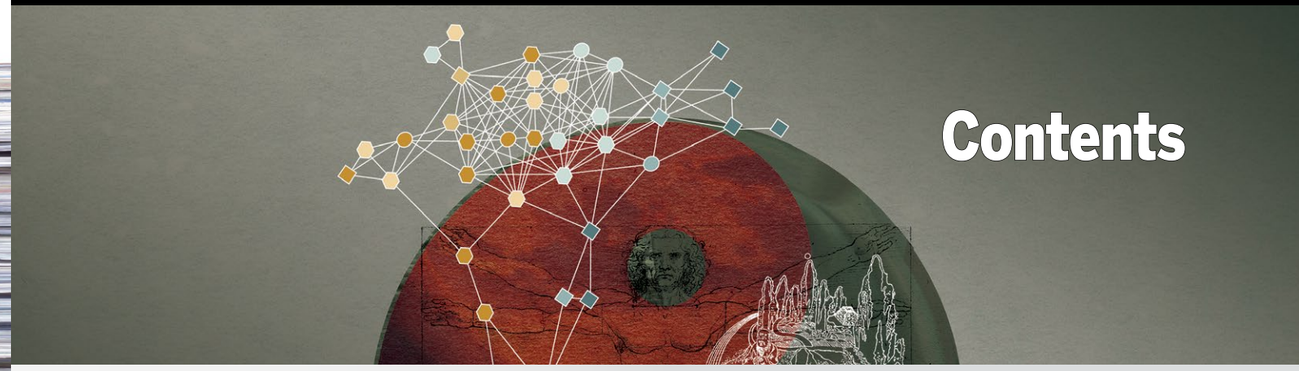
Produced by the *Science*/AAAS Custom Publishing Office

The Art and Science of Traditional Medicine
Part 3: The Global Impact of Traditional Medicine

Sponsored by

中医药规范研究学会
GP-TCM
Research Association

Produced by the *Science*/AAAS Custom Publishing Office



Contents

It is appropriate and timely that Chinese scientist Youyou Tu was awarded half of the 2015 Nobel Prize in Physiology or Medicine in recognition of her pioneering work on the antimalarial artemisinin, extracted from *Artemisia annua*, an ancient herbal remedy used to treat fever. This third issue in the Art and Science of Traditional Medicine series features another time-honored herb, ginseng. Also discussed are the systems and network pharmacology of TCM, pharmacognosy and regulation of traditional medicine in Europe, and how these best practices can be applied globally, but particularly in Africa. Attention garnered by the Nobel award hopefully will generate interest in traditional medicines from other parts of the world, including the Middle East, the Indian sub-continent, and the Americas.

Editorial Team

Tai-Ping Fan, Ph.D. (Guest project editor)
University of Cambridge, UK

Josephine Briggs, M.D.
National Center for Complementary & Alternative Medicine, NIH, USA

Liang Liu, M.D., Ph.D.
Macau University of Science & Technology, Macau SAR, China

Aiping Lu, M.D., Ph.D.
Hong Kong Baptist University, Hong Kong SAR, China

Jan van der Greef, Ph.D.
University of Leiden and TNO, The Netherlands

Anlong Xu, Ph.D.
Beijing University of Chinese Medicine, China

Editor: Sean Sanders, Ph.D.
Assistant Editor: Tianna Hicklin, Ph.D.
Proofreader/Copyeditor: Bob French
Designer: Amy Hardcastle

Bill Moran, Global Director
Custom Publishing
bmoran@aaas.org
+1-202-326-6438

Ruolei Wu, Associate Director, Asia
Custom Publishing
rwu@aaas.org
+86-186-0082-9345

Articles

ILLUSTRATION (FRONT) CHARLOTTE LOKIN

The content contained in this special, sponsored section was commissioned, edited, and published by the *Science*/AAAS Custom Publishing Office. It was not peer-reviewed or assessed by the Editorial staff of the journal *Science*; however, all manuscripts have been critically evaluated by an international editorial team consisting of experts in traditional medicine research selected by the project editor. The intent of this section is to provide a means for authors from institutions around the world to showcase their state-of-the-art traditional medicine research through review/perspective-type articles that highlight recent progress in this burgeoning area. The editorial team and authors take full responsibility for the accuracy of the scientific content and the facts stated. Articles can be cited using the following format: [Author Name(s)], *Science* **350** (6262 Suppl), Sxx-Sxx (2015).

Produced by the *Science*/AAAS Custom Publishing Office

Ginseng: A panacea linking East Asia and North America?

Authors:
Ran Joo Choi[1], Alice S. T. Wong[2], William Jia[3], Il-Moo Chang[4], Ricky N. S. Wong[5], Tai-Ping Fan[1*], Yeong Shik Kim[6*]

According to ancient Chinese medical literature and Korean history, ginseng has been used since around 2000 BCE. It has been regarded as a very precious medicinal plant, on par with poppy, aloe, and garlic, the use of which goes back to the same period in other parts of the world. It is not surprising that the name *Panax*—meaning "all healing" in Greek—has been applied to this plant, because it has been used to treat various diseases from ancient times, and is also recognized, especially in Asian countries, as a health supplement that can increase energy and instill a sense of well-being. To date, fourteen species belonging to the *Panax* genus have been identified, and three species are widely circulated on the global market: *Panax ginseng* C.A. Meyer, cultivated mainly in Korea and northeastern China; *Panax quinquefolius* L. (American ginseng), grown mainly in the Canadian provinces of Ontario and British Columbia and the American state of Wisconsin; and *Panax notoginseng* Burkill, found in southern China (*1*).

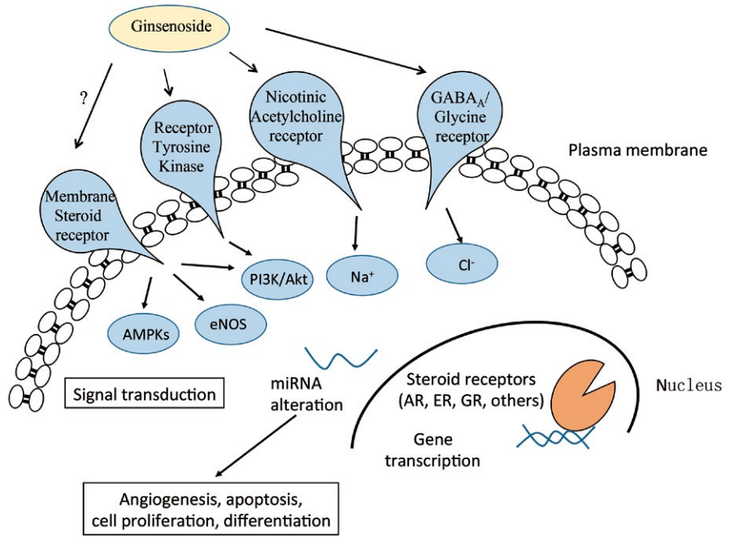


FIGURE 1. Schematic representation of genomic and nongenomic actions by ginsenosides. Ginsenosides can act through genomic effects by binding to steroid hormone receptors, such as androgen receptors (AR), estrogen receptors (ER), and glucocorticoid receptors (GR), to modulate gene expression. On the other hand, nongenomic activities, such as phosphoinositide 3-kinase/Akt (PI3K/Akt), adenosine monophosphate-activated protein kinases (AMPKs), and endothelial nitric oxide synthases (eNOS) that occur outside the nucleus can also be involved in the mechanisms of action (MOAs) of ginsenosides. Ginsenosides are also implicated in ion channel regulation that includes the nicotinic acetylcholine receptor that results in sodium ion (Na^+) influx and the $GABA_A$/glycine receptor that conducts chloride (Cl^-) ions. In addition, ginsenosides can be a regulator of microRNAs (miRNAs) that modulate angiogenesis, apoptosis, cell proliferation, and differentiation.

History and use

P. ginseng is likely to have originated in Manchuria (now the northeast part of China) and in the ancient Three Kingdoms of Korea (*2*). The first description of ginseng in the history of traditional Chinese medicine appeared in the pre-Han era (BCE 33–48), over 2,000 years ago. In 1713, the Royal Society published a letter from Father Jartoux, a Jesuit missionary in China, containing a description of ginseng's botany, habitat, and medicinal uses (*3*). *P. quinquefolius* was discovered by American settlers in the mid-1700's in New England. This plant had long been used by the Native Americans, who valued the root for its curative powers and life-enhancing capabilities. Ginseng has purported use for the treatment of cancer, diabetes, and cardiovascular dysfunctions, as well as for cognitive enhancement with an apparently low rate of adverse effects. In combination with other materia medica, *P. ginseng* and *P. notoginseng* have been used in complex Chinese formulations for treating angina pectoris (*4, 5*).

[1]Department of Pharmacology, University of Cambridge, Cambridge, United Kingdom
[2]School of Biological Sciences, University of Hong Kong, Hong Kong, China
[3]Davad Mowafaghian Centre for Brain Health, University of British Columbia, Vancouver, Canada
[4]R&D Headquarters, Korea Ginseng Corporation, Daejeon, South Korea
[5]Department of Biology, Hong Kong Baptist University, Hong Kong, China
[6]World Health Organization Collaborating Centre for Traditional Medicine, Natural Products Research Institute, Seoul National University, Seoul, South Korea
*Corresponding Authors: tpf1000@cam.ac.uk (T.P.F.), kims@snu.ac.kr (Y.S.K.)

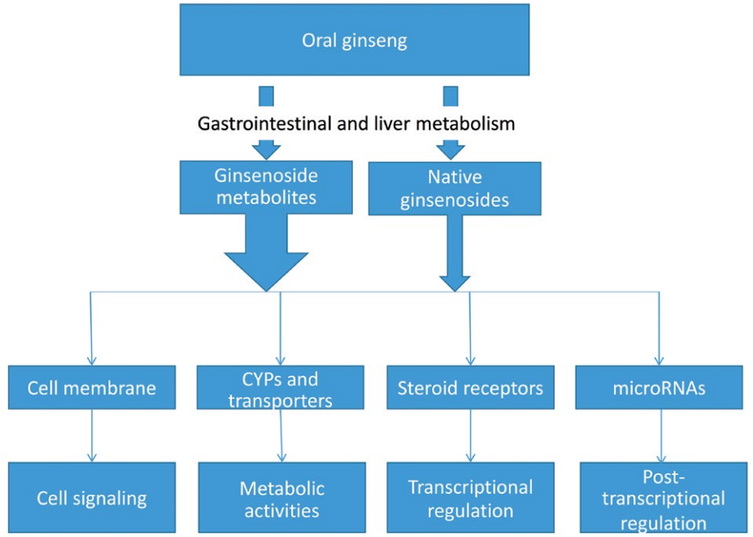


FIGURE 2. Metabolism of ginseng. Ginsenosides can be converted into their metabolites that may contribute the majority of bioactivities by regulating the transportation and metabolism of crucial substances in the human body. Metabolism mainly occurs in the intestine and the liver by adenosine triphosphate (ATP)-binding cassette transporters (ABC transporters), cytochrome P450 enzymes (CYPs), and others.

Processing, chemistry, and metabolism

Most ginseng in today's market is cultivated in the field for 4 to 6 years. Ginseng is classified into three types, depending on how it is processed after harvest: fresh ginseng (can be consumed in its fresh state), white ginseng (dried after peeling), and red ginseng, which requires special preparation skills, such as steaming and drying under specific conditions. Technology for the long-term storage of red ginseng was developed by pioneers in ginseng manufacture, securing the foundation for this form of the root. The process of steaming stabilizes the ginseng with regard to metabolism, and transforms the secondary metabolites into less polar phytosteroids that are thought to be both more active in the body and safer.

The active ingredients in ginseng include ginsenosides and polysaccharides. Ginsenosides belong to the saponin family and are divided into 20(*S*)-panaxadiols and 20(*S*)-panaxatriols, depending on the dammarane skeleton and the number of hydroxyl groups that can be substituted with other groups (*1*). The biological activities of these phytosteroids have been studied intensively with regard to their structure-activity relationships. Asian ginseng typically contains six types of ginsenosides: panaxadiols (Rb_1, Rb_2, Rc, and Rd) and panaxtriols (Re and Rg_1). In contrast, American ginseng contains high levels of Rb_1, Rd, and Re (*6, 7*).

Ginsenosides are extensively metabolized in the gastrointestinal tract after oral administration (*8*), with sugar moieties being removed to generate the aglycones, 20(*S*)-protopanaxadiol (aPPD), and 20(*S*)-protopanaxatriol (aPPT), and the partially deglycosylated ginsenosides. Since most native ginsenosides are either poorly absorbed in the intestines or are quickly metabolized by deglycosylation, oxidation, and esterification in the intestine or the liver, they could be regarded as "pro-drugs." Thus, understanding the pharmacokinetics and pharmacodynamics of native ginsenosides and their metabolites is critical for their clinical application.

Standardization

Currently, there are many ginseng products on the market and the quality control of these commodities is of paramount importance. Quality control of ginseng extracts and finished products is usually based on the determination of specific bioactive ginsenosides. Although the international standard ISO 17217-1:2014 specifies minimum requirements and test methods for ginseng seeds and seedlings (*9*), ginseng extract should also be standardized such that each batch contains an acceptable concentration range of active ingredients to guarantee quality and efficacy from product to product. Distinguishing between *P. ginseng* and *P. quinquefolius*, which have similar chemical and physical properties but seemingly different pharmacological activities, is a challenge. Recently, all known ginsenosides were identified by metabolomics using high-performance chromatography/mass spectrometry analysis, and this large data set was statistically analyzed. In a targeted analysis, ginsenoside Rf was confirmed as a chemical marker present in processed *P. ginseng*, but not in processed *P. quinquefolius* (*10*).

Diverse pharmacological activities via multiple mechanisms

Given the structural similarity between ginsenosides and steroid hormones, we hypothesized that ginsenosides function as receptor agonists, partial agonists, or antagonists depending on the microenvironment. As shown in Figure 1, ginsenosides act by binding to steroid hormone receptors, such as androgen, estrogen, and glucocorticoid receptors, to modulate gene expression (*11–14*). We have previously reported that the dominance of Rg_1 leads to angiogenesis, whereas Rb_1 exerts an opposing effect (*15*) through activation of glucocorticoid (*16*) and estrogen (*17*) receptors. In addition to their classic genomic effects, ginsenosides can also function through transcription-independent, nongenomic activation

of signaling cascades, such as phosphoinositide 3-kinase/Akt, adenosine monophosphate-activated protein kinase, and calcium signaling that occurs outside the nucleus (*18*–*23*) (Figure 1). Ginsenosides are also implicated in ion channel regulation, including voltage-dependent and ligand-gated ion channels, for the control of cardiovascular function and hypertension (*24*–*26*). Recent developments have also revealed ginsenosides to be an important regulator of microRNAs (miRNAs) (*27*–*30*). Moreover, messenger RNA-like, noncoding RNAs were identified in ginseng, suggesting that it might exert a regulatory role through miRNAs and small interfering RNAs (siRNAs) (*31*). Whether these small RNAs could affect our body function awaits further investigation (*32*). A number of studies have demonstrated that ginsenosides, and especially their metabolites, interact with cytochrome P450 enzymes (CYPs) and adenosine triphosphate (ATP)-binding cassette transporters (ABC transporters, including breast cancer resistance protein, BCRP) (*33*–*36*). Given the fundamental roles of ABC transporters and CYPs in the absorption, transportation, and metabolism of nutrients, hormones, and environmental toxins, it is plausible that ginseng may exert its wide-ranging biological effects and health benefits by modulating the transportation and metabolism of vital substances in the human body (Figure 2). Intriguingly, aPPD and aPPT are BCRP inhibitors and therefore potential chemosensitizers (*37*). Ginseng root also contains acidic polysaccharides that appear to play important roles in immune modulation (*38*). In addition, ginseng polysaccharides have shown antifatigue (*39, 40*) and antidiabetic (*41*) effects. However, although numerous studies have been done in vitro and in vivo, very few clinical studies exist.

Challenges and opportunities

Despite playing an important role as a health supplement and medicine in East Asia for millennia, the clinical efficacy of ginseng remains to be established through stringent evidence-based validation. The synthesis of ginsenosides, including the backbone and its glycosylated derivatives, is extremely challenging. This bottleneck limits the development of ginsenosides as drug candidates. Thus, developing novel techniques for enriching bioactive components in ginseng should be a top priority. For example, selective transformation of ginsenosides by specific intestinal microbes may provide a new opportunity for drug discovery. It is also an exciting prospect to obtain the full genome sequence of ginseng root as a precursor to manipulating the biosynthesis of specific ginsenosides and realizing a "ginsenoside factory" (*42*–*44*). A high-throughput, multidisciplinary approach should be developed to bring new insights into the molecular actions of ginsenosides and how the multiple, distinct signaling networks that it impacts are interconnected. Finally, more robust clinical trials should be designed and implemented. Only good clinical outcomes can instill faith in patients and the general public with regard to products derived from this time-honored treatment.

References

1. L. P. Christensen, *Adv. Food Nutr. Res.* **55**, 1 (2009).
2. "A History of Ginseng," in *Korean Ginseng*, H. W. Bae, Ed. (Korea Ginseng Research Institute, Republic of Korea, 1978), pp. 12–74.
3. J. H. Appleby, *Notes Rec. Roy. Soc.* **37**, 121 (1983).
4. X. Zhao *et al.*, *Science* **347**, S38 (2015).
5. R. Liu *et al.*, *Science* **347**, S40 (2015).
6. N. Fuzzati, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **812**, 119 (2004).
7. J. B. Wan *et al.*, *J. Sep. Sci.* **30**, 825 (2007).
8. L. W. Qi *et al.*, *Curr. Drug Metab.* **12**, 818 (2011).
9. International Standards Organization Online Browsing Platform, ISO 17217-1:2014(en), "Traditional Chinese Medicine—Ginseng Seeds and Seedlings—Part I: Panax Ginseng C.A. Meyer"; available at https://www.iso.org/obp/ui/#iso:std:iso:17217:-1:ed-1:v1:en.
10. H. W. Park *et al.*, *J. Ginseng Res.* **38**, 59 (2014).
11. Y. J. Lee *et al.*, *Mol. Cell Endocrinol.* **133**, 135 (1997).
12. Y. N. Lee *et al.*, *J. Steroid Biochem. Mol. Biol.* **67**, 105 (1998).
13. Y. Yu *et al.*, *Cancer* **109**, 2374 (2007).
14. B. Cao *et al.*, *Int. J. Cancer* **132**, 1277 (2013).
15. S. Sengupta *et al.*, *Circulation* **110**, 1219 (2004).
16. K. W. Leung *et al.*, *J. Biol. Chem.* **281**, 36280 (2006).
17. K. W. Leung *et al.*, *Br. J. Pharmacol.* **152**, 207 (2007).
18. K. Shinkai *et al.*, *Jpn. J. Cancer Res.* **87**, 357 (1996).
19. K. W. Leung *et al.*, *FEBS Lett.* **580**, 3211 (2006).
20. K. W. Leung *et al.*, *FEBS Lett.* **581**, 2423 (2007).
21. T. T. Hien *et al.*, *Toxicol. Appl. Pharmacol.* **246**, 171 (2010).
22. K. W. Leung *et al.*, *Angiogenesis* **14**, 515 (2011).
23. Y. Liu *et al.*, *Cell Death Dis.* **2**, e145 (2011).
24. T. Kimura *et al.*, *Gen. Pharmacol.* **25**, 193 (1994).
25. S. Y. Nah, *Front Physiol.* **5**, 98 (2014).
26. C. H. Lee *et al.*, *J. Ginseng Res.* **38**, 161 (2014).
27. K. O. Skaftnesmo *et al.*, *Curr. Pharm. Biotechnol.* **8**, 320 (2007).
28. L. S. Chan *et al.*, *Eur. J. Pharm. Sci.* **38**, 370 (2009).
29. N. Wu *et al.*, *Acta Pharmacol. Sin.* **32**, 345 (2011).
30. I. S. An *et al.*, *Oncol. Rep.* **29**, 523 (2013).
31. M. Wang *et al.*, *J. Integr. Plant Biol.* **57**, 256 (2015).
32. L. Zhang *et al.*, *Cell Res.* **22**, 107 (2012).
33. Y. Zhao *et al.*, *Planta Med.* **75**, 1124 (2009).
34. N. T. Chiu *et al.*, *Biopharm. Drug Dispos.* **35**, 104 (2014).
35. S. Deb *et al.*, *J. Steroid Biochem. Mol. Biol.* **141**, 94 (2014).
36. A. Kawase *et al.*, *J. Nat. Med.* **68**, 395 (2014).
37. J. Jin *et al.*, *Biochem. Biophys. Res. Commun.* **345**, 1308 (2006).
38. S. Kang *et al.*, *J. Ginseng Res.* **36**, 354 (2012).
39. J. Wang *et al.*, *Arch. Pharm. Res.* **37**, 530 (2014).
40. D. L. Barton *et al.*, *J. Natl. Cancer Inst.* **105**, 1230 (2013).
41. C. Sun *et al.*, *Food Funct.* **5**, 845 (2014).
42. K. J. Kim *et al.*, *DNA Res.* **11**, 247 (2004).
43. N. H. Kim *et al.*, *J. Ginseng Res.* **38**, 130 (2014).
44. S. Chen *et al.*, *Science* **347**, S27 (2015).

