Biological screening of natural products and drug innovation in China

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Natural products have been applied to human healthcare for thousands of years. Drug discovery in ancient times was largely by chance and based on clinical practices. As understanding of therapeutic benefits deepens and demands for natural products increase, previously serendipitous discoveries evolve into active searches for new medicines. Many drugs presently prescribed by physicians are either directly isolated from plants or are artificially modified versions of natural products. Scientists are looking for lead compounds with specific structures and pharmacological effects often from natural sources. Experiences and successes of Chinese scientists in this specialized area have resulted in a number of widely used drugs. The tremendous progress made in life sciences has not only revealed many pathological processes of diseases, but also led to the establishment of various molecular and cellular bioassays in conjunction with high-throughput technologies. This is advantageous and permits certain natural compounds that are difficult to isolate and purify, and compounds that are difficult to synthesize, to be assayed. The transition from traditional to empirical and to molecular screening will certainly increase the probability of discovering new leads and drug candidates from natural products.

Keywords: natural products; traditional Chinese medicine; drug screening; high-throughput technologies; clinical trial; therapeutics

1. INTRODUCTION

From the beginning, combating disease has been an important aspect of interactions between humans and the natural environment. In the process of understanding and treating diseases, man has discovered by trial and error a variety of natural products, mostly from plant sources, of therapeutic value. Many of these medicinal plants contain several active components and have, in one form or another, been in use for thousands of years by a significant fraction of the population, and are still used in healthcare in many countries or regions of the world. Plants have supplied virtually all ancient cultures with food, clothing, shelter and medicines. According to incomplete statistics, approximately 1% of the roughly 300 000 different species of higher plants that exist have a history of food use. By contrast, 10–15% have extensive documentation for application in traditional medicine. Although drug discovery in ancient times was largely by chance and based on practices in humans, it provides a precious legacy and knowledge that benefits us even today. Indeed, it is estimated that approximately 25% of the drugs prescribed worldwide at present come from plants and 60% of anti-tumour/anti-infectious drugs already on the market or under clinical investigations are of natural origin. The effectiveness of natural products in mitigating

illnesses has inspired pharmaceutical scientists to search for new directions in drug discovery and development. The transition from fortuitous discovery to systematic screening through validation in experimental models has taken place since the 1930s. High-throughput screening (HTS) and high-throughput chemistry technologies developed in the last two decades have significantly improved the speed, scale and quality of this process.

2. TRADITIONAL CHINESE MEDICINE: A NATIONAL HERITAGE

In primitive societies, certain materials were found to be able to alleviate pain or sickness. With accumulated experience and knowledge, the relationship between these materials and certain diseases or symptoms was gradually established. As understanding of therapeutic benefits deepened and demands for such materials increased, passive discovery evolved into active searches for new medicines (Wang 2005).

The story of the ancient Chinese doctor, Shen Nong, testing hundreds of plants may be regarded as the best example of an active search for medicines and the earliest record of drug screening. Through thousands of years of clinical practice and through constant screening and evaluation, people have discovered a variety of plant resources possessing medicinal value. In China, from the end of the Spring and Autumn Period (770–476 BC) through the Warring States (472–221 BC) to the Qin Dynasty (221–207 BC) and the early Han Dynasty (206 BC–AD 220),

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medicinal plants were not only specially recorded and described in several medical monographs, but also mentioned in historical literature such as *Lu Shi Chun Qiu* (AD 239), *Huai Nan Zi* (AD 139) and *Shan Hai Jing* (the Warring States), etc.

In ancient China, medicines were called 'Ben Cao' (Chinese materia medica). The oldest herbal medicine book, *Shen Nong Ben Cao*, was written in the late Han Dynasty. It recorded 365 types of herbs, including 252 plants, 67 animal parts and 46 minerals. Some related medical indications were also described, such as *Herba Ephedrae* for asthma, *Radix et Rhizoma Rhei* for thyroid enlargement and *Herba Artemisiae Annuae* for malaria, etc. Furthermore, the author divided these herbs into three different categories in accordance with their properties, applications and toxicities. In the *Handbook of Prescriptions for Emergency* (approx. AD 333), the method to treat malaria was described: 'a handful of *Herba Artemisiae Annuae* is added to two litres of water and pounded into juice to take'; in the Tang Dynasty (AD 618–907), *The Newly Revised Materia Medica* recorded a plant extraction approach by pounding *Indigo naturalis* into pieces, then 'immersing in water for a whole night, evaporating and drying in air'. The final product, in powder form called 'Qingdai', is for treatment of bacterial infections. From then on, the speed of herbal use greatly accelerated and the application range constantly expanded. From the eastern Han Dynasty (AD 25–220) to the end of the Song Dynasty (AD 960–1279), for *ca* 1000 years, both the varieties and sources of medicinal plants were greatly increased. The classical herbal pharmacopoeias of the period include: *Prescription in Jade Box* written by Hong Ge, *Essential Prescriptions Worth a Thousand Gold* written by Simiao Sun, who at that time was regarded as the 'