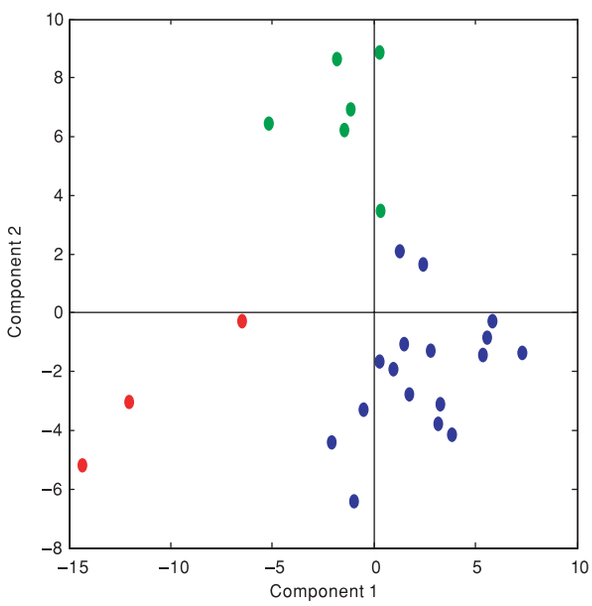

Figure 8. Effect of Ginkgo on human volunteers. Score plot (red, no effect upon Ginkgo treatment; blue, descending blood flow; green, ascending blood flow) showing urinary NMR spectra of volunteers treated with Ginkgo (each point represents a complete NMR spectrum). Subjects with an increasing blood flow after treatment have a different urinary NMR spectrum, and thus metabolic composition, than subjects with a decreasing blood flow respectively subjects showing no effect upon treatment.

TCM INTERVENTION FOR METABOLIC SYNDROME

Metabolic syndrome is characterized by insulin resistance and is closely linked to excess body fat and a sedentary lifestyle. Also known as syndrome X, the condition can lead to serious medical problems, including severe obesity, type 2 diabetes, high blood pressure, heart disease and stroke. Because the condition itself typically causes no symptoms, most people are unaware they have it until, like the Trojan horse of Greek mythology, it starts to attack its host from within, with devastating results.

APOE*3Leiden (E3L) transgenic mice exhibit elevated plasma cholesterol and triglyceride levels, mainly confined to the VLDL/LDL lipoprotein fraction

(Van Vlijmen *et al.*, 1996). Extensive previous research showed that, in contrast to wild-type mice, E3L mice are highly responsive to sucrose, fat and cholesterol feeding as far as the effects on plasma VLDL and chylomicron levels are concerned. In addition, E3L mice are also sensitive to high fat-induced insulin resistance (e.g. Muurling *et al.*, 2002). In contrast, the plasma cholesterol and triglyceride levels are very low in normal wild-type mice and (almost) not responsive to diet and hypolipidaemic drugs. Since the APOE*3-Leiden transgenic mice are highly responsive in their plasma lipids to dietary treatment, their plasma total cholesterol levels can easily be titrated to a desired level by varying the amount of cholesterol in the diet. Upon high fat and cholesterol feeding, these mice develop various stages of atherosclerotic lesions depending on plasma total cholesterol levels and resembling those found in humans. The male mice are used as a diabetes type II model while the female mice are used as an arteriosclerosis model. In conclusion, the E3L mouse represents a suitable animal model for the investigation of the following aspects (Jong *et al.*, 2001; Van der Hoek *et al.*, 2004; Duivenvoorden *et al.*, 2004):

- the effects of drugs and dietary compounds on plasma cholesterol and triglyceride levels;
- The effect of cholesterol-lowering and anti-atherosclerotic drugs and nutrients on the development of atherosclerosis;
- The effects of drugs and dietary compounds on insulin sensitivity.

Therefore, this mouse model can be used for studying metabolic syndrome.

Effects of three TCM formulas (A, B and C) in the male APOE*3Leiden (E3L) transgenic mouse have been investigated for their effects on insulin resistance, an early stage of metabolic syndrome, combining two sophisticated techniques/platform: (1) *euglycaemic clamp* (to measure insulin resistance, Voshol *et al.*, 2001; (2) *metabolomics platform* (to identify biomarker fingerprints, e.g. Van der Greef *et al.*, 2002; Morel *et al.*, 2004). Figure 9A shows the readout of the experiment as the glucose infusion rate (GIR) and when this value is low, it translates in a high insulin resistance. The control group has a high insulin resistance after the high feed period. Low ALAT values indicate low liver damage, so a favourable liver safety profile. The results demonstrated that both A and B TCM preparations showed a moderate effect on insulin resistance and C preparation was the most effective: no liver toxicity was observed (see ALAT values in Fig. 9A).