Acknowledgments

This work was supported by grants from the National Research Foundation of Korea to Y.S.K. (MRC2009-93146) and the Health and Medical Research Fund to A.S.T.W. (11121191)

Produced by the *Science*/AAAS Custom Publishing Office

Pharmacognosy in the United Kingdom: Past, present, and future

Authors: Melanie-Jayne R. Howes and Monique S.J. Simmonds*

For centuries, pharmacognosy has been instrumental in developing both conventional and herbal medicines in Europe. Isolated phytochemicals from natural sources have been the source for new pharmaceutical drugs and provided template chemical structures for drug discovery. In recent years, natural products have played a role in the development of approximately 50% of approved new chemical entities (*1*). Moreover, the majority of new small-molecule drugs of natural origin are derived from terrestrial microbes, with others coming from higher plants, marine organisms, and terrestrial animals (*2*). Juxtaposed with natural product drug discovery is the development of herbal medicines. These mixtures encompass medicinal plants that may contain diverse and biologically active phytochemicals; however, the active constituents of many herbal medicines are unknown and evidence for efficacy is often limited. Therefore, a major challenge for such medicines is quality control and standardization. In the European Union (EU), movements to harmonize the legislation surrounding traditional herbal medicines have aimed to improve their safety and quality. However, there are limitations and, in some respects, herbal medicines are still less well-regulated compared to conventional medicines. Although the use of herbal medicines in the United Kingdom (UK) is popular, detailed knowledge of their pharmacological and clinical effects is often lacking, as are data on their pharmacokinetic and pharmacodynamic properties. While EU legislation now provides standards for the quality and safety of many herbal medicines, research to establish the "science" behind their use is not progressing at the same pace. Moreover, pharmacovigilance reporting practices could be improved to assist practitioners in gaining a better understanding of appropriate uses and safety.

Herbal medicine use in the UK

Although herbal medicines are relatively popular (used by 35% of the population in the UK), there is a general lack of understanding about what herbal medicines are (or are not); however, there is a broad perception amongst the public that they are safe because they are "natural" (*3*). The EU Directive (2004/24/EC) on Traditional Herbal Medicinal Products (HMPs) introduced regulatory standards for herbal medicines in April 2004. The directive requires EU member states to implement regulatory arrangements for HMPs that can be used without medical supervision and that have evidence for traditional use (*4*). In response to this directive, the UK's Medicines and Healthcare products Regulatory Agency (MHRA) launched the Traditional Herbal Medicines Registration Scheme in 2005. Following a transition period to allow HMP manufacturers time to comply with the directive's requirements, all such products intended for sale in the UK require either a full marketing authorization or a traditional herbal registration (THR). These regulations mean that THR HMPs that are intended for minor conditions and are suitable for self-diagnosis must meet the required standards for quality and safety [with respect to European Pharmacopoeia (EP) monographs and community herbal monographs that are evaluated by the Committee on Herbal Medicinal Products]. THR HMPs are required to have been used medicinally for at least 30 years (prior to THR application), with at least 15 years of use relating to the EU.

While these regulations have made advances in improving the safety and quality of registered HMPs, several issues still need to be addressed for the use of herbal medicines. Evidence for "traditional use" currently takes the place of hard scientific data from pharmacological tests and clinical trials. Therefore, evidence for efficacy, the scientific or pharmacological basis to explain the reputed activity, and knowledge of the active constituents and their mechanisms of action are limited for many herbal medicines. There are relatively few robust clinical trials assessing efficacy for the majority of herbal medicines. Clinical evaluation and comparability are major issues for trials that investigate inadequately authenticated or standardized herbal medicines not subject to THR standards. Furthermore, Regulation 3 of The Human Medicines Regulations 2012, commonly referred to as the "herbalist exemption," permits unlicensed herbal remedies to be prepared and supplied by an herbal practitioner to meet the needs of an individual patient following a one-to-one consultation. Although this practice enables herbal practitioners to meet the needs of patients by supplying tailored herbal medicines, a current regulatory loophole allows anyone to practice herbal medicine, regardless of their qualifications or experience. This clearly has implications for public health, from receiving an appropriate diagnosis and treatment to dealing with the safety and quality issues of the remedies that may occur with unlicensed herbal medicines.

Herbal medicines used in the UK include traditional European medicines such as sage (*Salvia officinalis* L.), described in a 16th-century herbal by Gerard, who says it is "singularly good for the head and brain and quickenethe the nerves and memory," and by Culpeper 50 years later, who states that sage "also heals the memory, warming and quickening the senses" (*5*). There are limited clinical trial data to suggest that *S. officinalis* extracts can improve cognitive function for healthy subjects and patients with dementia (*5*). Although traditionally used to aid memory, *S. officinalis* HMPs are unlikely to gain THR status for cognitive disorders such as dementia, because THR HMPs must comply with permitted indications, which entail conditions that are suitable for self-medication without the need for medical supervision. However, *S. officinalis* THR HMPs are available in the UK to

Royal Botanic Gardens Kew, Richmond, Surrey, United Kingdom
*Corresponding Author: m.simmonds@kew.org

relieve some conditions that do not normally require medical intervention, which include menopausal and cold symptoms (based on traditional use). In addition to traditional European herbal medicine, other practices of traditional herbal medicine from a variety of cultures are increasingly being used in the UK, including traditional Chinese medicine (TCM) and those from Ayurvedic, African, and South American traditions. Some of these are supplied under the herbalist exemption and not controlled by THR regulations.

In 1864, the first edition of the British Pharmacopoeia (BP) was introduced, containing the official monographs for medicines in the UK. This collection of standards comprised the required characteristics and tests for numerous herbal medicines, including potentially toxic plants such as aconite, *Digitalis*, and belladonna, as well as other naturally derived remedies, such as purified ox bile and leeches (*6*). Over the last 150 years, the development of conventional pharmaceutical drugs has increased considerably, while the use of herbal medicines in conventional "Western" medicine has declined. This trend is reflected in the current BP, with fewer herbal medicine monographs included than pharmaceutical drug monographs (*7*). However, with the introduction of THR and HMP quality standard requirements, the number of monographs for herbal medicines is now increasing once again in the BP and European Pharmacopoeia. Moreover, a higher number of species monographs are included, reflecting the incorporation of different practices into UK medicine, such as TCM (e.g., *Salvia miltiorrhiza* Bunge root and rhizome) and Ayurvedic medicine (e.g., *Withania somnifera* (L.) Dunal root) (*7*).

Future directions

The introduction of the EU Directive (2004/24/EC) and THR scheme in the UK have enabled progress on the safety and quality control issues of many HMPs; however, the impact of these regulations on safeguarding public health remains to be determined. To evaluate these issues, thorough monitoring of adverse responses to HMPs, either due to intrinsic (i.e., effects inherent in the plant itself) or extrinsic (i.e., effects resulting from quality control issues such as adulteration or substitution of the intended species) are essential. In general, there is an underreporting (via pharmacovigilance schemes) of adverse drug reactions (ADRs) by health care professionals (HPCs) (*8*) as well as much variation between HCPs in the reporting of ADRs (*9*). The importance of this type of reporting is exemplified by St. John's wort (*Hypericum perforatum* L.). In this case, ADR reporting through pharmacovigilance schemes led to the identification of several clinically important drug interactions and potential safety issues (*10*). To promote herbal medicines' safe use, we recommend that HCPs improve their knowledge of such remedies and encourage them to report any ADRs and herb-drug interactions. Moreover, the preparation and supply of unlicensed herbal medicines as permitted under the herbalist exemption should also be further scrutinized to improve the regulation of this practice and address potential quality and safety issues, while maintaining access to trained herbal medicine practitioners, which many patients value.

Finally, the issue of efficacy needs to be addressed far more robustly for many herbal medicines. More research is needed to identify the active constituent(s) and their modes of action, and to determine their polyvalent nature, while understanding more about their pharmacokinetic and pharmacodynamic properties (similar to the process for conventional pharmaceuticals). It is essential to authenticate and standardize HMPs in order to define their safety, quality, and efficacy standards and to enable clinical trial data to be based on phytochemically characterized HMPs containing standardized levels of active constituents. Meanwhile, the fact that plants are incredible synthetic chemists and have already provided numerous lead chemical structures (e.g., paclitaxel and docetaxel) for the development of conventional pharmaceutical drugs, which may not have been discovered via synthetic compound libraries, should not be ignored. Plants have an important role in the future of medicine and, whether they are used as herbal medicines or in drug discovery programs, it is essential that they are cultivated from sustainable sources and that their medicinal products are designed to meet the appropriate standards for quality and public health safety.

References

1. D. J. Newman, G. M. Cragg, *J. Nat. Prod.* **75**, 311 (2012).
2. A. D. Kinghorn, L. Pan, J. N. Fletcher, H. Chai, *Nat. Prod. Rep*. **74**, 1539 (2011).
3. MHRA, "Public Perception of Herbal Medicines"; available at https://www.gov.uk/drug-safety-update/public-perception-of-herbal-medicines.
4. Council Directive 2004/24/EC, *O. J. L.* **136**, 85-90 (2004).
5. M-J. R. Howes, P. J. Houghton, *Curr. Alzheimer Res*. **9**, 67 (2012).
6. *British Pharmacopoeia* (General Council of Medical Education and Registration of the United Kingdom, London, 1864).
7. *British Pharmacopoeia* (The Stationery Office, London, 2014).
8. L. Hazell, V. Cornelius, P. Hannaford, S. Shakir, A. J. Avery, *Drug Saf*. **36**, 199 (2013).
9. G. Yamey, *Br. Med. J.* **319**, 1322 (1999).
10. Committee on Safety of Medicines, Medicines Control Agency, *Current Problems in Pharmacovigilance* **26**, 6 (May 2000).

Produced by the *Science*/AAAS Custom Publishing Office

Traditional herbal medicines in the European Union: Implementing standardization and regulation

Authors: Werner Knöss[1*] and Ioanna Chinou[2]

Medicinal plants have been used in Europe since ancient times. A variety of traditions for using herbal preparations have developed throughout the current member states of the European Union (EU), with a diverse set of regulations developing throughout the 20th century. Recently however, common legislation for the regulation of medicinal products in the EU has emerged (*1*, *2*). Providing a complete set of data to satisfy EU regulatory requirements for bringing herbal medicines to market has proven challenging because many products have had a long history of different traditional uses in the different states. A new legislative approach was therefore developed in 2004 to harmonize the assessment of and access to traditional herbal medicinal products (*3*). The new legislation worked to combine scientific evaluation with the large database of accumulated evidence collected over many years of herbal medicine use.

Legal provisions for herbal medicine in the EU

The approval of medicinal products in the EU is linked to the assessment of quality, safety, and efficacy by a regulatory authority. Basic definitions for herbal substances, herbal preparations, herbal medicinal products, and traditional herbal medicinal products have been provided in Community Directive 2001/83/EC, as amended by Directive 2004/24/EC (*1*, *2*). The legislation also describes the details of the documentation requirements for market access for the three main categories of herbal medicines: (1) marketing authorization for *new herbal medicinal products* based on a full set of new efficacy and safety data; (2) marketing authorization for *herbal medicinal products* based on well-established use documented in published literature (including clinical trials); and (3) registration for *traditional herbal medicinal products*, for which efficacy is based on plausibility and long-standing use.

In order to harmonize scientific evaluation in the EU, the Committee on Herbal Medicinal Products (HMPC) was established at the European Medicines Agency (EMA) in London in 2004 (*3*, *4*). This scientific committee is composed of 28 members with one scientific expert from each member state. Five co-opted members represent special fields of expertise: pediatrics, general medicine, pharmacology, clinical pharmacology, and toxicology. The core task of the HMPC is to standardize herbal medicinal products and traditional herbal medicine products in the EU by developing monographs and list entries for herbal substances and their preparation. The establishment of monographs and other regulatory documents is a fully transparent process starting with a public "call for data."

A rapporteur is nominated by the HMPC and is responsible for the evaluation of information provided from the public call for data, the results of a systematic literature research in the public domain, and market overviews provided by the member states. A draft monograph is established while the scientific evidence is evaluated and documented in an assessment report. Scientific discussions in the Working Party on European Union Monographs and List Entries (MLWP) and HMPC contribute to revision of both documents, which are published for comments together with a list of references. When a monograph is finally adopted by the HMPC, the entire set of documents, including an overview on the comments from the public consultation, is made available on the EMA's website. Since 2013, the agendas and minutes of the plenary meetings of the HMPC have also been published, and any interested party, applicant, or citizen can access the work of the HMPC (*5*).

Developing standards for herbal medicines

When creating monographs for herbal medicines in the EU, all of the available data is scientifically evaluated to create a unified view of the safety and efficacy of herbal substances and their preparations. Monographs may include two variations: well-established use and/or traditional use.

Well-established use is based on approval of a product for medicinal use in the EU market for at least 10 years. Efficacy must be proven by at least one published successful clinical trial together with published data that meet the further requirements for efficacy and safety.

For traditional herbal medicinal product registration, evidence for safety and efficacy are derived from the long-standing use of a traditional medicinal product. The criteria for a product's acceptance includes demonstrating its use as an herbal medicine for 30 years with at least 15 years of such use in the EU. Additional safety data may be requested by a national regulatory authority when deemed necessary. This approach to approving traditional herbal medicines is only appropriate for products that are very safe. Therefore, this avenue is restricted to products that are administered orally, externally, or by inhalation and that treat minor complaints. Ailments that require a medical prescription, diagnosis, or supervision by a medical doctor are excluded and traditional herbal medicines must comply with provisions for over-the-counter medicines.

The application of monograph standards

Within the last 10 years, the HMPC has released approximately 130 monographs (for examples, see Table 1), 12 list entries, 13 public statements, and about 40 guidance documents (*5*). Only 25 monographs have been approved based on well-established use. Public statements have been developed when a monograph could not be drafted for reasons such as

[1]Department of Complementary and Alternative Medicines and Traditional Medicinal Products at the Federal Institute for Drugs and Medical Devices (BfArM), Bonn, Germany
[2]University of Athens, Faculty of Pharmacy, Division of Pharmacognosy and Chemistry of Natural Products, Zografou Campus, Athens, Greece
[*]Corresponding Author: werner.knoess@bfarm.de

a lack of data or substance-specific concerns. The guidance documents address a broad set of issues related to quality, safety, and efficacy to support further harmonization among the member states.

The inventory of herbal substances that the monographs are intended to represent currently has about 180 entries. Creating such support for the safe and effective use of traditional herbal medicinal products in the EU is the primary goal of the HMPC. The committee conducts a review of the monographs every five years in order to provide a sustainable and reliable system that represents the current state of scientific knowledge. More than 1,300 traditional herbal medicinal products have been registered by the national regulatory authorities of the EU member states. The registrations were granted for individual applications that took the standards laid down in the legislation and described by the monographs into account. These registered traditional herbal medicinal products cover a broad spectrum of conditions, including coughs and cold, mood and sleep disorders, gastrointestinal disorders, urinary tract/gynecology disorders, and pain and inflammation. Approximately one-third of the approved products are composed of multiple herbal preparations.

TABLE 1. Selected examples of Committee on Herbal Medicinal Products (HMPC) monographs for herbal substances. TU, traditional use; WEU, well-established use.

Substance	TU	WEU
Harpagophyti radix	✓	
Hyperici herba	✓	✓
Pelargonii radix	✓	
Valerianae radix	✓	✓
Passiflorae herba	✓	
Ginseng radix	✓	
Ginkgo folium	✓	✓

Globalization of traditional medicines

The ongoing globalization of traditional medicines has brought with it a broad diversity of regulatory systems in different countries and regions. For example, there is a lack of internationally accepted definitions and standard requirements for quality, safety, and efficacy. Different concepts have been established to consider the particular characteristics of traditional medicines. Thus, companies face immense obstacles when trying to gain access to different markets for their herbal medicines. An international dialogue about scientific and regulatory issues is necessary to develop reasonable and adequate requirements. Such a conversation should also address topics such as translating indications into another cultural context or therapeutic environment (e. g., an additional diet or a parallel physical treatment), using material of a nonherbal origin, and classifying herbal products.

The European legislation was primarily designed to deal with traditional herbal medicinal products with a well-known origin in Europe. However, the existence of therapeutic systems and products from traditional Chinese medicine (TCM) or Ayurvedic medicine within Europe has prompted the HMPC to address issues related to non-European traditional medicines (*6*). A document was released in the spring of 2014 that explained the European regulatory framework and the options and limitations for traditional products originating from non-European regions (*7*). In addition, the HMPC has started a pilot project to create monographs for the herbal substances used in Asian traditional medicines, such as TCM and Ayurvedic medicine.

Conclusions

Harmonizing the process for evaluating and authorizing traditional herbal medicines in the 28 member states of the EU is an ongoing process. The legislation and practices over the last decade have demonstrated that it is possible to standardize the scientific and regulatory evaluation of traditional medicines. By considering their individual characteristics and long-standing uses, traditional medicines have been made available to citizens in a more regulated environment. HMPC monographs and monographs related to the quality of herbs from the European Pharmacopeia form the basis of the regulation standards (*8*). Admittedly, there are still challenging issues in the EU surrounding specific topics such as assigning well-established uses and classifying certain products. The EU's legislation is not specific regarding how to distinguish between (herbal) medicinal products, food supplements, medical devices, and cosmetics. On the global level, there is a need to discuss different legal frameworks and to develop harmonized solutions, which should take into account the specific indications for traditional medicines; the availability of marketed products with adequate quality, safety, and efficacy; and the means to provide reliable information to consumers and health care experts for the use of herbal medicinal products.

References

1. European Parliament and EU Council, Directive 2001/83/EC; available at http://ec.europa.eu/health/files/eudralex/vol-1/dir_2001_83_cons/dir2001_83_cons_20081230_en.pdf.
2. European Commission Health and Safety Directorate-General, "Marketing Authorisation, Notice to Applicants"; available at http://ec.europa.eu/health/files/eudralex/vol-2/a/vol2a_chap1_201507.pdf.
3. European Parliament and EU Council, Directive 2004/24/EC; available at http://ec.europa.eu/health/files/eudralex/vol-1/dir_2004_27/dir_2004_27_en.pdf.
4. European Parliament and EU Council, Regulation (EC) No. 726/2004; available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:136:0001:0033:en:PDF.
5. European Medicines Agency, "HMPC: Agendas, Minutes and Meeting Reports"; available at http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/document_listing/document_listing_000193.jsp&mid=WC0b01ac0580028e96.
6. Committee on Herbal Medicinal Products, "HMPC Work Programme for 2012–2015"; available at http://www. ema.europa.eu/docs/en_GB/ document_ library/Work_programme/2011/12/WC500119957.pdf.
7. Committee on Herbal Medicinal Products, "Questions & Answers on the EU Framework for (Traditional) Herbal Medicinal Products, Including Those from a 'Non-European' Tradition"; available at http://www.ema.europa.eu/docs/en_GB/ document_library/ Regulatory_and_procedural_guideline/2014/05/WC500166358.pdf.
8. *European Pharmacopeia,* 8th Ed. (European Directorate for the Quality of Medicines, Strasbourg, 2014).

Acknowledgments

The views expressed in this article are the views of the authors and may not be understood or quoted as being made on behalf of or reflecting the position of the European Medicines Agency or any of its Committees or Working Parties. The authors state no conflict of interest. The data and figures provided are based on data available in September 2014.

Produced by the *Science*/AAAS Custom Publishing Office

Traditional African medicine: From ancestral knowledge to a modern integrated future

Authors: Joseph Kahumba[1], Tsiry Rasamiravaka[2,3], Philippe Ndjolo Okusa[4], Amuri Bakari[1,4], Léonidas Bizumukama[5], Jean-Baptiste Kalonji[6], Martin Kiendrebeogo[7], Christian Rabemenantsoa[3], Mondher El Jaziri[2], Elizabeth M. Williamson[8]*, Pierre Duez[4]*

Traditional African medicine (TAM) is characterized by a belief in the supernatural as a cause of illness, divination as a diagnostic tool, and the ritualized use of a wide variety of plant- and animal-derived agents in its treatment (*1*). These are usually purchased from local markets (Figures 1A and 1B) and remain the primary source of health care for 80% of both rural and urban populations (*2*). In its strategy for 2014–2023, the World Health Organization (WHO) encourages the development and modernization of TAM as an integral part of emerging health care systems (*3*). However, some of the more exotic practices in TAM, which include the use of animal parts, especially in the *vodun* (voodoo) religion in West Africa, generate lurid headlines in the press and reduce the scientific credibility of TAM. Herbs may be used as part of a regimen in which physical characteristics (aroma, shape, color) and attendant rituals (incantation, song) are more important than pharmacological effects. However, effective strategies for using TAM herbal knowledge are available, as exemplified by a study on antimalarial plants used by traditional healers in Nigeria (*4*). The demand for medicinal plants in Africa is increasing dramatically due to population growth, resulting in the risk of extinction of certain species and an increasing likelihood of falsification of herbal materials. National policies need to be developed to protect both patients and endangered species (*3*) while protecting traditional knowledge and conservation policies (*5*).

TAM as a source of therapeutic agents

It is necessary to understand how individual plants are used in TAM in order to provide a context for exploration. Even though they have been the source of new drugs, poisonous species are rarely used for healing, since it is not possible to accurately control the dose. *Physostigma venenosum*, the Calabar bean, produces the alkaloid physostigmine (eserine), and its derivative rivastigmine, used to treat Alzheimer's disease. It has no traditional medical use and was in fact administered as an ordeal poison in Nigeria to those accused of witchcraft (*1*). From an estimated African biodiversity of ~45,000 plant species, only 5,000 have documented medicinal use (*6*). The list of drugs provided by the African flora (Table 1) is short compared with those from other traditional medical systems, suggesting an unrivaled opportunity for the discovery of new drugs. Ethnobotanically directed approaches are more successful than random selection, as demonstrated in studies using South

TABLE 1. Discoveries based on African medicinal plants.

Properties	Plant species	Constituents and therapeutics
Anticancer	*Catharanthus roseus* (L.) G. Don (Apocynaceae);	Vincristine, vinblastine, and others, to treat leukemias, Hodgkin's lymphoma
	Combretum caffrum (Eckl. & Zeyh.) Kuntze (Combretaceae)	Combretastatins – possible anti-angiogenic; induces apoptosis in proliferating endothelial cells
α_2 adrenergic antagonist	*Pausinystalia johimbe* (K.Schum.) Pierre ex Beille (Rubiaceae)	Yohimbine, to treat erectile dysfunction and hypotension
Cholinesterase inhibitor	*Physostigma venenosum* Balf. (Fabaceae)	Physostigmine derivatives, to treat myasthenia gravis (neostigmine) and Alzheimer's disease (rivastigmine)
Antihypertensive/antipsychotic	*Rauvolfia vomitoria* Afzel. (Apocynaceae)	Reserpine‡, occasionally used clinically to treat hypertension and experimentally to deplete catecholamines
Anti-HIV	*Sutherlandia frutescens* (L.) R. Br. (Fabaceae)	Antiretroviral effects under investigation
Cardiotonic	*Strophanthus gratus* (Wall. & Hook.) Baill. (Apocynaceae)	Ouabain (formerly for heart failure), used experimentally to block Na-K-ATPase
Hallucinogenic	*Tabernanthe iboga* Baill. (Apocynaceae)	Ibogaine, possible treatment for narcotic addiction

‡Reserpine had, however, been isolated 2 years earlier from *Rauvolfia serpentina* (L.) Benth. ex Kurz, found in India.

[1]Laboratoire de Pharmacognosie, Université de Lubumbashi, Lubumbashi, Democratic Republic of the Congo
[2]Laboratoire de Biotechnologie Végétale, Université Libre de Bruxelles, Belgium
[3]Laboratoire de Biodiversité et de Biotechnologie, Institut Malgache de Recherches Appliquées (IMRA), Antananarivo, Madagascar
[4]Department of Therapeutic Chemistry and Pharmacognosy, Université de Mons (UMONS), Mons, Belgium
[5]Department of Chemistry, Université du Burundi, Bujumbura, Burundi
[6]Laboratoire de Pharmacie Galénique et Analyse des Médicaments, Université de Lubumbashi, Lubumbashi, Democratic Republic of the Congo
[7]Laboratoire de Biochimie et Chimie Appliquées, Université de Ouagadougou, Ouagadougou, Burkina Faso
[8]School of Pharmacy, University of Reading, Whiteknights Campus, Reading, United Kingdom
*Corresponding Authors: e.m.williamson@reading.ac.uk (E.M.W) and pierre.duez@umons.ac.be (P.D.)

Produced by the *Science*/AAAS Custom Publishing Office

A

FIGURE 1A. A traditional medicine stall in Madagascar. Key: (**1**) *Eucalyptus citriodora* leaves (respiratory antiseptic), (**2**) *Aloe vahombe* leaves (immunostimulant), (**3**) *Cedrelopsis grevei* bark (tonic, aphrodisiac), (**4**) *Zea maïs* (silk) (diuretic), (**5**) *Aphloia theiformis* leaves (antipyretic), (**6**) *Combretum albiflorum* seeds (deworming), (**7**) *Curcuma longa* rhizome powder (against jaundice), (**8**) *Raventsara aromatica* bark (respiratory antiseptic, antibiotic), (**9**) *Mollugo nudicaulis* leaves (antitussive), (**10**) Tallow molded into balls.

B

FIGURE 1B. A traditional medicine stall in Lubumbashi, Democratic Republic of the Congo. Key: (**1**) *Pterocarpus angolensis* D.C. Stem (hemorrhoids, nappy/diaper rash), (**2**) *Solanum incanum* L. fruits (gonorrhea and hernia), (**3**) Shell from Lualaba River (welding fontanel), (**4**) Tortoise shell (burns treatment), (**5**) *Albizia adianthifolia* stem bark (aphrodisiac and perianal swelling treatment), (**6**) *Diplorhynchus condylocarpon* (Müll.Arg.) Pichon stem (abdominal pain, wound healing), (**7**) *Cassia sieberiana* D.C. roots (hemorrhoids, skin irritation), (**8**) *Mucuna poggei* Taub seeds (nappy/diaper rash; analgesic in pelvic pain).

African Cape flora (*7*) and African plants that contain effective antihyperglycaemic agents (*8*).

Parasitic infections are a major cause of death in Africa, and TAM herbs are widely used to treat them. However, like many diseases in developing countries, these diseases remain underresearched as they do not promise a good commercial return on investment. Nevertheless, new lead antiprotozoal compounds have emerged from herbs used in TAM to treat malaria, and include cowaxanthone (from *Garcinia cowa*), which has comparable antiplasmodial activity to pyrimethamine (*9*), and cryptolepine (from *Cryptolepis sanguinolenta*) (*10*). Clinical trials of *Nauclea pobeguinii* extracts have also shown promising results in the treatment of malaria (*11*). There are still no vaccines for leishmaniasis, and the toxicity of antimony- and pentamidine-based drugs means that interest in plant-derived leads for potential antileishmanial drugs remains high. These include chelerythrine derivatives (from *Garcinia lucida*), gibbilimbol B (*Piper malacophyllum*), warifteine (*Cissampelos sympodialis*), and flavonoids from *Baccharis retusa* and *Kalanchoe pinnata* (*12*). African trypanosomiasis (sleeping sickness) is usually fatal if left untreated, but *Momordica balsamina*, *Securidaca longipeduncula*ta, and *Quassia africana* have yielded compounds with potent activity (*13*). Medicinal plants also contain compounds with activities unrelated to traditional use, which must be borne in mind and may also be exploited. Indeed the antileukemia *Vinca* alkaloids were discovered serendipitously when the Madagascar periwinkle *Catharanthus roseus* was being investigated for its antidiabetic properties.

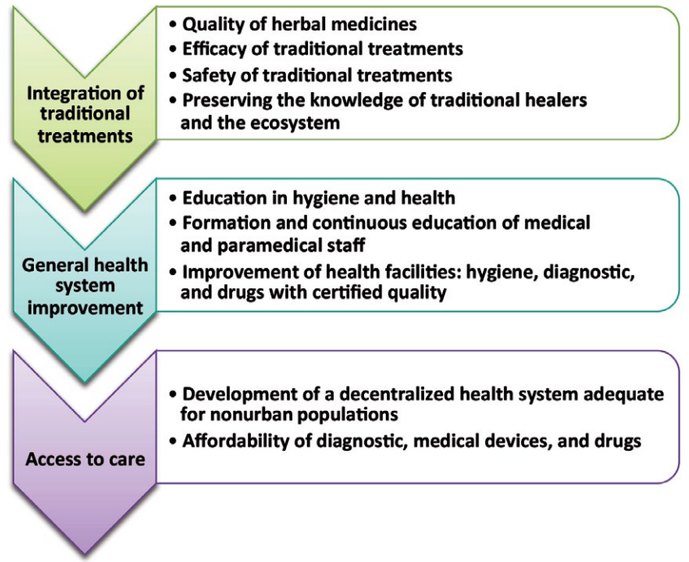


FIGURE 2. Africa's challenge: A future modern health care system integrating traditional medicine.

The alarming incidence of bacterial multidrug resistance to antibiotics requires an urgent search for new antibacterials. The expression of virulence factors in many pathogens requires the full activation of quorum sensing (QS) processes: cell-to-cell bacterial communication mechanisms that detect critical cell numbers by producing and recognizing diffusible signal molecules termed "autoinducers." The compounds coordinate the expression and regulation of virulence factors, biofilm formation, and motility. QS presents a promising series of targets to antagonize virulence in pathogens and/or disturb biofilm formation. For example, catechin and naringenin inhibited the production of virulence factors in *Pseudomonas aeruginosa* PAO1, a consequence of reduced expression of QS- (*lasB* and *rhlA)* and QS-regulatory (*lasI*, *lasR*, *rhlI* and *rhlR)* genes (*14*, *15*). Recently, the Malagasy species *Dalbergia trichocarpa*, traditionally used to treat diarrhea and laryngitis, was shown to inhibit a wide variety of virulence factors in *P. aeruginosa* PAO1; its constituent coumarate esters interfere with the QS system's *rhl* and *las* gene expression (*16*). Extracts of *Kigelia africana*, used topically on wounds and abscesses, have been shown to interfere with the response of bacteria to autoinducers, and to modulate their synthesis in *Chromobacterium violaceum* and *Agrobacterium tumefaciens* (*17*).

Conclusions

TAM currently supports the medical needs of millions of Africans. Based on experience gained from other traditional medicine systems, its modernization and integration with conventional medicine may offer a new and holistic view of health care, contributing to better universal health coverage in Africa, as advocated by the World Health Organization. This remains quite a challenge, as depicted in Figure 2, despite the rich source of new active compounds to be found in African flora. This flora is ripe for exploration, as long as traditional medical uses and methods of administration are interpreted with caution, and the rights of local people and the environment are respected.

References

1. D. T. Okpako, *Trends Pharm. Sci.* **20**, 482 (1999).
2. O. M. J. Kasilo, J. M. Trapsida, C. N. Mwikisa, P. Lusamba-Dikassa, *The African Health Monitor* **14**, 7 (2010).
3. *WHO Traditional Medicine Strategy: 2014–2023* (World Health Organization, Geneva, 2013).
4. I. P. Dike, O. O. Obembe, F. E. Adebiyi, *J. Ethnopharmacol*. **144**, 618 (2012).
5. WHO Regional Office for Africa, "Intellectual Property Approaches to the Protection of Traditional Knowledge in the African Region"; available at http://www.aho.afro.who.int/en/ahm/issue/13/reports/intellectual-property-approaches-sprotection-traditional-knowledge-african.
6. M. F. Mahomoodally, *Evid. Based. Complement. Alternat. Med.* **2013**, 617459 (2013).
7. C. H. Saslis-Lagoudakis *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **109**, 15835 (2012).
8. R. N. Ndip, N. F. Tanih, V. Kuete, in *Medicinal Plants Research in Africa* (Elsevier, London, UK, 2013), pp. 753–776.
9. J. Bero, M. Fréderich, J. Quentin-Leclercq., *J. Pharm. Pharmacol.* **61**, 1401 (2009).
10. L. F. Rocha e Silva *et al*., *Phytomed.* **20**, 71 (2012).
11. K. Mesia *et al*., *Planta. Med*. **78**, 853 (2012).
12. M. Wink., *Molecules* **17**, 12771 (2012).
13. D. Musuyu Muganza *et al., J. Ethnopharmacol.* **141**, 301 (2012).
14. O. M. Vandeputte *et al., Appl. Environ. Microbiol*. **76**, 243 (2010).
15. O. M. Vandeputte *et al*., *Microbiology* **157,** 2120 (2011).
16. T. Rasamiravaka *et al*., *Microbiology* **159**, 924 (2013).
17. H. Y. Chenia, *Sensors* **13,** 2802 (2013).

Produced by the *Science*/AAAS Custom Publishing Office

Traditional Chinese herbal medicine preparation: Invoking the butterfly effect

Authors: Helen Sheridan[1], Brigitte Kopp[2], Liselotte Krenn[2], Dean Guo[3], Jandirk Sendker[4*]

The metaphor of the "butterfly effect"—in which the proverbial butterfly's flapping wings contribute to a tornado across the other side of the globe—is based in chaos theory and encapsulates the concept that a small change at one place in a complex system can have large effects elsewhere (*1*). Such an effect could be construed as contributing to the unique nature of Chinese herbal medicines (CHMs), whereby several specific variables that initially may have minor effects can have a significant downstream impact on the quality, potency, and therapeutic efficacy of the final product (*2*). Two of these factors are the pharmaceutical practices of *paozhi* processing of herbal drugs and the formation of hot-water decoctions from single or multiple herbal drugs (formulae) based on ancient tradition. These two factors act on the chemical composition and biological activity of the resulting *tang* decoction that is finally consumed (*3, 4*).

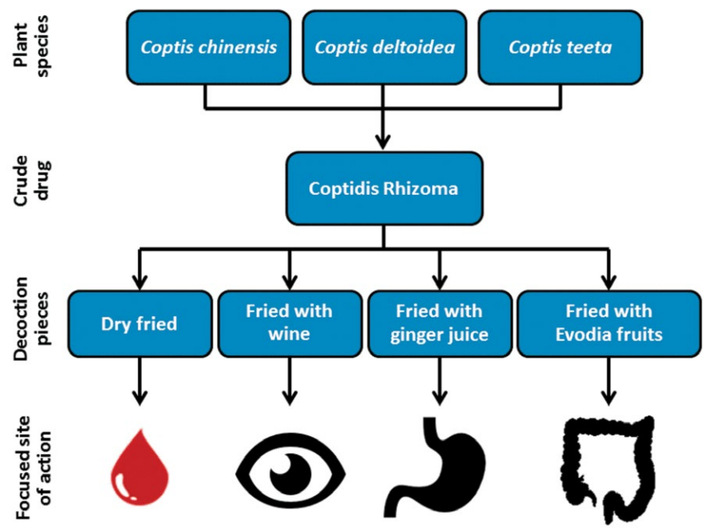


FIGURE 1. According to TCM theory, *paozhi* processing yields decoction pieces with variable therapeutic properties (*3, 7*).

The art of *paozhi*

According to traditional Chinese medicine (TCM) theory, *paozhi* processing transforms raw herbal drugs into "decoction pieces," thus instilling them with the desired properties for their medical application, including improved flavor and detoxification or alteration of their therapeutic efficacy. *Paozhi* encompasses techniques such as cutting, crushing, calcining, or frying with or without liquid adjuvants such as vinegar or honey (*3*). A prominent example is the highly toxic crude root of *Aconitum carmichaelii* (*Fuzi*) which, after detoxification by *paozhi* processing, is incorporated into numerous TCM formulae used to treat joint pain and rheumatic disease (*5, 6*). Also, different kinds of decoction pieces can be derived from the same raw material by processing in different ways. For example, the Chinese pharmacopeia describes four different decoction pieces that may be derived from raw rhizomes of the species *Coptis* (*7*). These pieces, from the same source, have distinct activity and different sites of action within the human body (Figure 1). Despite its long tradition, it is only recently that the effects of *paozhi* have been systematically studied. The current understanding is that *paozhi* processing can alter the qualitative and quantitative chemical composition of herbal materials and can thus impact the final pharmacological or toxicological properties of the decoction pieces (*3*).

Chinese herbal decoctions

TCM formulae are typically composed of two or more processed herbal drugs that are jointly decocted. Traditional decoctions (*tang*) are prepared by repeated boiling of decoction pieces in water for 1 or more hours. The method may also require soaking in cold water before heating, or the introduction of single herbal components later in the process. The composition of the *tang* decoction can be changed by simple actions such as an initial soaking in cold water, which initiates innate enzymatic activity resulting in the alteration of chemical composition, as demonstrated by the formula of *Fuzi Xiexin Tang* (FXT) (*8*). In addition, studies of the simple two-herb formula *Danggui Buxue Tang* (DBT), composed of *Astragalus membranaceus* root and *Angelica sinensis* root, demonstrate how multiple parameters like decoction time, initial temperature, *paozhi* processing, or the ratio of the two herbal ingredients may impact the chemical composition and activity of the resulting *tang* decoction (Figure 2) (*4, 9–11*). In particular, in the examples of DBT and FXT, as well as other studies, the practice of joint decoction of herbal materials itself was found to affect the properties of the final product. With DBT, joint decoction showed a significantly improved cardioprotective effect on isolated rat hearts (*12*) and osteoblast differentiation (*13*) when compared to a mixture of individually prepared decoctions of *Angelica* and *Astragalus* roots. Significantly, the concentrations of some of DBT's phytochemicals were found to be increased by 10% to 4,900% in the same studies due to coextraction. It was concluded that the observed synergism results from physicochemical interactions between the chemical constituents of both herbal ingredients. Such interactions have been observed in several studies with other formulae (see *8, 14–16*).

Physicochemical interactions

Physicochemical interactions may affect the solubility of phytochemicals in simpler environments than a Chinese *tang* decoction. It has been observed that ubiquitous herbal constituents like sugars, amino acids, or small organic acids can function singly or in combination as natural deep eutectic solvents, which are able to dissolve phytochemicals and biological macromolecules up to 460,000-fold better than water (*17*). The solubility of phytochemicals in water itself can also be affected by the presence of other small organic molecules, as exemplified by hypericine from St. John's wort, the solubility of which increases 120-fold in the presence of tannins (*18*). In contrast, a reduction in the solubility of different toxic alkaloids was observed in the presence of rhubarb root, a process believed to be linked to the formation of insoluble sediments (*8*).

An exciting new finding is that traditional *paozhi* processing techniques may also augment a decoction's therapeutic efficacy based on physicochemical interactions. Preparing DBT with *Angelica sinensis* root that has been processed with rice wine according to the traditional protocol not only resulted in modified concentrations of *Angelica* phytochemicals, but also significantly increased the concentrations of the observed *Astragalus* phytochemicals; the qualitative phytochemical changes were accompanied by an increase in estrogenic and osteogenic activity (*19*). Some of these physicochemical interactions have been recently modeled using ferulic acid, a constituent of *Angelica sinensis*. The acid increased the concentrations of *Astragalus* phytochemicals and displayed a dose-dependent effect on the estrogenic and osteogenic activity of a decoction from *Astragalus* roots, but only when added before the decoction process. Ferulic acid alone was completely inactive in these models (*20*). This example demonstrates that such complex physicochemical interactions may account for synergistic effects on the biological activity of CHMs and thus contribute to other possible synergisms that may occur due to pharmacokinetic or pharmacodynamic effects (*14*).

Conclusions

Modern scientific study of TCM is leading to an increased understanding of the complex interactions occurring between herbal components during the processing and extraction of these medicines. The examples given here indicate that the evolution of these ancient processes over millennia may actually have improved the therapeutic efficacy and safety of the resulting *tang* decoctions. The increased knowledge of these relationships provides support for the proper use of traditional procedures in the preparation of CHMs.

As discussed above, subtle changes in the complex production chain of CHMs can influence the composition and efficacy of *tang* decoctions through specific interactions between their constituents. The extent of such interactions may be influenced by a single detail like the *paozhi* impact on one ingredient, thus invoking a butterfly effect.

Unlike the proverbial butterfly, however, the application of modern scientific methodologies allows the source of the disruption to be traced by correlating the chemical profile (metabolome) of the herbal preparation with its bioactivity. This approach can also effectively aid the identification of chemical features that indirectly influence an herbal medicine's therapeutic efficacy (*21*). Knowledge about the role of particular herbal ingredients or phytochemicals within a CHM is a prerequisite for the development of meaningful quality control assays, and thus a requirement for the international registration of TCM products. Without fully understanding the subtle contributing factors,

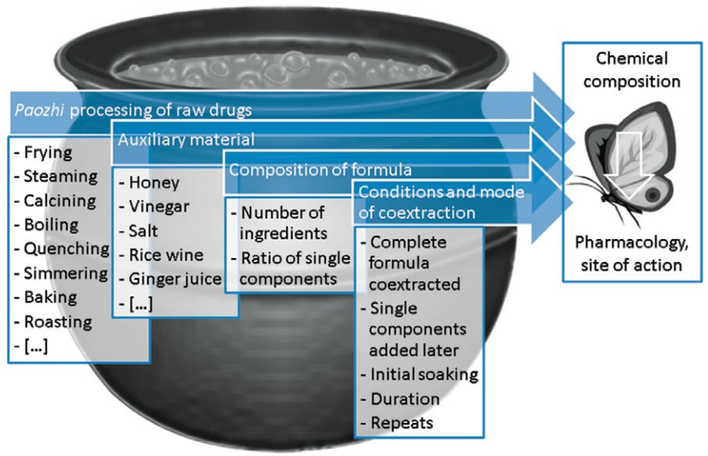


FIGURE 2. Selection of factors affecting the chemical composition of a *tang* decoction.

[1]Trinity College Dublin, School of Pharmacy and Pharmaceutical Sciences, and Trinity International Development Initiative, Trinity Biomedical Sciences Institute, Dublin, Ireland
[2]University of Vienna, Department of Pharmacognosy, Vienna, Austria
[3]Shanghai Research Center for Modernization of Traditional Chinese Medicine, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China
[4]University of Münster, Institute of Pharmaceutical Biology and Phytochemistry, Münster, Germany
[*]Corresponding Author: jandirk.sendker@uni-muenster.de

modernization of TCM could negatively impact the unique properties and therapeutic activity of these medicines. Modern technologies and international collaborations will provide an excellent platform to fully explore and elucidate the complex interactions in herbal medicines in the future and thus aid the development of modernized CHMs that maintain the therapeutic properties of their ancestors.

References

1. K. J. Dooley, *Nonlinear Dynamics Psychol. Life Sci.* **13**, 279 (2009).
2. Q. Xu *et al., J. Altern. Complement. Med.* **13**, 132 (2013).
3. Z. Zhao *et al., Planta Med.* **76**, 1975 (2010).
4. W. L. Zhang *et al., Evid. Based Complement. Alternat. Med.* **2014** (2014), doi: 10.1155/2014/608721.
5. Y. Li, Y. Wang, L. Su, L. Li, Y. Zhang, *Chem. Cent. J.* **7**, 36 (2013).
6. J. Singhuber, M. Zhu, S. Prinz, B. Kopp, *J. Ethnopharmacol.* **126**, 18 (2009).
7. State Pharmacopeia Committee, *Pharmacopoeia of the People's Republic of China* (China Medical Science and Technology Press, Beijing, 2010).
8. Q. Zhang, C. Wang, Y. Ma, E. Zhu, Z. Wang, *Biomed. Chrom.* **27**, 1079 (2013).
9. Z. H. Song *et al., Planta Med.* **70**, 1222 (2004).
10. Q. Gao *et al., Chin. Med.* **2**, 12 (2007).
11. Y. Z. Zheng *et al., Planta Med.* **76**, 439 (2010).
12. D. H. F. Mak, P. Y. Chiu, T. T. X. Dong, K. W. K. Tsim, K. M. Ko, *Phytother. Res.* **20**, 561 (2006).
13. R. C. Y. Choi *et al., Evid. Based Complement. Alternat. Med.* **2011** (2011), doi: 10.1093/ecam/nen085.
14. Y. Yang *et al., Fitoterapia* **92**, 133 (2014).
15. S. S. Zhou *et al., J. Chin. Mass Spec. Soc.* **34**, 88 (2013).
16. M. Xu, G. Wang, H. Xie, Q. Huang, W. Wang, *J. Ethnopharmacol.* **115**, 483 (2008).
17. Y. Dai, J. van Spronsen, G. J. Witkamp, R. Verpoorte, Y. H. Choi, *Anal. Chim. Acta* **766**, 61 (2013).
18. A. Nahrstedt, V. Butterweck, *J. Nat. Prod.* **73**, 1015 (2010).
19. T. T. X. Dong *et al., J. Agric. Food Chem.* **54**, 2767 (2006).
20. K. Y. Z. Zheng *et al., Planta Med.* **80**, 159 (2014).
21. H. Sheridan *et al., J. Ethnopharmacol.* **140**, 482 (2012).