A mass spectrometry based method (LC-MS) was capable of generating robust fingerprints of complex blood samples. The lipid platform measures a wide range of lipids in plasma and the outcome is visualized in Fig. 9B. It is a principal component analysis of the LC-MS profiles obtained at different time points: 0, 4 and 7 weeks. It reflects the longitudinal changes and the control (disease state) can be used as a reference. Clearly preparation C demonstrates the strongest effect as a function of time, which has been shown to detect differences—biomarkers—in lipid patterns. Other multivariate analyses have demonstrated in more detail the time profiles related to the effect (not given here). The platform yields information on the system effects related to lipid metabolism, time effects, and time-dose effects and is an easy tool to compare different herbal mixtures.

CONCLUSIONS AND PERSPECTIVES

The impressive developments in genomics, transcriptomics, proteomics, metabolomics and bio-informatics now enable an in-depth scientific approach of TCM using the integrative approach of systems biology. Moreover, recent developments show that TCM and Western medicine may have more in common than previously assumed.

Systems biology will prove to be a valuable tool in TCM research by providing the following deliverables:

1. Proof of concept and efficacy/safety studies with TCM preparations
2. Mechanistic studies and development of biomarkers and surrogate endpoints
3. Fingerprinting of TCM-products, to guide quality control and production
4. Discovery and validation of new drug targets
5. Discovery of new leads

The important next step will be the recognition that any mental change is followed by a physical change and mapping human physiology in relation to psychology

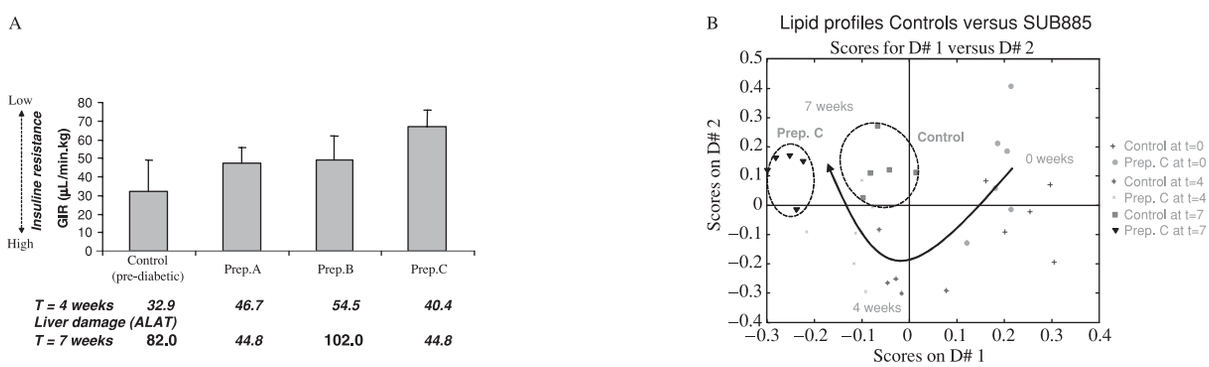

Figure 9. Effects of three TCM formulae on insulin sensitivity and the profile of lipid metabolites. Male heterozygous APOE*3Leiden mice were fed with a high fat diet for 10 weeks. At week 10, the mice were randomized on the basis of plasma cholesterol and triglyceride levels into different groups of 10 mice each. Then the mice were further fed with high fat plus TCM mixtures (preparation A, preparation B and preparation C) for 8 weeks. The control was without addition of TCM mixture. A: Glucose infusion rate was a measure of insulin resistance (Voshol *et al.*, 2002). B: Longitudinal principal component discriminant analysis (PCDA) of lipid fingerprints during a high fat diet with preparation C at 0, 4 and 7 weeks.

will be an important step forwards in starting to understand the body–mind interactions and appreciating the underlying mechanisms of placebo-effects. Not only strong mental changes as stress factors but also more subtle hidden aspects might be discovered, which would really be a step forward towards a true holistic approach in life sciences. Studying homeostasis especially linked to its dynamic and non-linear character will be the core of future systems biology research. In many clinical studies the availability of samples is often at the body fluid level only, but this is exactly the source of the communication and control signals at the biochemical level which can provide the systems characteristics necessary to understand health and disease. The coupling of this information with non-invasive imaging techniques opens the way to couple physiological mapping with neurosciences. Ultimately the full bioactivity of phytomedicine in terms of the interplay of the body–mind interaction can be studied, which will be a base for further studying personalized medication.

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