Bridging the seen and the unseen: A systems pharmacology view of herbal medicine

Authors: Yan Schroën[1,2], Mei Wang[1,3], Herman A. van Wietmarschen[1,4], Renger Witkamp[5], Thomas Hankemeier[6], Tai-Ping Fan[7], Jan van der Greef[1,3,4,6*]

The human body functions as a dynamic ecosystem consisting of innumerable interacting systems, creating emerging properties and synergetic effects and extending beyond the physical barriers of the human organism, encompassing interactions with the environment. Understanding the human organism in its full complexity requires consideration of its different levels of organization (Figure 1, left) (*1*).

Medical questions regarding how a disease develops and how to prevent and intervene are amenable to a system-oriented paradigm in which interventions include multitarget pharmacological strategies that can influence processes across systems (*2*, *3*).

Although Western medicine has provided a very successful disease management system based on intervention at a single target, further improvements will rely heavily on new diagnostic tools to differentiate between disease subtypes and individual biological patterns.

Recognition of the uniqueness of each human entails differentiation at higher levels of organization, which requires a systems approach and expanded diagnostic insights (*4*). A better understanding of the biology and the influence of multitarget approaches on regulatory pathways could provide new perspectives for system-level interventions (*5*). Understanding system resilience to a multitude of environmental stressors will shed light on personalized health and prevention options within a biopsychosocial context.

In medical plant research, isolates of single components are primarily used, which does not reveal the synergetic properties and full impact of the natural product. This was elegantly demonstrated in studies of *Berberis fremontii* (Frémont's mahonia), which showed that the antimicrobial effects of the bioactive compound berberine were enhanced >100-fold when combined with an inactive component, 5′-methoxyhydnocarpin, isolated from the same plant (*6*). Reverse pharmacology, wherein a traditional preparation is taken as a starting point, holds promise for studying the synergetic nature of herbal medicine (*5*), especially when combined with subtyping based on modern 'omics technologies. Combining phenomenological descriptions of a system from TCM with experimental data can provide a top-down guide that includes a wealth of information and may even facilitate novel insights.

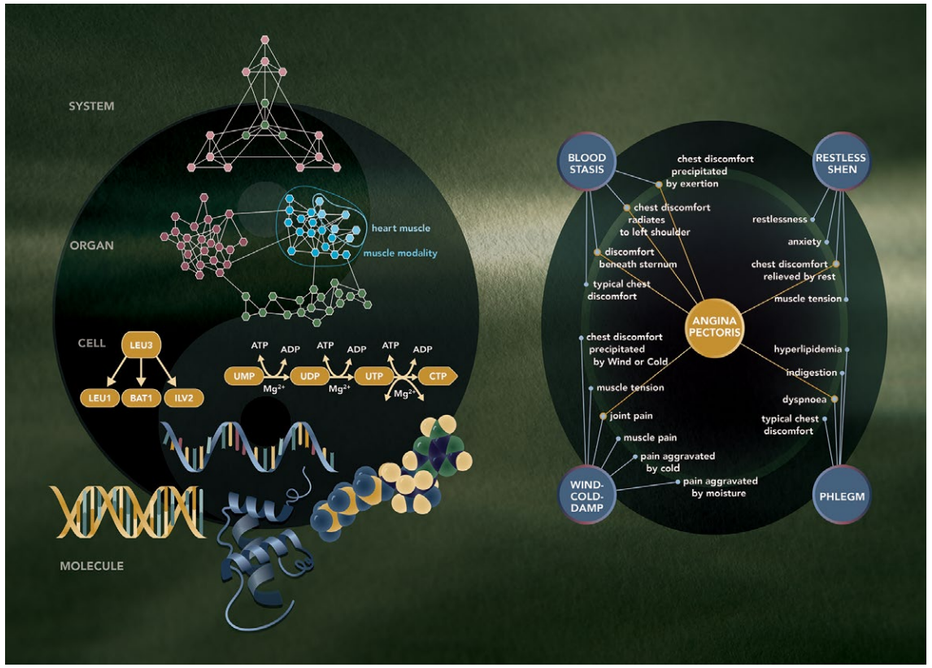


FIGURE 1. An example of systems pharmacology in herbal medicine. Left, a systems view of human biology, with selected effects of *Diao Xin Xue Kang* (DXXK). Right, the four traditional Chinese medicine (TCM) symptom clusters that are the main intervention targets for DXXK in China are illustrated for angina pectoris.

DXXK as an example

An example of the application of a systems pharmacology perspective in multitarget pharmacology research can be illustrated by *Diao Xin Xue Kang* (DXXK), the first traditional Chinese herbal medical product registered in Europe and produced in China according to the European Traditional Herbal Medicinal Products legislation. DXXK is an extract of rhizomes from *Dioscorea nipponica* Mankino, a plant from the Dioscoreaceae (yam) family. Over 300 papers have been published on the extract's pharmacology, safety, and mechanisms of action, and DXXK has been subjected to phase 1, 2, and 3 clinical trials with an estimated 16,000 patients enrolled (*7*). The main focus in these studies has been its use in the treatment of myocardial dysfunction, an indication included in the TCM description of the plant.

To obtain a systems view of the biochemical and functional effects of DXXK, pharmacological studies have examined various biochemical pathways, ranging from molecular to organ-level assessments. Analysis of DXXK's phytopharmacological constituents revealed that its bioactivity could be attributed to a group of steroidal saponins, namely dioscin, diosgenin, prosapogenin A, and prosapogenin C (*8*–*12*). Saponins influence oxidative stress (*12*, *13*), which is a major risk factor for vascular endothelial cell apoptosis, a process that is implicated strongly in the pathogenesis of cardiovascular disorders (*14*, *15*). Steroidal saponins also exhibit vasodilator and protective effects on human vascular endothelial cells (*16*, *17*). Clinical studies have shown that these saponins have protective effects against hyperlipidemia, including inhibition of platelet aggregation and reductions in cholesterol and triglyceride levels (*18*–*20*).

Studies at the cellular level have revealed that DXXK affects the renin-angiotensin-aldosterone system in a manner that is consistent with its antihypertensive effects (*21*). At the organ level, the phytoestrogen diosgenin, which is also found in DXXK, acts as a vasodilator and modulates vascular smooth muscle function by regulating cell viability, migration, and calcium homeostasis (*22*, *23*). Recent studies have revealed that the significant anti-inflammatory effect may be attributed to its inhibitory effect on the NF-κB/COX-2 pathway and relevant inflammatory mediators including prostaglandin 2, nitric oxide, tumor necrosis factor α, interleukin (IL) 1β and IL-6 (*24*).

In TCM, DXXK is used to treat a variety of conditions, including myocardial dysfunction, atherosclerosis, hypertension, migraine, and muscle spasms. From a Western perspective, these disparate applications suggest that there may be

[1]Sino-Dutch Centre for Preventive and Personalized Medicine, Zeist, the Netherlands
[2]Oxrider, Education and Research, Hilvarenbeek, the Netherlands
[3]SU BioMedicine BV, Utrechtseweg, Zeist, the Netherlands
[4]TNO, Department of Microbiology and Systems Biology, Zeist, the Netherlands
[5]Division of Human Nutrition, Wageningen University, Wageningen, the Netherlands
[6]Division of Analytical Biosciences, Leiden Academic Centre for Drug Research, Leiden University, Leiden, the Netherlands
[7]Department of Pharmacology, University of Cambridge, Cambridge, United Kingdom
*Corresponding Author: jan.vandergreef@tno.nl

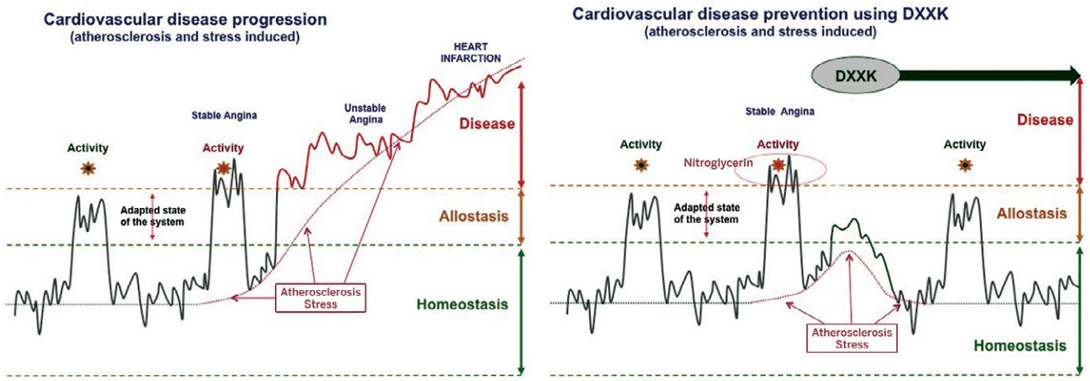


FIGURE 2. Conceptual depiction of the preventive effect of *Diao Xin Xue Kang* (DXXK) on the progression from a healthy to a diseased condition over time. The graph on the left illustrates a loss of resilience and the allostatic response. The graph on the right illustrates how intervention with DXXK can bring the system back to a healthy, resilient state, reducing the long-term influence of stressors.

shared regulatory pathways related to these conditions. In a more general sense, TCM offers attractive ways to generate a systems view on interdisease relationships owing to its unique knowledge of symptom patterns, which can be translated into Western concepts.

However, some important and intrinsic characteristics underlying the complexity of the TCM concepts can be lost in translation. Elucidating this missing information can build a bridge between Western and Chinese medicine, providing insights into large-scale organization (Figure 1, left). In particular, symptom relationships can help to bridge Chinese and Western perspectives on disease states (Figure 1, right) and can point to associations among regulatory pathways, a likely level at which major synergetic effects can be uncovered.

Where East meets West

Closer examination of points of interconnectedness between Chinese disease subtypes and Western pharmacology suggests that key elements in DXXK bioactivity involve the musculature. This is consistent with DXXK's ability to induce relaxation of vascular muscles (*25–28*) and reduce stress-related tension in intestinal, cardiac, and skeletal muscles (the latter involving the neck), as well as to reduce muscle spasms in the lower back and legs (*29*). Interestingly, Leino-Arjas et al. demonstrated a relationship between cardiovascular risk factors such as atherosclerosis and lower back pain (*30*).

A dynamic systems view of the effects of DXXK on cardiovascular disease progression is illustrated schematically in Figure 2. A healthy system can respond to and exchange information with its environment efficiently. Stressors can move a resilient system into a temporary state of allostasis. Systems should return to homeostasis when the offending stressors have been alleviated. The development of an allostatic load leads to the loss of ability to cope with stressors within the boundaries of a healthy condition (*31*), resulting in a stable angina. Eventually, the system may fall into a state in which it is unable to return to normal stasis conditions, even after direct stressors have been alleviated. That is, a person may develop unstable angina and even cardiac infarction (Figure 2, left). Clinical observations and phase 3 clinical study findings suggest that DXXK may prevent the system from progressing toward the diseased state (Figure 2, right) (*32*). The multitude of pharmacological effects related to the relaxation of vascular muscles observed with administration of DXXK can be explained by a putative systems-level organization change wherein an underlining dysfunctional regulatory process may be influenced. If so, then DXXK may be achieving an improvement in the muscle function at a higher system level, resulting in reduction of vascular tension and, thereby, increases in the oxygen flow to active tissues. The effect of DXXK on muscles relates directly to DXXK's TCM symptom treatment pattern, namely muscle cramps in the neck, lower back, and legs as well as dysfunction of cardiac muscle. Moreover, this association is consistent with known manifestations of stress in the musculature, such as lower back pain (*33*) and heart attacks (*34*). The physical manifestations of chronic stress highlight an important aspect of integrating physiological and psychological determinants in both the diagnosis and intervention, a key perspective in psychoneuroendocrinology (*35–37*).

Future perspectives

Looking to the future, further studies are needed to obtain a more detailed accounting of system level actions, particularly with respect to the dynamics of higher organization systems and elucidation of biochemical variations among different clinical subgroups. Furthermore, enhancing our knowledge of biological rhythmicity and dynamics will be important for attaining a fuller understanding of systems biology in medicine (*38, 39*). Indeed, the notion of dynamic system rhythms being reflected in the manifestation of symptoms over time is key in TCM. The TCM view of dynamics resonates with the classical idea of *Panta rhei*, or "everything flows," credited to the Greek philosopher Heraclitus. Major knowledge gaps remain in our understanding of how psychological and environmental factors influence health and in our discernment of higher system-level organization (*40*). A systems pharmacology approach that connects TCM symptom descriptions with biochemical pathway knowledge has the potential to bridge these gaps.

References

1. I. Prigogine, G. Nicolis, *Ann. N.Y. Acad. Sci*. **231**, 99 (1974).
2. L. Von Bertalanffy, *Acad. Manag. J*. **15**, 407 (1972).
3. J. van der Greef, R. N. McBurney, *Nat. Rev. Drug Discov*. **4,** 961 (2005).
4. J. van der Greef *et al*., *Planta Med*. **76,** 2036 (2010).
5. J. van der Greef *et al*., in *Metabolic Profiling: Its Role in Biomarker Discovery and Gene Function Analysis,* G.C. Harrigan, R. Goodacre, Eds. (Kluwer, Boston, 2003), pp.171–198.
6. F. R. Stermitz *et al*., *Proc. Natl. Acad. Sci U.S.A*. **97**, 1433 (2000).
7. Z. R. Liu *et al*., *China J. Traditional Chin. Med. Pharmacy* **19**, 620 (2004)
8. S. Du, W. Liu, T. Fu, B. Li, Z. Xia, *Acta Medica Sinica* **37**, 267 (2002).
9. X. G. Liang, Q. X. Pang, *Chin. J. Geriatr. Heart Brain Vessel Dis.* **6**, 129 (2004).
10. L. Ni, P. Xu, X. S. Wu, F. Chen, *Shanghai Journal of Traditional Chinese Medicine* **41** (11), 76 (2007).
11. K. Ning, Y. Li, H. Cao, L. Li, *Trad. Chin. Drug Res. Clin. Pharm*. **19**, 1 (2008).
12. T. Wang *et al*., *J. Ethnopharmacol*. **139**, 214 (2012).
13. J. S. Jaswal *et al*., *Biochim. Biophys. Acta* **1813**, 1333 (2011).
14. C. Cardillo, J. A. Panza., *Vasc. Med*. **3**, 138 (1998).
15. M. Cattaruzza, T. J. Guzik, W. Słodowski, *Circ. Res*. **95**, 841 (2004).
16. K. L. G. Dias *et al*., *Eur. J. Pharm*. **574**, 172 (2007).
17. G. Gong, *Chem. Biol. Interact.* **184**, 366 (2010).
18. Y. Liu, Y. Lin, *Fujian Medical J.* **19**, 65 (1997).
19. Z. Feng *et al*., *New Drugs Clin. Rem.* **13**, 152 (1994).
20. S. Xie, J. Zhang, *New Drugs Clin. Rem*. **13**, 161 (1994).
21. Y. P. Zhu, B. G. Li, in *Diao Xin Xue Kang Jiao Nang* (Science Press, Beijing, 2004), p. 172.
22. A. L. S. Au *et al., Eur. J. Pharm.* **502**,123–133 (2004).
23. M. Esfandiarei *et al*., *J. Pharm. Exp. Therapeutics* **336**, 925 (2011).
24. L. Wang *et al*., *Int. J. Clin. Exp. Pathol.* **8**, 4830 (2015)
25. Y. Y. Guan *et al*., *Acta Pharmacol. Sin.* **15**, 392 (1994).
26. H. B. Arcasoy *et al*., *Boll. Chim. Farm*. **137**, 473 (1998).
27. C. Y. Kwan, *Acta Pharmacol. Sin*. **21**, 1101 (2000).
28. Y. H. Wang *et al*., *J. Natural Products (India)* **2**, 123 (2009).
29. Chinese Pharmacopoeia Commission, *Pharmacopoeia of the People's Republic of China 2010*, ISBN 9780119207798 (2010).
30. P. Leino-Arjas, S. Solovieva, J. Kirjonen, A. Reunanen, H. Riihimaki, *Scand. J. Work Environ. Health* **32**, 12 (2006).
31. P. Sterling, *Physiol. Behav*. **106**, 5 (2011).
32. Z. Zhou, *New Drugs Clin. Rem*. **13**, 84 (1994)
33. L. A. Päivi *et al*., *J. Epidemiol. Community Health* **43**, 293 (1989).
34. E. Mostofsky *et al*., *Heart J*. **35**, 1404 (2014).
35. P. D. Gluckman *et al*., *Lancet* **373**, 1654 (2009).
36. B. S. McEwen, *Proc. Natl. Acad. Sci. U.S.A.* **2012**, 1 (2012).
37. B. L. Fredrickson *et al., Proc. Natl. Acad. Sci. U.S.A.* **110**, 13684 (2013).
38. L. Glass, *Nature* **410**, 277 (2001).
39. J. Bass, J. S. Takahashi, *Science* **330**, 1349 (2010).
40. J. van der Greef, *Nature* **480**, S87 (2011).

Acknowledgments
The authors thank Charlotte Lokin for producing the artwork in Figure 1.

Produced by the *Science*/AAAS Custom Publishing Office

Hypothesis-driven screening of Chinese herbs for compounds that promote neuroprotection

Authors:
Thomas Friedemann[1],
Min Li[2†],
Jian Fei[3†],
Udo Schumacher[4],
Juxian Song[2],
Sven Schröder[1*]

Protection against the loss of neurons or the retardation of disease progression is the major challenge for the treatment of neurodegenerative disorders like Alzheimer's disease (AD) and Parkinson's disease (PD). Current established drug therapies treat mainly symptoms, leading to cognitive enhancement in AD or improved movement in PD. However, neuronal repair or prevention of further degeneration has not been convincingly demonstrated in humans (*1*). Common mechanisms of neuronal damage include, among others: oxidative stress, mitochondrial dysfunction, autophagy dysfunction, excitotoxicity, protein aggregation, and genetic defects (*1–3*). Practically all drugs for AD that were neuroprotective in both in vitro and in vivo preclinical models failed in large clinical trials. Due to this failure, the therapeutic potential of traditional Chinese medicine (TCM) has recently received increased attention. Multiple herbs have been tested in cell cultures or animal models. However, in a situation similar to that of synthetic drugs, the evidence of neuroprotection in clinical studies is still unsatisfactory, most likely due to the fact that the paradigm of treatment with a single chemical entity is not easily applicable to the complexity of TCM prescriptions (*4*).

The screening modality bottleneck

In recent decades the search for novel plant-derived drugs has relied on hypothesis-free, high-throughput screening (HTS) using metabolomic, proteomic, and genomic methodologies (*5*). The professed goal has been to identify isolated single-target small molecular chemicals based on compound libraries. However, even the largest plant compound libraries represent only a small fraction of possible chemical diversity of natural products (*6*). Further, in vitro HTS hits often lack efficacy in vivo (*7*). One instructive example is Huperzine A, an alkaloid isolated from *Huperzia serrata*, which showed multiple beneficial effects in preclinical models, but failed in a phase 2 clinical study for AD (*8*). Research that primarily focuses on monocompounds isolated from plants carries a high risk that the observed effects will not be transferable from in vitro or animal models to clinical practice.

Neurodegeneration is a complex process involving multiple pathophysiological mechanisms; therefore it seems only rational to apply a multitargeted approach to a multifactorial

Produced by the *Science*/AAAS Custom Publishing Office

disease. Accordingly, multicomponent medicines may prove to be more potent by virtue of multiple bioactive components (*9*).

TCM herbal mixtures have long been used to treat AD and PD. Examples include modified *Huanglian-Jiedu-Tang* (*10*) and *Fu-Zhi-San* (*11*) for AD and *Jia-Wei-Liu-Jun-Zi-Tang* (*12*) or *San-Huang-Xie-Xin-Tang* (*13*) for PD. Even if controlled clinical trials show efficacy, elucidating the mechanisms of action is an onerous challenge due to the complex chemical composition of herbal extracts.

Hypothesis-driven screening

The philosophy and practice of physiology and pathology vary significantly between TCM and Western medicine in that similar pathophysiological features are often described using different terminologies. Therefore, the application of traditional clinical knowledge to the Western system requires an interdisciplinary and intercultural validation process to identify effective herbal candidates and develop the optimal experimental design.

Cell and animal models used to validate drug candidates from classical screening processes can mimic human pathophysiology to a limited extent. By contrast, the candidate herbs from a bedside-to-bench-to-bedside approach have already been tested successfully in humans. This latter, hypothesis-driven approach (as opposed to the hit-and-miss high-throughput approach) reduces the risk of running into cost-intensive dead ends due to inefficacy or unexpected side effects discovered during clinical trials. The process begins when candidate herbs are systematically reviewed in the scientific and medical literature for their in vitro, in vivo, and clinical actions, and discussed by an interdisciplinary panel of experts. A substantiated working hypothesis is then established by analyzing and integrating the traditional medicinal usage and current scientific data of individual herbs and their known bioactive compounds. Based on this knowledge, one can carefully select in vivo and in vitro models for the primary screening and efficacy assay steps. After initial screening, transcriptomic, proteomic, and metabolomic analysis can be performed to further substantiate mode-of-action hypotheses (*14*).

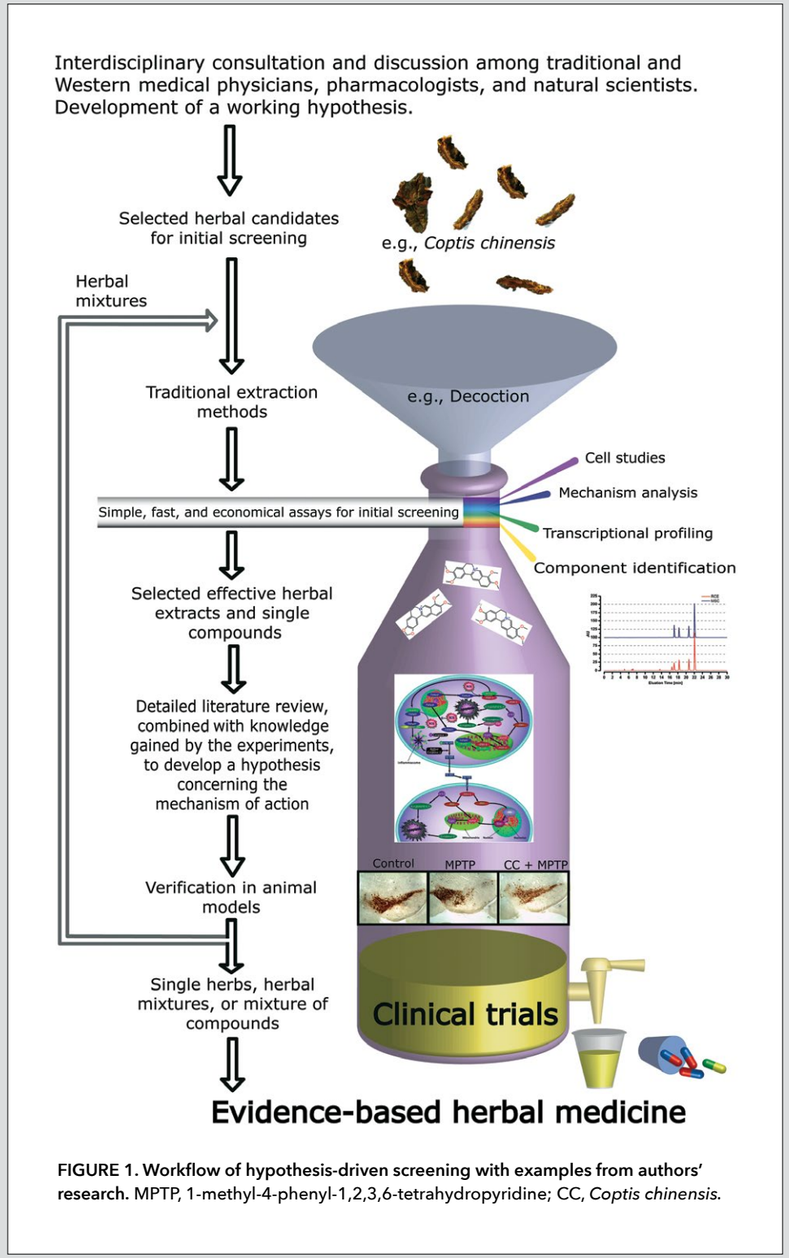


FIGURE 1. Workflow of hypothesis-driven screening with examples from authors' research. MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; CC, *Coptis chinensis*.

[1]HanseMerkur Center for Traditional Chinese Medicine at the University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[2]Mr. & Mrs. Ko Chi-Ming Centre for Parkinson's Disease Research, School of Chinese Medicine, Hong Kong Baptist University, Hong Kong, China
[3]School of Life Sciences and Technology, Tongji University, Shanghai, and Shanghai Research Center for Model Organisms, Shanghai, China
[4]Institute of Anatomy and Experimental Morphology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[†]Contributed equally to this work.
[*]Corresponding Author: schroeder@tcm-am-uke.de

These hypothesis-based screenings should be followed by mechanistic studies to identify the mode of action of the drug as a prerequisite for the preparation of clinical trials. Figure 1 represents a hypothesis-driven screening process for the evidence-based evaluation of a TCM product. The aim is not to find just one single compound for a single pathway, but rather to apply the procedure to combinations of herbs or substances, thus enabling the discovery of additive and synergistic effects, reflecting the current practice of TCM. Substantial optimization of this process is still required, but it provides a potentially valuable alternative to current, suboptimal classical screening methods.

Test case: Finding herbs for PD

Following careful consideration, the traditional formula *Jia-Wei-Liu-Jun-Zi-Tang* was chosen to test our hypothesis-based screening methodology. It has previously been shown to improve symptomatology and communication ability in PD patients (*12*). We screened a series of extracts and compounds from this formula and identified several autophagy enhancers with neuroprotective effects (*15–17*). Two representative compounds isolated from *Uncaria rhynchophylla* (Miq.) Jacks (Gouteng), corynoxine (Cory) and corynoxine B (Cory B), were found to promote the degradation of α-synuclein (the main component of Lewy body fibrils) and protect dopaminergic neurons by enhancing autophagy in cell culture and *Drosophila* models of PD. Cory enhances autophagy by inhibiting the mechanistic target of rapamycin (mTOR) pathway, while Cory B elicits that same effect by targeting HMGB1-Beclin 1 interaction (*18*) and restores autophagy inhibited by α-synuclein (*15*). These two active compounds may exert synergistic effects, accounting for the neuroprotective activity. Proteomic/metabolomic analysis and animal studies are ongoing to clarify the molecular mechanisms of action and potential preclinical efficacy.

In a second study, hypothesis-driven screening guided us to *Coptis chinensis* Franch. (CC) and coptisine (Cop, a component of CC), both of which showed neuroprotective effects against oxidative stress-induced cytotoxicity (*19*). However, a crude extract of CC was more effective than Cop alone (*20*). Subsequently, we extended our research to in vitro and in vivo models for PD, using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mouse experiments to create a subchronic PD model, and its active metabolite 1-methyl-4-phenylpyridinium (MPP^+) in cell experiments. Furthermore, we found that CC and Cop protected cells from MPP^+-induced cytotoxicity and CC also protected MPTP-treated mice from movement disorders and loss of dopaminergic cells in the substantia nigra. Our data suggested that the neuroprotective effect of CC or Cop might at least in part be caused by transcriptional regulation (*18*). Microarray analyses of the transcriptome of CC-treated cells revealed only two differentially regulated genes, MTND1 and TXNIP, which could possibly explain the neuroprotective effect (*19*, *20*).

It is apparent from our work that combining single compounds or herbs, which act via different modes, has the potential to generate additive or synergistic effects. Comparing the efficacy of the crude herb, its active compounds, and traditional mixtures using the herb as an ingredient, may help us to better understand the scientific principles behind herbal compositions. Validation of this promising approach will not be an easy path, and can only be achieved through the concerned efforts of a collaborative network of scientists and medical professionals.

Conclusions

TCM herbal mixtures have been used successfully for millennia, but their mode of action remains poorly understood. Nevertheless, they may have an enormous potential due to their multitarget mode of action for treating multifactorial complex diseases, including AD and PD, for which satisfactory conventional treatments do not exist. The classical screening approach using shotgun methods has not been as successful as hoped, despite considerable cost and effort. The development of hypothesis-driven screening methods is therefore essential and should result in valuable outcomes.

References

1. C. Holscher, *Neural Regen. Res.* **9**, 1870 (2014).
2. J. L. Cummings *et al.*, *Alzheimers Res. Ther.* **6**, 37 (2014).
3. A. L. Petraglia *et al.*, *Surg. Neurol. Int.* **2**, 146 (2011).
4. D. Normile, *Science* **299**, 188 (2003).
5. A. L. Harvey *et al.*, *Nat. Rev. Drug Discov.* **14**, 111 (2015).
6. D. H. Drewry *et al.*, *Curr. Opin. Chem. Biol.* **14**, 289 (2010).
7. J. L. Medina-Franco *et al.*, *Drug Discov. Today* **18**, 9 (2013).
8. M. S. Rafii *et al.*, *Neurology* **76**, 1389 (2011).
9. B. M. Schmidt *et al.*, *Nat. Chem. Biol.* **3**, 360 (2007).
10. S. S. Durairajan *et al.*, *PLOS ONE* **9**, e92954 (2014).
11. M. Bi *et al.*, *Neurosci. Lett.* **501**, 35 (2011).
12. W. F. Kum *et al.*, *Evid. Based Complement. Alternat. Med.* **2011**, 724353 (2011).
13. Y. C. Lo *et al.*, *Evid. Based Complement. Alternat. Med.* **2012**, 501032 (2012).
14. X. Wang *et al.*, *Mol. Cell. Proteomics* **12**, 1226 (2013).
15. J. X. Song *et al.*, *Autophagy* **10**, 144 (2014).
16. J. H. Lu *et al.*, *Autophagy* **8**, 98 (2012).
17. L. L. Chen *et al.*, *J. Neuroimmune Pharmacol.* **9**, 380 (2014).
18. D. Tang *et al.*, *J. Cell Biol.* **190**, 881 (2010).
19. T. Friedemann *et al.*, *J. Ethnopharmacol.* **155**, 607 (2014).
20. T. Friedemann *et al., Evid. Based Complement. Alternat. Med.* **2015,** 827308 (2015).

Acknowledgments

The authors would like to acknowledge Prof. Wolfgang Schwarz for the founding and establishment of a scientific Sino-German Network on TCM research. Thanks to Sarah Mirza for the illustration. This work was supported by the HanseMerkur Insurance Group and the Innovation Foundation Hamburg (721.230-002), and by grants from the General Research Fund (HKBU 121009/14), the Innovation and Technology Fund (ITS/274/12), and the Health and Medical Research Fund (HMRF 12132091) from the Hong Kong Government (For M. Li).

Produced by the *Science*/AAAS Custom Publishing Office

Mapping ancient remedies: Applying a network approach to traditional Chinese medicine

Author:
Shao Li*

Over the thousands of years that traditional medicine has been practiced, a wealth of clinical experience and a large number of herbal formulae have been accumulated to support the practice of traditional Chinese medicine (TCM). It is challenging to assess TCM therapies that are mechanistically unclear, in particular because many ingredients in an herbal formula may exert their effects on the body through low affinity binding to multiple different targets. This is at odds with the current "one target, one drug" approach most often associated with Western therapies, which is committed to the pursuit of drugs that bind to a single target with high affinity and specificity. At the same time that the single target-based, high-throughput screening assays that are the hallmark of reductionist research are being questioned due to high failure rates (*1*), network pharmacology is evolving as a systematic paradigm for drug discovery and development (*2*, *3*). Network pharmacology adopts a network approach to represent and analyze the complex biological systems underlying diseases and drug actions. It thus aids in drug discovery, drug design, and drug development, sharing a holistic perspective that is characteristic of TCM (*2*–*5*). Today, the integration of TCM and network pharmacology (TCM-NP) provides an innovative research perspective for proponents of both reductionist and holistic medicine.

Treating a network as a therapeutic target

TCM-NP highlights a "network target, multicomponent therapeutics" approach (*6*). The core principle of a network target is to construct a biological network that can be used to decipher complex diseases. The network is then used as the therapeutic target, to which multicomponent remedies, such as herbal formulae, are applied (*5*, *6*). Here, a network-based model incorporating an "effect-on" and "effect-off" switch can be proposed as a means to understand how herbal medicine might work. For the model to be "on," multiple ingredients (or a single ingredient as a special case) in an herb or herbal formula should induce additive or synergistic effects on a set of interacting targets within a given network, such that the final outcome reaches a threshold to produce a measureable pharmacological result by network propagation and integration in both space (spatial extension) and time (temporal duration) (Figure 1A). In this way, multiple low-affinity actions can achieve a significant effect. By contrast, in the "off" scenario, herbal ingredients that exert opposite or antagonistic actions on a target network (Figure 1B), or only weakly affect decentralized targets in a network (Figure 1C), may not produce effects that reach the measureable threshold. This model can help to explain why the actual efficacy of herbal ingredients can be greater than the sum of the effects of individual ingredients (*7*, *8*). For example, a recent study demonstrated that the classic *Liu-Wei-Di-Huang* formula can exert diverse therapeutic actions on metabolic and immune disorders by regulating a set of networked targets through different groups of bioactive ingredients (*9*).

According to the proposed effect-switch model, an optimal combination of herbal ingredients from herbal formulae can be considered worth pursuing if it satisfies the criteria for a network-based effect switch: turning on desirable effects and turning off undesirable effects (including side effects and toxicity).

TCM-NP methodologies

Through the development of computational and experimental methods, TCM-NP aims to map both disease genes (including gene products) and herb targets in a network, and provide information on bioactive compounds, synergistic combinations, mechanisms of action, and modern indications for herbal formulae by measuring the network association (e.g., modularity, connectivity, feedback, and dynamics) between disease genes and herb targets (Figure 2A). Representing complex biological systems as networks provides a foundation for the exchange of scientific and clinical data between modern and traditional forms of medicine. Now, 'omics technologies, knowledge databases, and bioinformatics are providing more actionable data and increasingly sophisticated analysis tools, thus accelerating the understanding of biological networks, a situation that will undoubtedly speed TCM-NP progress. For example, by exploiting the available data pool, a computational method, drugCIPHER, has been developed to predict an herbal compound's target profile by integrating chemical, target, and network information from current FDA-approved drugs (*10*). A sibling method, CIPHER, also performed well in predicting disease genes (*11*). In recent years, the use of systems biology and bioinformatics technologies in TCM has been growing rapidly, as has the generation of TCM-NP data and our understanding of multilayer networks. Through this work, associations have been elucidated between herbs, compounds, molecules, microbes, phenotypes, and diseases and/or TCM syndromes, generating fresh insights into holistic traditional medicine.

Not only does network pharmacology reflect the holistic properties of herbal medicine, but the rich trove of data on the use of TCM as herbal combinations can assist in refining the network. Considering that we still have much to learn regarding biological systems and drug action/interactions, the field of network pharmacology can undoubtedly benefit by combining top-down and bottom-up strategies. Since certain herbal formulae have been shown to be clinically effective, the inclusion of this empirical knowledge of multicomponent therapeutics may permit exciting advancements in network pharmacology.

Ministry of Education Key Laboratory of Bioinformatics and Bioinformatics Division, Tsinghua National Laboratory for Information Science and Technology/Department of Automation, Tsinghua University, Beijing, China
*Corresponding Author: shaoli@mail.tsinghua.edu.cn

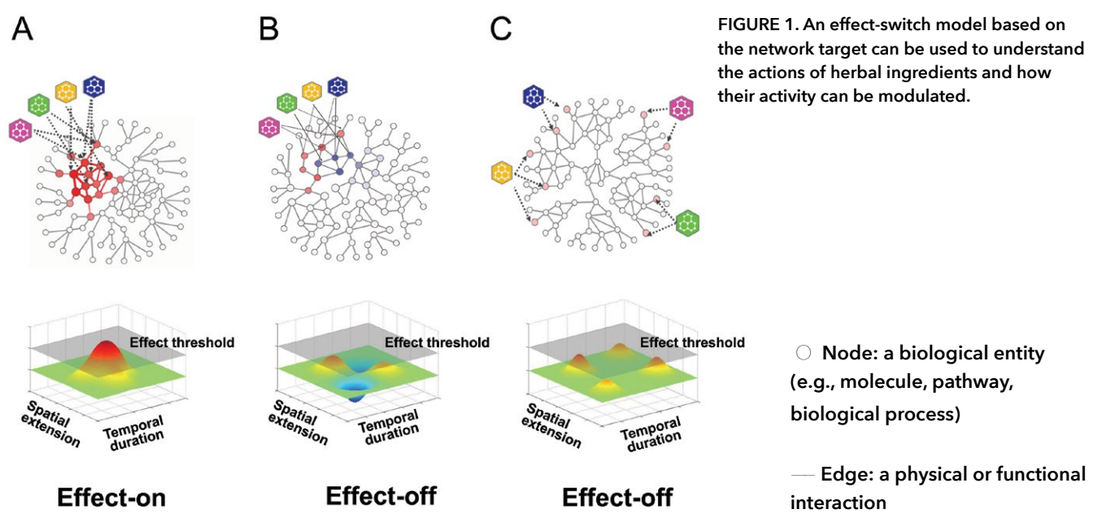


FIGURE 1. An effect-switch model based on the network target can be used to understand the actions of herbal ingredients and how their activity can be modulated.

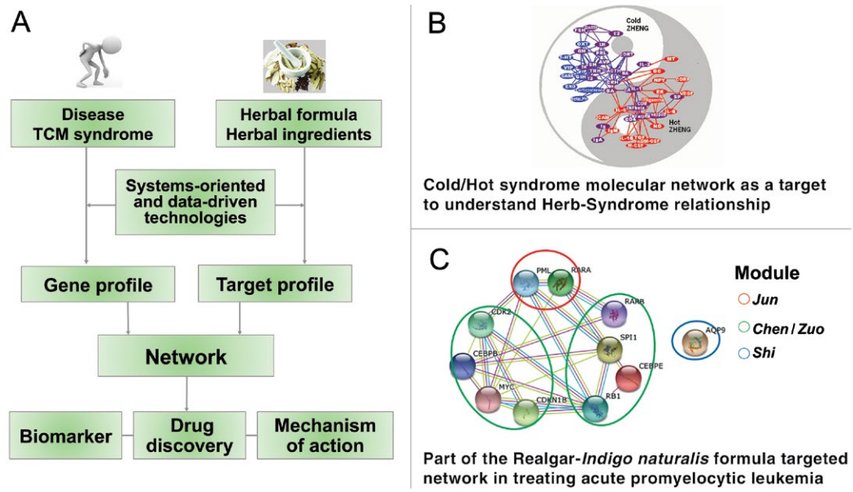


FIGURE 2. (A) Schematic of traditional Chinese medicine-network pharmacology (TCM-NP) methodology. (**B**) A representation of a Cold/Hot Syndrome molecular network (*12*). (**C**) Part of the Realgar-*Indigo naturalis* components targeted network. PML, promyelocytic leukemia; RARA, retinoic acid receptor, alpha; RB, retinoblastoma; MYC, v-*myc* avian myelocytomatosis viral oncogene homolog; CDK2, cyclin-dependent kinase 2; SPI1, a gene encoding transcription factor PU.1; CDKN1B, cyclin-dependent kinase inhibitor 1B; CEBPB, cytidine-cytidine-adenosine-adenosine-thymidine (CCAAT)/enhancer binding protein, beta; CEBPE, CCAAT/enhancer binding protein, epsilon; RARB, retinoic acid receptor, beta; AQP9, aquaporin 9 (*15*).

Application of TCM-NP in traditional medicine

TCM-NP promises to help elucidate the complex molecular mechanisms underlying the actions of traditional therapies as well as explore new indications for their use. Herbal formulae are traditionally used to treat so-called TCM syndromes (*Zheng*). Most medicinal herbs can be categorized as cold, cool, warm, or hot, based on their composition and nature. One of the earliest TCM-NP studies showed that Cold and Hot Syndromes are closely associated with a number of networked

Produced by the *Science*/AAAS Custom Publishing Office

neuroendocrine-immune molecules, indicating a metabolism-immune imbalance. Meanwhile certain so-called cold herbs can target hub nodes in the Hot Syndrome molecular network, and vice versa, to restore the corresponding network balance (*12*) (Figure 2B). It was further found that active compounds in a cold herbal formula, *Qing-Luo-Yin*, could synergistically suppress the cytokine and vascular endothelial growth factor pathways in a hot network to treat disorders involving inflammation and angiogenesis (*13*).

Moreover, TCM-NP may provide a network-based interpretation for the *Jun-Chen-Zuo-Shi* (emperor-minister-assistant-courier) theory of combining herbal formulae. A disease molecular network could accordingly be divided into *Jun-Chen-Zuo-Shi* target modules to aid in the determination of the optimal combination therapy (*14*). For instance, in a Realgar-*Indigo naturalis* formula, tetraarsenic tetrasulfide as a *Jun* can target the promyelocytic leukemia (PML)-retinoic acid receptor alpha (RARA), a fusion protein involved in acute PML. Indirubin and tanshinone IIA can act as *Chen* and *Zuo*, respectively, by targeting the network immediately adjacent to, and interacting with, PML-RARA, while the *Shi* targets the membrane channel transporter, aquaporin-9, to aid arsenic transportation (*15*) (Figure 2C; the target interactions are extracted by using the Search Tool for the Retrieval of Interacting Genes/Proteins, http://string-db.org/). Additional TCM-NP case studies have recently been published (*16*).

Clearly, the merging of TCM and network pharmacology is in its early stages. A more in-depth analysis of TCM-NP will require more powerful computational or experimental methodologies and technologies, together with more comprehensive TCM data. Although the task is challenging, there is much optimism for the future, particularly with the arrival of the big data and precision medicine era. Moving forward, TCM-NP promises to be an innovative way to explore the application and efficacy of TCM, and can contribute to narrowing the gap between Eastern and Western medical practices.

References

1. E. C. Butcher, *Nat. Rev. Drug Discov*. **4**, 461 (2005).
2. A. L. Hopkins, *Nature Chem. Biol.* **4**, 682 (2008).
3. S. Li, *J. Chin. Integrat. Med.* **5**, 489 (2007).
4. A. L. Barabási, N. Gulbahce, J. Loscalzo, *Nat. Rev. Genet.* **12**, 56 (2011).
5. S. Li, B. Zhang, *Chin. J. Nat. Med.* **11**, 110 (2013).
6. S. Li, B. Zhang, N. B. Zhang, *BMC Syst. Biol.* **5**(Suppl. 1), S10 (2011).
7. C. T. Keith *et al*., *Nat. Rev. Drug Discov.* **4**, 71 (2005).
8. T. Xue, R. Roy, *Science* **300**, 740 (2003).
9. X. Liang *et al*., *Mol. BioSyst.* **10**, 1014 (2014).
10. S. Zhao, S. Li, *PLOS ONE* **5**, e11764 (2010).
11. X. Wu *et al*., *Mol. Syst. Biol.* **4**, 189 (2008).
12. S. Li *et al*., *IET Syst. Biol.* **1**, 51 (2007).
13. B. Zhang *et al*., *Evid. Based Complement. Alternat. Med.* **2013**, 456747 (2013).
14. S. Li *et al*., *BMC Bioinformatics* **11**(Suppl. 11), S6 (2010).
15. L. Wang *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **105**, 4826 (2008).
16. S. Li, T. P. Fan, W. Jia, A. Lu, W. Zhang, *Evid. Based Complement. Alternat. Med.* **2014**, 138460 (2014).

Acknowledgments

The author thanks Dr. Tai-Ping Fan and his colleagues for their review of this work and their valuable comments and suggestions. This work is supported by the National Natural Science Foundation of China (81225025 and 91229201).

Drug discovery in traditional Chinese medicine: From herbal *fufang* to combinatory drugs

Authors: Bing He†, Cheng Lu†, Maolin Wang, Guang Zheng, Gao Chen, Miao Jiang, Xiaojuan He, Zhaoxiang Bian, Ge Zhang*, Aiping Lu*

Today, drug discovery is a critical issue in the pharmaceutical industry. Although global spending on drug discovery and development has risen sharply in the last decade, the approval rate for new drugs is declining. This situation is mainly due to drug failure caused by lack of efficacy and/or safety. One important reason for this is that common single-drug therapeutics are rarely able to fully address the complex nature of most human diseases (*1*). Producing combinatory drugs—combinations of multiple drugs against multiple disease targets—is an appropriate approach to address this issue (*2*).

Traditional Chinese medicine (TCM), a medical system based on natural products, has been widely used in East Asia for thousands of years to provide treatments and cures for disease. The long history and extensive documentation of TCM clinical practices have accumulated a considerable number of *fufang* (herbal compound prescriptions) that exhibit in vivo efficacy and safety, and provide a unique resource for combinatory drug discovery.

TCM: Synergy of multiple ingredients

The documented history of TCM dates back more than four thousand years to the times of Shennong (Yan Emperor), while mature TCM theory was established during the Song dynasty (960–1279 CE). TCM theory is based on a holistic, interconnected view of the world. The patient is considered as a system in which the normal balance of Yin/Yang has been disrupted.

The first step in the TCM diagnosis process is to determine the particular *Zheng* (pattern or syndrome) afflicting the patient (*3*). In our studies, we analyzed the molecular networks of *Han Zheng* (cold pattern) and *Re Zheng* (heat pattern) in rheumatoid arthritis patients. The results indicated that *Han Zheng* is related to the Toll-like receptor signaling pathway, while *Re Zheng* impacts the calcium and peroxisome proliferator-activated receptor signaling pathways (*4*). Characteristic molecular signatures for each *Zheng* were also identified (*5*).

Based on the particular *Zheng* and characteristics of the patient, a suitable *fufang* was chosen for treatment. *Fufang* were formulated based on the TCM principle of *Jun-Chen-Zuo-Shi,* with *Jun* (literally "emperor") being the principal ingredient that targets the primary causes and symptoms of the disease, *Chen* ("minister") targeting the underlying causes of the disease and potential protective mechanisms, *Zuo* ("assistant") helping the *Jun* and *Chen* ingredients to achieve their optimal curative effects by counteracting any potential adverse side effects and by treating any secondary symptoms of the disease, and finally, *Shi* ("courier") ensuring that all ingredients are properly absorbed and delivered to the target organs.

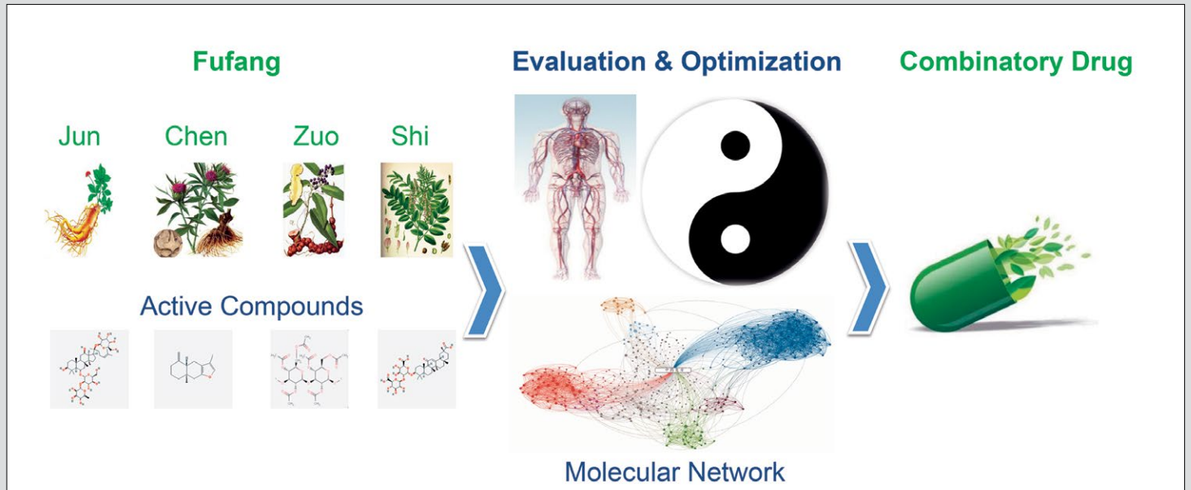


FIGURE 1. Overview: From herbal *fufang* to combinatory drugs.

We will use a well-known and clinically tested *fufang* for leukemia therapy as an example to illustrate this principle. The formula, known as Realgar–Indigo formula (RIF), contains realgar, indigo minerals, and red sage root. Molecular analyses showed that arsenic in realgar works as *Jun* by directly attacking the receptor oncoprotein in leukemia cells. Tanshinone, the active ingredient in red sage root, acts as *Chen* by partially restoring those pathways that stop leukemia spreading. Indirubin, the active ingredient in indigo, works as *Zuo* by antagonizing the toxicity of arsenic and slowing the growth of leukemia cells. Lastly, indirubin and tanshinone work as *Shi*, as these ingredients can enhance the cellular uptake of arsenic by increasing the gene expression and synthesis of carrier pore proteins in the cell membrane (*6*). Such multiple synergetic ingredients in *fufang* offer a unique opportunity to attack multiple disease-causing mechanisms simultaneously, and make it a unique resource for the discovery of new combinatory drugs. Arsenic, the *Jun* ingredient mentioned above, is now the primary drug in a combination therapy for acute promyelocytic leukemia (*7*). Generally, understanding the pharmacology network of *fufang* will be useful in TCM-based combinatory drug discovery. The recent development of 'omics technologies and in silico methods for analyzing signaling pathways provide useful tools for understanding the pharmacology network of various *fufang*.

The application of 'omics and in silico technologies

'Omics technologies such as genomics, transcriptomics, proteomics, and metabonomics are high-throughput technologies used to analyze a variety of molecules simultaneously. These technologies have facilitated the study of the molecular pharmacology of *fufang* at multiple levels (*8*). However, the high cost of such studies has thus far limited the number of *fufang* studies using 'omics technologies. As a lower cost alternative, in silico methods using computational algorithms and cheminformatics can virtual screen large numbers of drug-target interactions in order to construct pharmacology networks of *fufang* activity (*9*). In one example, the active compounds and mechanisms of actions of *Gegen-Qin-Lian-Tang* for the treatment of type 2 diabetes were determined by an in silico approach (*10*).

A network-based evaluation approach

A primary advantage of *fufang* is the ability to simultaneously target multiple points within the complex network of a disease. We established an evaluation approach to examine the interaction between a *fufang* and a human disease network to facilitate the translation of a *fufang* into a combinatory drug (Figure 1).

This approach evaluated three effects of the drug: the major therapeutic effect (MTE), the associated therapeutic effect (ATE), and any ancillary effects (AEs). MTE is the ability of the drug to target the affected disease network and recover normal function, similar to the role of *Jun* ingredients. ATE is the drug's ability to enhance the effects of the MTE and provide protection against negative side effects, as provided by *Chen* and *Zuo*. AEs refer to any additional assistive mechanisms, similar to the role of *Shi* ingredients.

Institute for Advancing Translational Medicine in Bone & Joint Diseases, School of Chinese Medicine, Hong Kong Baptist University, Hong Kong, China
†Contributed equally to this work.
*Corresponding Authors: zhangge@hkbu.edu.hk (G.Z.) and aipinglu@hkbu.edu.hk (A.L.)

Produced by the *Science*/AAAS Custom Publishing Office

The evaluation of ingredients considers three aspects: coverage of the *fufang* target network, the ability of the formula to alter the target's function, and the impact of this alteration on the disease network. Here, we provide an example to illustrate the general evaluation process and its contribution to combinatory drug discovery. *Bizheng-Tang*, the decoction of eight ingredients, could help overcome the low response of leflunomide in rheumatoid arthritis (RA) treatment. To simplify *Bizheng-Tang*, we studied the gene expression profiles of low-response RA mice before and after *Bizheng-Tang* administration, as well as the effect of each separate ingredient in the formula. The results suggested that *Rhizoma Ligustici Chuanxiong* plays an essential role in *Bizheng-Tang*. Further clinical trials confirmed that ligustrazine, the active component of *Rhizoma Ligustici Chuanxiong*, in combination with leflunomide effectively overcame the low response to RA treatment (*11*).

This simplified approach for evaluation of *fufang* ingredients demonstrates a potential way to discover combinatory drugs, although further testing and verification of this process is still required.

Future work

To date, only a small number of *fufang* have been studied using advanced 'omics technologies and in silico methods. Although 'omics technologies are powerful, results are susceptible to variability caused by the use of nonstandardized research materials. Proper standards must therefore be established to better control study-to-study variation. In silico methods used for virtual screening have been developed mainly for Western chemical medicine and a one-drug, one-target system. They are often not sufficiently powerful to handle the complex multidrug, multitarget nature of *fufang*. New algorithms therefore need to be developed specifically for *fufang*. When trying to develop combinatory drugs from *fufang*, one of the most challenging steps is deciding on a short list of effective formulae from the extensive ancient and contemporary literature, as there are over 11,000 plant species used in more than 100,000 *fufang* in China. The future discovery of combinatory drugs from *fufang* will benefit from the development of a research platform that contains biological information on *fufang* herbs and compounds, and on data from standardized 'omics studies, all integrated using TCM-specific in silico tools.

References

1. J. W. Scannell, A. Blanckley, H. Boldon, B. Warrington, *Nature Rev. Drug Dis.* **11**, 191 (2012).
2. J. Woodcock, J. P. Griffin, R. E. Behrman, *New Engl. J. Med.* **364**, 985 (2011).
3. M. Jiang *et al.*, *J. Ethnopharmacol.* **140**, 634 (2012).
4. C. Lu *et al.*, *Rheumatol. Int.* **32**, 61 (2012).
5. C. Lu *et al.*, *Evid. Based Comp. Alternat. Med.* **2012**, 203043 (2012).
6. L. Wang *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 4826 (2008).
7. H. H. Zhu, X. J. Huang, *New Engl. J. Med.* **371**, 2239 (2014).
8. X. Cheng *et al.*, *Sci. Reports* **4**, 5147 (2014).
9. S. Li, T. P. Fan, W. Jia, A. Lu, W. Zhang, *Evid. Based Comp. Alternat. Med.* **2014**, 138460 (2014).
10. H. Li *et al.*, *Evid. Based Comp. Alternat. Med.* **2014**, 495840 (2014).
11. M. Jiang *et al.*, *Drug Discovery and Therapy World Congress 2014,* PO-34, 123 (2014); available at http://www.ddtwc.com/pdf/Abstract-Book(2014).pdf.

The polypharmacokinetics of herbal medicines

Authors: Wei Jia[1,2,3]*, Tai-ping Fan[4], Xiaoning Wang[3], Guoxiang Xie[2]

The pharmacokinetics (PK) of multicomponent herbal medicines (HMs) is a long-standing bottleneck for botanical drug and traditional medicine research. There are a number of reasons for this. One is the sheer number of plant-derived molecules that are typically present in HMs, which presents a substantial challenge to chemical and pharmacological evaluation. This is further complicated by the wide concentration range of the components. Another factor is the dynamic nature of chemical interactions between the plant-derived molecules and endogenous molecules. These interactions shape the PK of an HM and, consequently, the treatment outcome for individual patients. Monitoring the chemical components is made still more challenging by a lack of authenticated standards, by the complexity of both botanicals and biological sample matrices, and by the need for cross-disciplinary expertise involving 'omics sciences, biochemistry, pharmacology, bioinformatics, and systems modeling. As a result, current research on the PK of HMs is still in its infancy. It is largely focused on in vivo characterization of one or two key HM components, the results of which may be difficult to link to the holistic treatment effects that result from drug-drug interactions (*1*).

A Poly-PK Approach

The traditional approach to understanding the pharmacology of a multicomponent agent is to study the effects of single active components on well-defined targets, such as specific enzymes or genes. However, it has proven impractical to integrate the results obtained using these reductive approaches to generate a systems understanding of concerted pharmacological interventions (*2*). The attempts to characterize the PK of multicomponent natural products have, however, demonstrated that the PK behavior of a given phytochemical is altered by coexisting constituents (*3*–*5*).

The advent of comprehensive profiling technologies offers tremendous new opportunities for understanding multicomponent PK. Phytochemical profiling and metabolomics can be coupled to multivariate statistical tools to generate multiparametric assessments. These allow us to create a concentration–time profile of a multicomponent HM, which we call a "Poly-PK," as well as other health determinants associated with the intervention.

We recently proposed an integrated profiling approach. It uses tandem mass spectrometry (MS) to provide quantitative dynamic concentration profiles of bioavailable xenobiotic molecules that result from in vivo absorption, and hepatic and gut bacterial metabolism, of herbal agents (*6, 7*). This Poly-PK approach takes into account both the diversity of the HM's chemical composition and its complex effects on the metabolic pathways of the mammalian system. When HMs enter our body, there are significant metabolite profile changes over time, which fall into three categories as illustrated in Figure 1: (1) HM-derived compounds absorbed into the circulation, (2) new metabolites generated by the chemical transformation of HM compounds by hepatic enzymes and gut microbes, and (3) endogenous metabolites that are altered in response to the HM intervention.

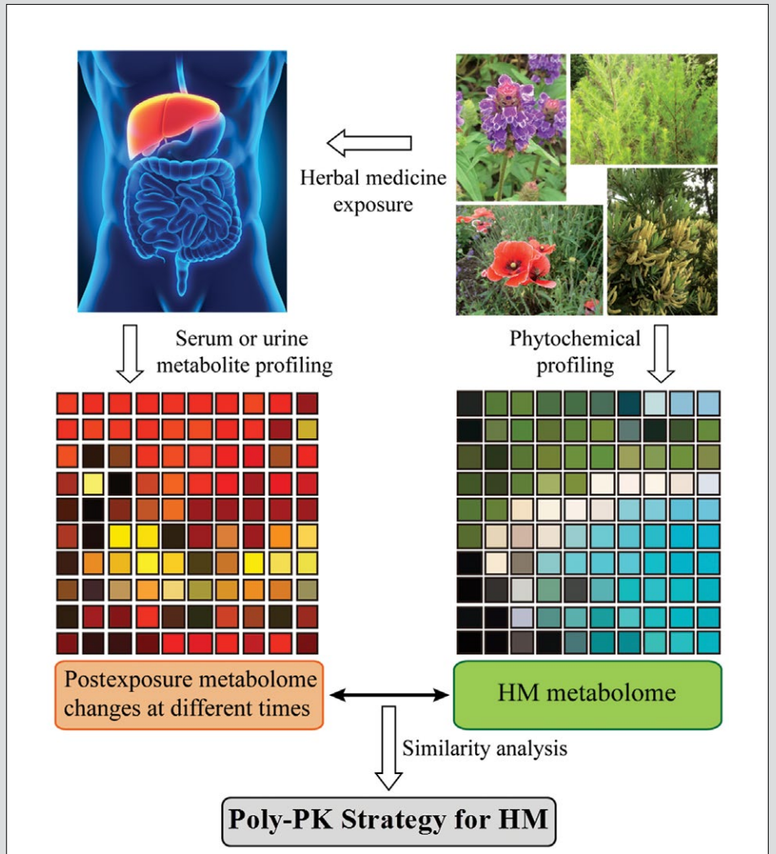


FIGURE 1. A Poly-PK strategy. The pharmacokinetic (PK) study of multicomponent herbal medicines (HMs) that integrates phytochemical and metabolite profiling.

Certain essential PK variables, such as maximum plasma concentration (C_{max}) and the time to reach C_{max} (t_{max}), can be obtained directly from the measured concentration data, while parameters such as the area under the concentration–time curve and the elimination half-life ($t^1/_2$) can be generated using PK modeling software.

Poly-PK in Action

We recently provided proof-of-concept for the above strategy (*8*). The study characterized the in vivo absorption and metabolism in humans of the phytochemicals of Pu-erh, a fermented tea produced in Southwest China. Pu-erh, which contains a large array of polyphenolic constituents, has a range of pharmacological properties, including the ability to reduce blood levels of triacylglycerol and total cholesterol (*9, 10*). Urine samples were collected from volunteers at 0, 1, 3, 6, 9, 12, and 24 hours following consumption of tea, and once a day during a six-week period that included a two-week baseline phase, a two-week daily Pu-erh tea ingestion phase, and a two-week "wash-out" phase. Volunteers were provided with standard meals for six weeks.

The Pu-erh tea water extraction and urine samples collected at the different time points were analyzed using ultraperformance liquid chromatography-quadrupole time-of-flight (TOF) MS and gas chromatography-TOF MS. This analysis generated 1,075 detected features from Pu-erh tea and 6,028 from urine samples (n = 12). The urinary metabolome dataset was subjected to univariate statistical analysis, yielding 2,652 variables that were altered by Pu-erh tea intake ($P < 0.05$). Using multivariate similarity analysis to compare the altered variables to the Pu-erh tea metabolome or the predose urine metabolome, we identified 19 absorbed tea polyphenols, 26 metabolites of the absorbed polyphenols, and 118 endogenous metabolites altered due to tea intake. Subsequent analysis demonstrated, for the first time, a correlation among the dynamic concentration profiles of bioavailable tea components and the human metabolic response profile (Figure 2). This type of approach, in which scientists simultaneously monitor the PK behaviors of multiple phytochemicals in vivo, will lead to the direct elucidation of the pharmacological and molecular mechanisms underlying HMs (*8*).

Perspectives

Over the past two decades HMs have been used increasingly as therapeutic interventions against a number of conditions (*2, 11, 12*). The pharmacology of HMs involves a "network" in which multiple components interact with multiple targets in vivo to exert a holistic treatment effect. The Poly-PK strategy described here uses an integrated phytochemical

[1]Shanghai Key Laboratory of Diabetes Mellitus and Center for Translational Medicine, Department of Endocrinology and Metabolism, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China
[2]University of Hawaii Cancer Center, Honolulu, Hawaii
[3]E-institute of Shanghai Municipal Education Committee, Shanghai University of Traditional Chinese Medicine, Shanghai, China
[4]Angiogenesis and Traditional Chinese Medicine Laboratory, Department of Pharmacology, University of Cambridge, Cambridge, United Kingdom
*Corresponding Author: wjia@cc.hawaii.edu

Produced by the *Science*/AAAS Custom Publishing Office

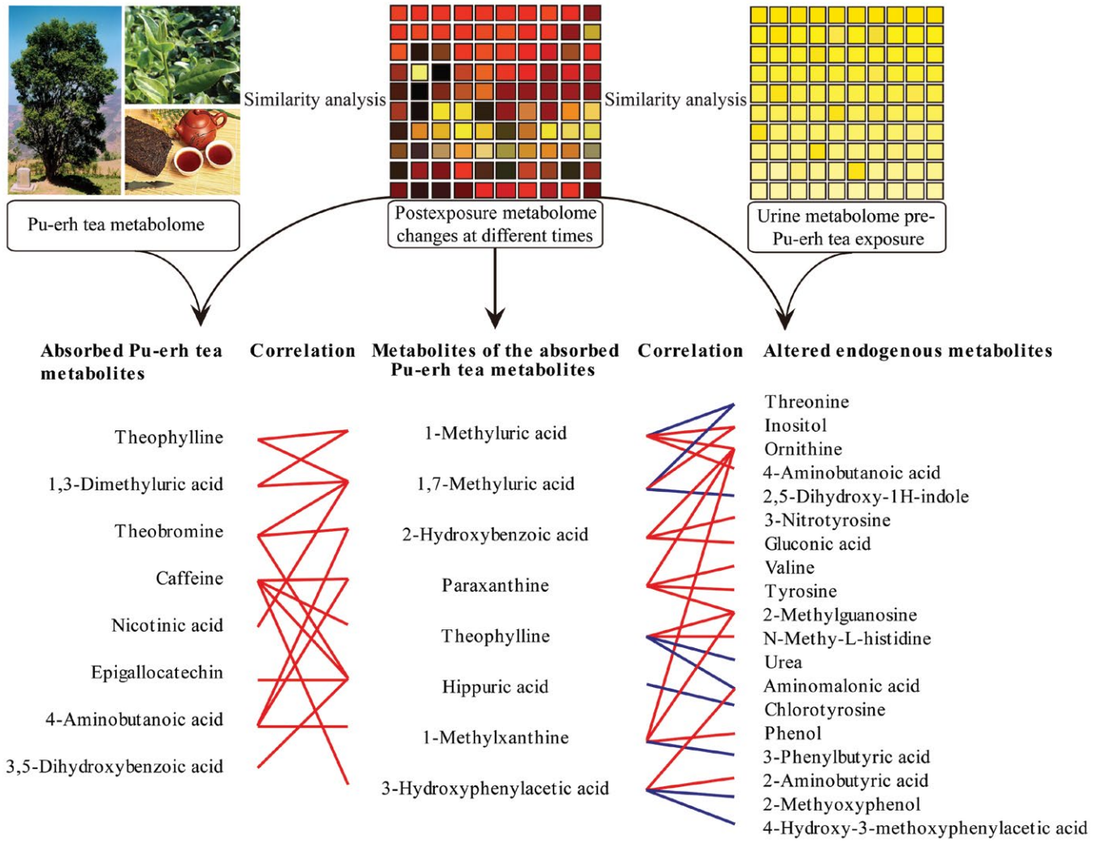


FIGURE 2. Poly-PK metabolomic profiles. The relationships among the three groups of metabolites associated with Pu-erh tea are visualized using correlation maps, as shown by red (positive) or blue (negative) lines.

and metabolomic profiling strategy coupled with multivariate statistical analysis to simultaneously monitor multiple HM components for pharmacological evaluation. This approach reveals the interrelationships between xenobiotics and endobiotics as well as the metabolic impact [using pharmacodynamic (PD) endpoints] of HM agents, providing an unprecedented level of insight into the mechanisms of action for HMs.

Most HMs are administered orally and are therefore exposed to microorganisms in the gut. The symbiotic gut microbiota performs a wide variety of biochemical transformations in which phytochemical compounds are selectively metabolized into active or absorbable components by microbial enzymes. Thus, two sets of genomes—our genome and gut microbiome—comodulate the absorption, distribution, metabolism, and excretion of HM compounds, generating a patient-specific PK profile. Many HM ingredients that were believed to be nonabsorbable and nonactive, such as polysaccharides and lignans, may have significant activities in vivo after oral administration, highlighting the important role that the human gut microbiota plays in HM pharmacology (*13*–*15*). A Poly-PK strategy can facilitate the development of personalized pharmacological evaluation of HMs, linking different patient responses to HM interventions. PK is often studied in conjunction with PD, and the Poly-PK strategy proposed here can simultaneously monitor PD markers through the measurement of multiparametric metabolic changes and other pharmacological endpoints (*6*). To achieve the desired HM therapeutic effect, each of the multiple components of the remedy will require a complete and dynamic panel of PK parameters. This information is essential for minimizing a drug's toxicity, reducing the chances of overdosing a patient or inducing drug complications, and, ultimately, improving patient compliance—and the quality of patients' lives.

We each possess a unique metabolic phenotype, known as a metabotype, that is characterized by endogenous metabolites and a panel of exogenous metabolites acquired from food consumption and/or drug treatments. This metabotype affects our individual metabolism of, and response to, any given HM. The Poly-PK strategy can unravel the complex interactions between the multiple components in HMs and in mammalian metabolic systems. The advent of the Poly-PK technology will greatly accelerate the holistic pharmacological evaluation of HM candidates and advance novel therapeutic developments. Furthermore, understanding the metabolic fate of a multicomponent drug is also a critical step toward developing the next generation of combinatorial chemical drugs, which will maximize the synergistic effects of certain drug components and help to prevent their undesirable metabolic side effects.

References

1. K. Ito *et al.*, *Pharmacol. Rev.* **50**, 387 (1998).
2. T. Xue, R. Roy, *Science* **300**, 740 (2003).
3. C. Xiang *et al.*, *Drug. Metab. Dispos.* **39**, 1597 (2011).
4. X. Qiao *et al.*, *J. Chromatogr. A* **1258**, 84 (2012).
5. S. M. He, E. Chan, S. F. Zhou, *Curr. Pharm. Des.* **17**, 357 (2011).
6. K. Lan, W. Jia, *Curr. Drug. Metab.* **11**, 105 (2010).
7. K. Lan, G. Xie, W. Jia, *Evid. Based Complement. Alternat. Med.* **2013**, 819147 (2013).
8. G. Xie *et al.*, *J. Proteome Res.* **11**, 3449 (2012).
9. J. K. Lin, S. Y. Lin-Shiau, *Mol. Nutr. Food Res.* **50**, 211 (2006).
10. K. L. Kuo *et al.*, *J. Agric. Food Chem.* **53**, 480 (2005).
11. R. L. Blaylock, *J. Am. Nutraceut. Assoc.* **2**, 19 (1999).
12. J. M. Hollander, J. I. Mechanick, *J. Am. Diet. Assoc.* **108**, 495 (2008).
13. E. D. Sonnenburg *et al.*, *Cell* **141**, 1241 (2010).
14. X. Xu *et al.*, *Biotechnol. Adv.* **31**, 318 (2013).
15. M. Blaut, T. Clavel, *J. Nutr.* **137**, 751S (2007).

The bioavailability barrier and personalized traditional Chinese medicine

Authors:
Lin-Lin Lu[1],
Xiao-Hong Liu[1],
Elaine Lai-Han Leung[3],
Ying Wang[1,2],
Jian Shi[1,2],
Ming Hu[1,4],
Liang Liu[3*],
Zhong-Qiu Liu[1,2*]

Traditional Chinese medicine (TCM) focuses on disease prevention and treatment using personalized therapies. The bioavailability barrier (BB) determines the concentration of drug being taken up by the human body, controlled by efflux transporters (ETs) and drug-metabolizing enzymes (DMEs), which are primarily regulated by nuclear receptors (NRs). Hence, polymorphisms of DMEs, ETs, and NRs can affect the pharmacokinetics of drugs, which ultimately influences the efficacy and/or toxicity of Chinese herbal formulas (CHFs). This paper presents the reconstruction of a BB-based network with new insights that help elucidate the therapeutic mechanisms of CHFs.

Western medicine focuses on molecular target-based therapy; however, there are limitations in transforming genotype-based or disease-oriented medicine into personalized and network-based clinical therapy (*1*). In contrast to Western medicine, CHFs achieve their effect through personalized modulation of a patient's health status. However, CHFs have not been widely accepted because their treatment mechanism has not yet been well defined (*2*). Determining how the components of CHFs will behave in the body is a pivotal aspect in determining treatment mechanisms of TCM. The BB has a key function in controlling absorption, biotransformation, and clearance of drugs in vivo (*3*). Therefore, a BB-based approach together with biological, biochemical, 'omics, and computational technologies is a powerful driver for establishing today's personalized TCM model.

The composition and characteristics of the BB network

The BB can be defined as a physiological defense network, because it plays a central role in preventing xenobiotic interference in the human body (*3*). The network is composed mainly of ETs and DMEs that are distributed in the liver and intestine, responsible for drug distribution and elimination (Figure 1A). ETs and DMEs are regulated by nuclear receptors (NRs) that respond to the endogenous and/or exogenous ligands (*4*). DMEs include cytochrome P450 and conjugating enzymes such as uridine 5′-diphospho-glucuronosyltransferases (UGTs) and sulfotransferases (SULTs). ETs refer to the transmembrane adenosine triphosphate (ATP)-binding cassette

Produced by the *Science*/AAAS Custom Publishing Office

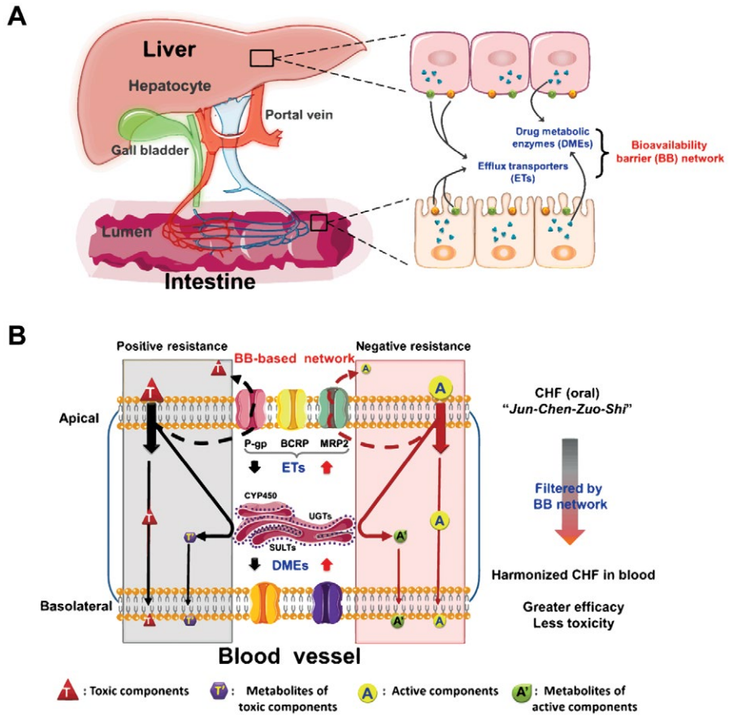


FIGURE 1. Composition of the BB network and its functions. (**A**) The molecular composition of the bioavailability barrier (BB) network in the liver and intestine. (**B**) The bidirectional activity of the BB network during harmonization, indicated by positive (left) and negative (right) resistance to active (yellow circle) and toxic (red triangle) components of Chinese herbal formulas (CHFs). P-gp, p-glycoprotein; BCRP, breast cancer resistance protein; MRP2, multidrug resistance protein 2; UGTs, UDP-glucuronosyltransferases; SULTs, sulfotransferases; ETs, efflux transporters; DMEs, drug-metabolizing enzymes.

efflux transporters (*3*). P-glycoprotein (P-gp), multidrug resistance protein 2 (MRP2), and breast cancer resistance protein (BCRP) on the apical membrane are the most important ETs, delivering or excreting drug metabolites. MRP1 and MRP3 on the basolateral membrane regulate the entry of drugs into the bloodstream. Multiple ETs and DMEs couple to create a complex network regulating disposition of drugs, particularly natural polyphenols abundant in CHFs (*3*).

Drug bioavailability depends not only on the activity of DMEs, but is also influenced by ETs (*3*). Therefore, variations in levels and activity of DMEs and ETs can markedly influence the pattern or pathway coupling in the BB network. For example, genetic variants of CYP2C19 and CYP2D6 are associated with reduced responses to the antiplatelet clopidogrel and the antiestrogen tamoxifen, respectively. The antibiotic doxorubicin exhibits individual differences that maximize therapeutic efficacy and minimize side effects on the basis of genetic variants of the regulatory pregnane X NR receptor (PXR), ETs (ABCB1, ABCG2, ABCC5, ABCB5, and RLIP76), and DMEs (CBR1, CBR3) (*5*). Therefore, the BB network is a critical determinant for implementing personalized medicine.

The BB network and harmonizing CHF efficacy and toxicity

A personalized treatment paradigm is central to the holistic and integrated approach of TCM. CHFs provide a valuable way to study the underlying multitarget mechanisms of personalized TCM treatments. In TCM, the most important principle of formulating CHFs is the *Jun-Chen-Zuo-Shi* (emperor-minister-assistant-courier) principle, which holds that each herb has its own diverse function (*6*). The biological functions of a CHF are harmonized by BB filtration in the body to achieve ideal therapeutic efficacy with minimal toxicity (Figure 1B).

BB filtration can optimize absorption and biotransformation of active and toxic components in CHFs to create a "reharmonized" formulation. The BB mainly exhibits this harmonization effect by bidirectionally driving the bioavailability of active and/or toxic components. Specifically, the bioavailability of active components in CHFs can be enhanced by inhibiting the functions of DMEs and/or ETs in the BB, with consequent improvement in positive pharmacological effects. In contrast, the BB prevents the overabsorption of toxic compounds in CHF (Figure 1B). For example, *Scutellaria baicalensis* contains abundant amounts of diverse polyphenols that possess anticancer and antiaging effects (*3*). MRP2/BCRP and UGTs/SULTs block the bioavailability of polyphenols, resulting in a reduction in pharmacological effects (*7*). However, ETs can act as molecular switches that facilitate the bioavailability of polyphenols (*3*).

Another example is *Radix aconite*, an herb considered to be clinically unsafe. Toxic aconitum alkaloids like aconitine have low bioavailability because of the resistance produced by the BB that limits their toxicity (*8*). In particular, CYP3A4, coupled with P-gp, BCRP, and MRP2 in the BB, blocks the entry of specific toxins into the blood (*9*). Thus, the rational use of such toxic herbs could be controlled by limiting the final dosage to a relatively safe level, not beyond the "resistance" capacity of the BB network. Notably, NRs could interact with the active/toxic components to alter the functions of DMEs and ETs, and consequently affect BB filtration. For example, *Radix glycyrrhizae*, popularly used as a *Shi* herb in CHF, activates PXR (*10*).

In summary, the BB-based network manipulates disposition of the active/toxic components in CHF via dual-directional regulation to achieve the maximal efficacy and minimal side effects of CHF. As such, the BB-based network is able to act as an intelligent, adaptive system for self-defense, while genomic variations of ETs and DMEs result in an individualized BB, which ultimately personalizes TCM treatment by controlling the transport behaviors of CHF (Figure 2).

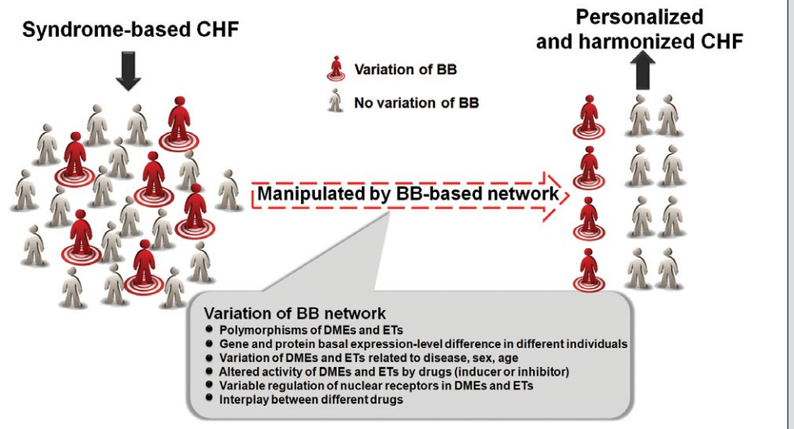


FIGURE 2. Personalized CHF therapy manipulated by the BB network.

Perspectives

The BB network is a complex system, largely because of the interplay of its key elements of DMEs, ETs, and NRs. It differs markedly among different individuals due to their unique polymorphisms and genotypes (*11*). The BB can be treated as a personalized system that induces the same drug to produce a variety of actions and toxicities in different individuals. In the future, characterization of personalized BB-based networks will bring a new era in both TCM and conventional medicine.

The essence of TCM is an individualized therapeutic system using CHF (*12*), which is consistent with the principles of personalized BBs. By taking into account BB filtering, CHF can be optimized to produce harmonized, multicomponent, multitarget formulae to achieve optimal effectiveness and low toxicity. We therefore recommend that future CHF research should be implemented together with evidence-based, personalized, and advanced BB research methodologies. The precise molecular mechanisms underlying each personalized BB need to be elucidated. Applying 'omics-related technologies such as metabolomics, proteomics, genomics, and computational prediction to profile individual BB network differences caused by polymorphisms or BB interaction factors could help to assess the unique effects of CHFs in different individuals (Figure 2). In conclusion, the BB network works not only as an indispensable tool for clarifying the mechanisms underlying CHF, but can also be used for characterizing and optimizing personalized TCM therapies.

[1]International Institute for Translational Chinese Medicine, Guangzhou University of Chinese Medicine, Guangzhou, China
[2]School of Pharmaceutical Sciences, Southern Medical University, Guangzhou, China
[3]State Key Laboratory of Quality Research in Chinese Medicine, Macau University of Science and Technology, Macau (SAR), China
[4]Department of Pharmacological and Pharmaceutical Sciences, College of Pharmacy, University of Houston, Houston, TX, USA
*Corresponding Authors: lliu@must.edu.mo (L.L) and liuzq@gzucm.edu.cn (Z.L.)

References

1. E. E. Schadt, *Nature* **461**, 218 (2009).
2. T. P. Fan *et al.*, *J. Ethnopharmacol.* **140**, 568 (2012).
3. M. Hu, *Mol. Pharm.* **4**, 803 (2007).
4. M. Wagner, G. Zollner, M. Trauner, *Hepatology* **53**, 1023 (2011).
5. K. M. Giacomini *et al.*, *Sci. Transl. Med.* **4**, 153ps118 (2012).
6. T. P. Fan *et al.*, *Trends Pharmacol. Sci.* **27**, 297 (2006).
7. E. Wenzel, V. Somoza, *Mol. Nutr. Food Res.* **49**, 472 (2005).
8. L. Ye *et al.*, *Toxicol. Lett.* **216**, 86 (2013).
9. Z. Cai, Y. Wang, L. J. Zhu, Z. Q. Liu, *Curr. Drug Metab.* **11**, 197 (2010).
10. Y. Mu *et al.*, *J. Pharmacol. Exp. Ther.* **316**, 1369 (2006).
11. P. E. Ferreira *et al.*, *Ther. Drug Monit.* **30**, 10 (2008).
12. L. Liu, E. L. Leung, X. Tian, *Nature* **480**, S100 (2011).

Acknowledgments

This work was supported by the National Natural Science Foundation of China (81120108025) and the Macau Science and Technology Development Fund (092/2012/A3).

Produced by the *Science*/AAAS Custom Publishing Office

Transdermal treatment with Chinese herbal medicine: Theory and clinical applications

Authors: Qing Wu[1*], Ling Dong[1], Jianping Liu[2], Dan Jiang[3]

Transdermal treatment with Chinese herbal medicine (CHM) has a long history of clinical application and theory in China. The earliest record of its use can be found in the ancient classic, *Huang Di Nei Jing* (227 BCE). The practice of transdermal treatment continued to evolve, reaching its highest popularity during the Qing dynasty, as elaborated in the book *Li Yue Pian Wen* (Wu Shi-Ji, 1864). It was emphasized in this book that the principles of treatment for both external and internal application of CHM were the same (*1*). This statement was the forerunner of the theory of transdermal administration for CHM, and modern transdermal drug delivery systems (TDDS) use the same concepts, although the precise delivery method is different.

The process of applying transdermal herbal medicine is not as simple as putting it directly on the skin. It should be applied specifically at the relevant acupuncture points (acupoints). According to Wu Shi-Ji, "If a disease is due to an external factor, you should apply herbs to release it on location; however, when the disease has spread into the body, you should apply herbs on the relevant acupuncture points to treat it." (*1*). Thus, transdermal treatments exert their therapeutic actions not only by absorption of active ingredients from herbs, but also the stimulation of acupoints. This concept is one of the distinctive differences between Chinese transdermal herbal treatments and modern TDDS (Figure 1).

Acupoints for treatment

The theory of acupoints and meridians is an important part of traditional Chinese medicine (TCM). The meridian system (or channel network) is believed in TCM theory to be the path along which the "*qi,*" or life energy, flows. According to this theory, *qi* and blood fill the meridian system, the channels, and are transported throughout the body via these meridians, feeding the organs. Modern biologists have discovered that there are convergent points of the organs' *qi* and blood along meridians (*2*). Placing an herbal patch directly on the acupuncture point therefore helps to maximize its therapeutic effects, with the aggregate effect being a combination of herbal action plus the acupoint response acting synergistically. The curative effect of an herbal patch placed on an acupoint is commonly regarded as superior to that of a patch placed on a non-acupoint (*3*). Reports on comparing responses of acupoints with non-acupoints indicate noticeable differences (*4*, *5*).

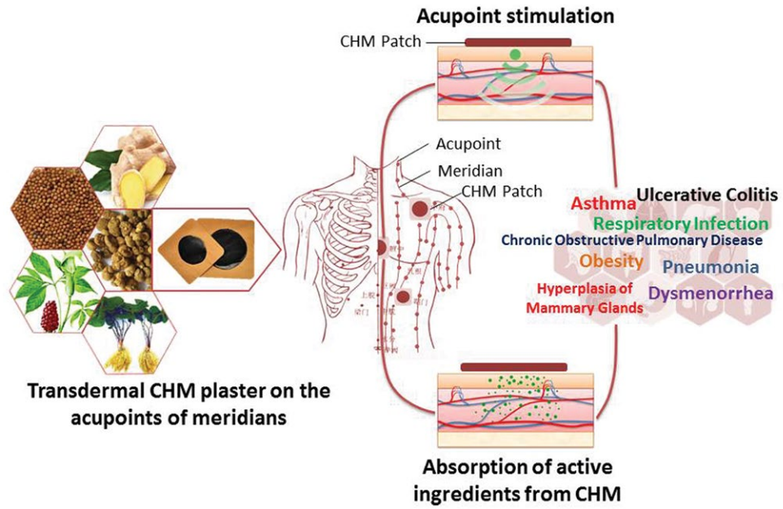


FIGURE 1. A variety of systemic diseases can be treated by application of transdermal Chinese herbal medicine (CHM) patches on meridian acupoints. A combination of acupoint stimulation and the active ingredients in the CHM elicit a healing response.

[1]School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing, China
[2]Centre for Evidence-Based Chinese Medicine, Beijing University of Chinese Medicine, Beijing, China
[3]Hallam Institute of Chinese Medicine, Sheffield, United Kingdom
*Corresponding Author: qwu@vip.sina.com

TABLE 1. Diseases and the corresponding acupoints for treatment (*6–18*).

Disease	Acupoints
Asthma in children	FS (BL13); XS (BL15); GS (BL17)
CODP (SP)*	FS (BL13); XS (BL15); GS (BL17)
Allergic rhinitis	DZ (DU14); FS (BL13); PS (BL20); SS (BL23)
Pneumonia in children	FS (BL13); GS (BL17); JL (EX-HN15); GH (BL43); Ashi
Respiratory infection in children	FS (BL13); DC (EX-B01); GH (BL43)
Brady arrhythmia	NG (PC6); XS (BL15)
Insomnia	SQ (RN8); NG (PC6); YQ (KI1)
Dysmenorrhea caused by endometriosis	ZJ (RN3); GY (RN4); ZG (RN19)
Dysmenorrhea	ZJ (RN3); GY (RN4); QH (BL24)
Ulcerative colitis	SJX (ST37); TS (ST25); ZSL (ST36); MM (DU4); GY (RN4)
Chronic renal failure	SQ (RN8)
Simple obesity	ZW (RN12); GY (BL26); QH (RN6); TS (ST25); SD (ST28); DH (SP15)

***Chronic obstructive pulmonary disease (stable phase).** DC, *Ding Chuan*; DH, *Da Heng*; DZ, *Da Zhui*; FS, *Fei Shu*; GH, *Gao Huang*; GS, *Ge Shu*; GY, *Guan Yuan*; GYS, *Guan Yuan Shu*; JBL, *Jing Bai Lao*; MM, *Ming Men*; NG, *Nei Guan*; PS, *Pi Shu*; QH, *Qi Hai*; SD, *Shui Dao*; SJX, *Shang Ju Xu*; SQ, *Shen Que*; SS, *Shen Shu*; TS, *Tian Shu*; XS, *Xin Shu*; YQ, *Yong Quan*; ZG, *Zi Gong*; ZJ, *Zhong Ji*; ZSL, *Zu San Li*; ZW, *Zhong Wan*.

Doctors practicing TCM use their extensive knowledge and experience of syndrome differentiation in clinical practice to diagnose patients before choosing which acupoints to stimulate. How each acupoint relates to a disease is based on both TCM meridian theory and many hundreds of years of empirical knowledge. For example, a transdermal herbal patch could be applied on acupoint *Shen Que* (RN8) for treating diarrhea, menstrual pains, or indigestion; whereas a patch on acupoint *Yong Quan* (KI1) treats high blood pressure, neurasthenia, or the common cold. Some common diseases and the corresponding treatment acupoints are summarized in Table 1 (*6–18*). All have been carefully selected from published clinical research papers using controlled trials and at least 100 cases. Liu and colleagues systematically reviewed the use of an acupoint herbal patch for treating allergic rhinitis and chronic obstructive pulmonary disease (COPD) in the stable phase using a meta-analysis. They included 21 randomized controlled trails (RCTs) involving a total of 2,327 participants (allergic rhinitis) and 20 RCTs involving 2,438 participants (COPD). The authors concluded that an herbal patch alone, or in combination with Western medicine (2011 Global Initiative for Chronic Obstructive Lung Disease guidelines), appeared to be effective for treating these diseases (*19*, *20*).

Recent advances in transdermal herbal preparations

Historically, the most common way to apply transdermal herbal preparations was using a black plaster. To prepare the plaster, herbs were fried in edible oil and red lead oxide (Pb_3O_4) was added to the refined herb oil to form a sticky mass. In recent years, however, since the advent of medicinal polymers, use of a black plaster has gradually given way to adhesive plasters, gel plasters, or patches, which have the significant advantage of reducing skin irritation. The technologies for extraction have also improved, allowing more concentrated extracts of active herbal ingredients to be made, thus facilitating percutaneous absorption of the multiple components of the herbal formula. Inclusion of carrier compounds such as microemulsions (*21*), liposomes (*22*), and cyclodextrin (*23*) can improve the compatibility of complex components and polymer materials. The latest transdermal herbal preparations can be more easily prepared, undergo improved quality control checks, and possess better stability than in the past (*23*). Moreover, pharmaceutical scientists are experimenting with the use of aromatic herbs that can act as natural transdermal uptake enhancers (*24*), which will potentially broaden their clinical application in the future.

References

1. S. Wu, *Li Yue Pian Wen,* 2nd Ed. (China Press of Traditional Chinese Medicine, Beijing, 2007).
2. L. Zheng, *Chin. Acupunct. Moxibustion* **4**, 222 (2003).
3. Y. Sui, *China J. Found. TCM* **9**, 53 2003).
4. X. Guo, X. Liu, *China Journal of Chinese Materia Medica* **37**, 1035 (2012).
5. L. Xu, Z. Cai, *J. TCM External Treat.* **14**, 6 (2005).
6. Y. Liang, *Chin. J. Integr. Tradit. Western Med.* **10**, 1424 (2013).
7. G. Li, L. Wang, Y. Lin, *Chin. J. Integr. Tradit. Western Med. Intens. Crit. Care* **9**, 1187 (2011).
8. M. Zhao, F. Qiao, X. Shen, *J. Tradit. Chin. Med.* **19**, 1661 (2012).
9. Y. Liu, J. Shen, R. Wang, *J. Tradit. Chin. Med.* **10**, 620 (1994).
10. L. Sun, S. Lin, S. Tong, *Chin. Acupunct. Moxibustion* **5**, 21 (1995).
11. L. Xu, Y. Zhang, Y. Zheng, *Chin. Acupunct. Moxibustion* **3**, 192 (2010).
12. J. Hu, F. Yang, *Lishizhen Med. Materia Medica Res.* **6**, 1445 (2013).
13. M. Chen, H. Zhang, J. Li, *Chin. Acupunct. Moxibustion* **9**, 725 (2012).
14. S. Wang, D. Lu, Y. Li, *Chin. Acupunct. Moxibustion* **4**, 265 (2009).
15. L. Hang, Z. Cai, Y. Zhu, *Chin. Acupunct. Moxibustion* **7**, 577 (2013).
16. X. Wu, Z. Liu, X. Zhang, *Chin. J. Integr. Tradit. Western Med. Intens. Crit. Care* **1**, 39 (2004).
17. L. Yin, Y. Li, S. Wang, *Chin. Acupunct. Moxibustion* **6**, 402 (2008).
18. N. Jiao, *Chin. J. Exp. Tradit. Med. Formulae* **12**, 293 (2011).
19. F. Zhou, P. Liu, *Integrative Medicine Research* **4**, 144 (2015).
20. F. Zhou *et al., World Journal of Traditional Chinese Medicine* **1**, 45 (2015).
21. X. Li, Q. Wu, *China Materia Medica* **38**, 37 (2013).
22. Y. Yuan, P. Huang, X. Yang, *Chinese Pharmacist* **7**, 17 (2014).
23. X. Xiong *et al.*, *Chinese Journal of Experimental Traditional Medical Formulae* **17**, 22 (2011).
24. Y. Lan *et al.*, *J. Zhejiang Univ.-Sci. B* **15**, 153 (2014).

Acknowledgments
Thanks to Dr. Yi Lan, Bochen Zhao, and Wenping Wang for their contributions to this article.

Produced by the *Science*/AAAS Custom Publishing Office

Acupuncture as a potential treatment for insomnia

Authors:
Wing-Fai Yeung[1],
Ka-Fai Chung[2],
Stephanie Tjen-A-Looi[3],
John Longhurst[3],
Lixing Lao[1,4*]

Insomnia—difficulty falling and staying asleep—is a frequent complaint, with about one-third of the general population worldwide presenting with symptoms (*1*). Although the neural mechanisms underlying chronic insomnia are poorly understood, substantial evidence has shown that it is a disorder of physiological hyperarousal involving both the central nervous system (CNS) and autonomic nervous system (ANS) (*2*, *3*).

Acupuncture has been widely used for the treatment of insomnia in Asia. According to the theory of traditional Chinese medicine (TCM), the mind (or *shen*) is situated in the heart region; insomnia is considered to be a disorder of the heart, so acupuncture points on the heart and pericardium are often used in treatment (*4*). Recently, several systematic reviews have hinted that acupuncture may be an effective treatment for insomnia. However, deficits in study design and quality have meant that definitive conclusions could not be drawn (*5*).

Other studies have shown that acupuncture may be able to increase β-endorphin production and μ-receptor activity (*6*), both of which are associated with enhanced non-rapid eye movement (NREM) sleep. Acupuncture also appears to regulate various neurotransmitters and hormones involved in sleep regulation, including β-endorphin, serotonin, acetylcholine, nitric oxide, melatonin, dopamine, gamma-aminobutyric acid (GABA), and neuropeptide Y (NPY) (*7–9*). Further reports have suggested that acupuncture may be related to a significant increase in secretion of melatonin, a hormone involved in regulation of day-night cycles, in insomnia patients (*10*). In both animal and human clinical studies, evidence indicates that acupuncture inhibits sympathetic nervous system activity and regulates the hypothalamic-pituitary-adrenal (HPA) axis (*11*), which may contribute to its mechanism of counteracting insomnia. This review summarizes the evidence of the possible mechanisms through which acupuncture may modulate insomnia by acting on hyperarousal of the ANS and regulation of HPA activation.

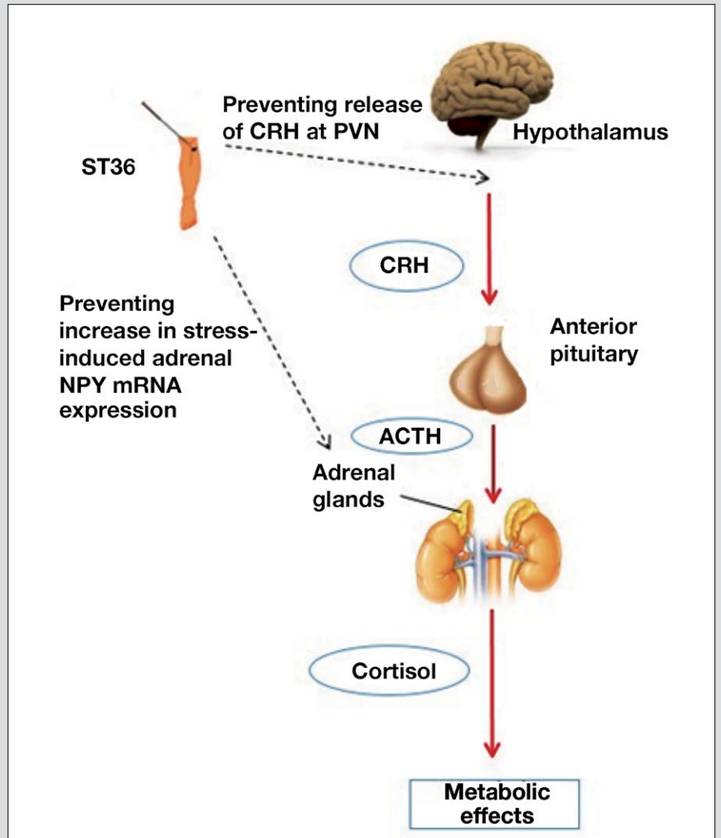


FIGURE 1. The possible pathway describing the effect of acupuncture on hypothalamic-pituitary-adrenal (HPA) activation. The stimulation of Zusanli (ST36) inhibits the HPA axis at or above the level of the paraventricular nucleus (PVN) through corticotropin-releasing hormone (CRH), thereby preventing the stress-induced elevations in circulating adrenocorticotropin hormone (ACTH) and corticosterone levels. It may also prevent increases in stress-induced adrenal neuropeptide Y (NPY) messenger RNA (mRNA) expression.

FIGURE: ADOPTED FROM THE PUBLIC DOMAIN AND FREEDIGITALPHOTOS.NET

Possible mechanism of action

Inhibition of sympathetic activity

Acupuncture is believed to modulate sympathetic and parasympathetic activity, as evidenced by its effects on the regulation of cardiovascular function, including lowering blood pressure in patients with hypertension (*12*) and decreasing the heart rate as well as skin blood flow in healthy subjects (*13*). An experimental study in healthy subjects found that needling on the Sishencong (EX-HN1) acupoint, commonly used in the treatment of insomnia, decreases the low-frequency component of the heart rate variability spectrum, which is an indicator of the balance between sympathetic and parasympathetic activities, suggesting that acupuncture enhances cardiac vagal tone and suppresses sympathetic activity (*14*). Acupuncture may alleviate insomnia symptoms and significantly decrease heart rate variability in poststroke patients (*15*), suggesting that improvement in subjective insomnia symptoms results from reducing sympathetic nervous system activity.

The pathophysiological pathway by which acupuncture may facilitate the sleep-wake transition through inhibition of sympathetic activity is not fully understood. Nevertheless, the effects of acupuncture on the excitatory cardiovascular reflexes may provide some hints. A long-loop pathway involving the arcuate nucleus (ARC) and ventrolateral periaqueductal gray (vlPAG), that modulates cardiovascular sympathoexcitatory bulbospinal neurons in the rostral ventrolateral medulla (RVLM) has been suggested as a possible explanation for an acupuncture mechanism. Electroacupuncture stimulation at acupoints Neiguan (PC6), a commonly used acupoint for insomnia, and Jianshi (PC5), activates ARC neurons in the ventral hypothalamus, which, in turn, provides excitatory projections to the midbrain vlPAG. Activation of neurons in the vlPAG stimulates cells in the raphe nuclei, which inhibit activity of cardiovascular premotor sympathoexcitatory neurons in the RVLM via endorphin, enkephalin, GABA, and serotonin (*16*). Since insomniacs apparently show elevated cardiovascular activity associated with ANS hyperarousal, the effects of acupuncture on sleep may involve this long-loop pathway.

Regulation of HPA axis

Acupuncture may improve sleep by regulating the HPA axis. Studies have shown that acupuncture reduces adrenocorticotropin hormone (ACTH), also known as corticotropin, and corticosterone/cortisol levels in animal models of stress (*17*) and in human subjects (*18*). However, precisely where in the HPA pathway acupuncture exerts its effect is not clear. More recently, an experimental study found that electroacupuncture at Zusanli (ST36) prevents chronic stress-induced activation of the HPA axis, as well as elevated sympathetic nervous system-related adrenal NPY (*19*). The study found that corticotropin-releasing hormone (CRH) levels were significantly reduced in acupuncture-treated animals. Findings suggest that acupuncture inhibits the HPA axis activity at or above the level of paraventricular nucleus (PVN) CRH, thereby preventing stress-induced elevations in circulating ACTH and corticosterone levels. Another study demonstrated that electroacupuncture at Zusanli (ST36) prevents an increase in stress-induced adrenal NPY messenger RNA (mRNA) expression (*20*). The increased adrenal NPY expression may result from central signals from either CRH or NPY, which are elevated in the PVN of stressed rats (Figure 1), suggesting that electroacupuncture inhibits the sympathetic NPY pathway by activating neurons in the PVN.

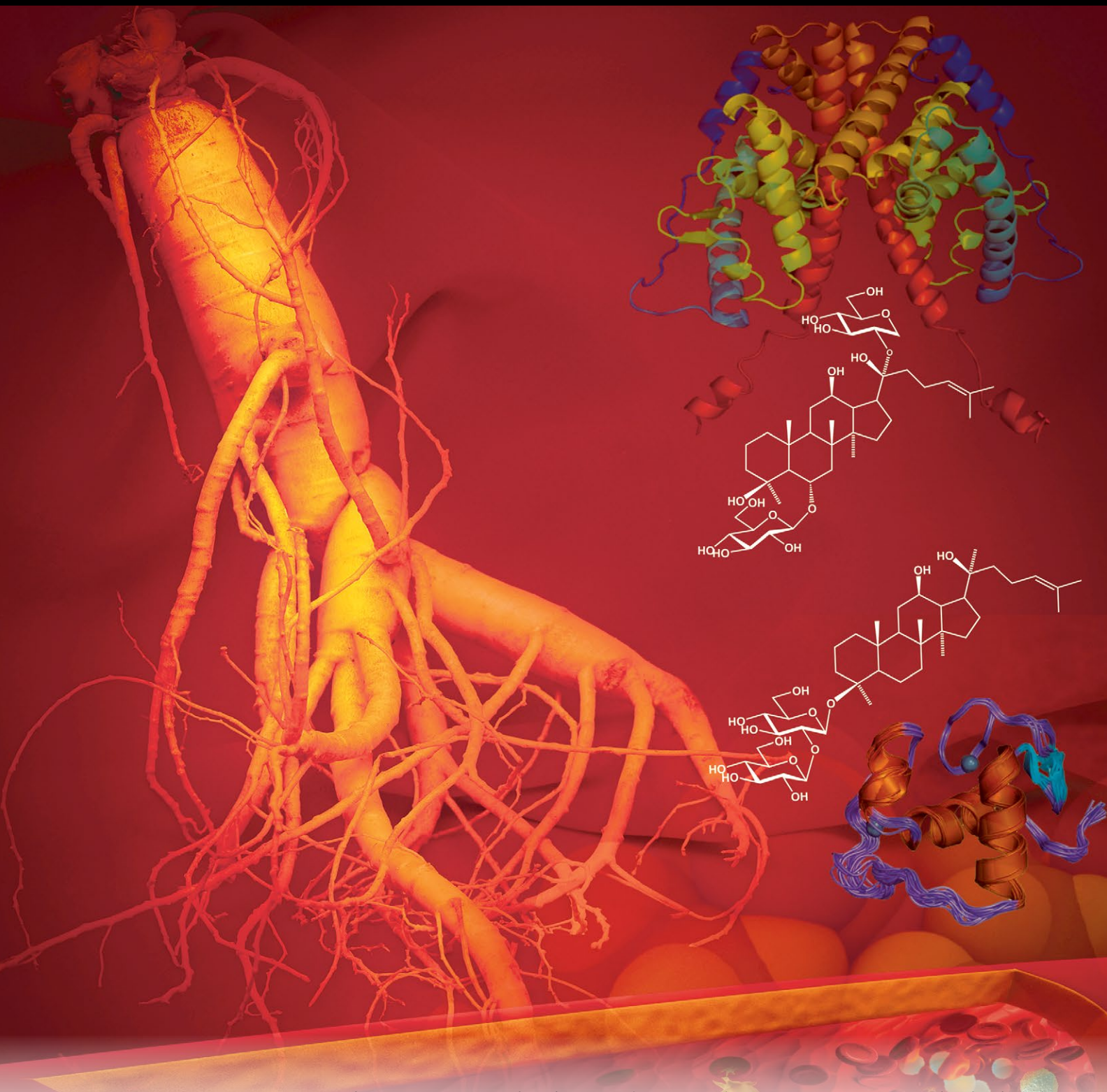
Conclusions

Emerging evidence suggests that acupuncture treatment counteracts insomnia by reducing hyperarousal of the ANS and through regulation of HPA activation. However, the mechanisms underlying acupuncture's actions in insomnia are still far from clear. Further research measuring anatomical location and physiological function are warranted to better understand the mechanisms of acupuncture in the management of insomnia.

References

1. M. M. Ohayon, *Sleep Med. Rev.* **6**, 97 (2002).
2. M. H. Bonnet, D. L. Arand, *Sleep Med. Rev*. **14**, 9 (2010).
3. D. Riemann *et al*., *Sleep Med. Rev*. **14,** 19 (2010).
4. T. J. Kaptchuk, *The Web That Has No Weaver: Understanding Chinese Medicine* (Contemporary Books, Chicago, IL, 2000).
5. E. Ernst, M. S. Lee, T. Y. Choi, *Eur. J. Gen. Pract.* **17**, 116 (2011).
6. C. H. Cheng, P. L. Yi, J. G. Lin, F. C. Chang, *Evid. Based Complement Alternat. Med.* **2011**, 159209 (2011).
7. X. Y. Gao *et al., Zhonggou Zhen Jiu* **27**, 681 (2007).
8. Q. Zhou *et al.*, *Int. J. Clin. Acupunct.* **17**, 79 (2008).
9. N. Samuels, C. Gropp, S. R. Singer, M. Oberbaum, *Behav. Med.* **34**, 55 (2008).
10. D. W. Spence *et al*., *J. Neuropsychiatry Clin. Neurosci.* **16**, 19 (2004).
11. W. Huang, N. Kutner, D. L. Bliwise, *J. Clin. Sleep Med*. **7**, 95 (2011).
12. P. Li *et al., Med. Acupunct.* **27**, 1 (2015).
13. S. Ballegaard *et al*., *Acupunct. Electrother. Res*. **18**, 103 (1993).
14. J. D. Wang, T. B. Kuo, C. C. Yang, *Auton. Neurosci*. **100**, 90 (2002).
15. S. Y. Lee *et al*., *Am. J. Chin. Med*. **37**, 1013 (2009).
16. S. C. Tjen-A-Looi, P. Li, J. C. Longhurst, *J. Appl. Physiol*. **106**, 1793 (2009).
17. H. J. Park *et al*., *Cell. Mol. Neurobiol*. **31**, 1123 (2011).
18. H. Harbach *et al*., *Eur. J. Anaesthesiol*. **24**, 370 (2007).
19. L. Eshkevari, E. Permaul, S. E. Mulroney, *J. Endocrinol*. **217**, 95 (2013).
20. L. Eshkevari *et al*., *Exp. Biol. Med*. **237**, 18 (2012).

[1]School of Chinese Medicine, the University of Hong Kong, Hong Kong, China
[2]Department of Psychiatry, the University of Hong Kong, Hong Kong, China
[3]Susan Samueli Center for Integrative Medicine, School of Medicine, University of California, Irvine, Costa Mesa, California, USA
[4]Center for Integrative Medicine, School of Medicine, University of Maryland, Baltimore, Maryland, USA
*Corresponding Author: lxlao1@hku.hk



The American Association for the Advancement of Science
1200 New York Avenue NW
Washington, DC 20005

Science
AAAS

The content contained in this special, sponsored section was commissioned, edited, and published by the *Science*/AAAS Custom Publishing Office. It was not peer-reviewed or assessed by the Editorial staff of the journal *Science*; however, all manuscripts have been critically evaluated by an international editorial team consisting of experts in traditional medicine research selected by the project editor. The intent of this section is to provide a means for authors from institutions around the world to showcase their state-of-the-art traditional medicine research through review/perspective-type articles that highlight recent progress in this burgeoning area. The editorial team and authors take full responsibility for the accuracy of the scientific content and the facts stated. Articles can be cited using the following format: [Author Name(s)], *Science* **350** (6262 Suppl), Sxx-Sxx (2015).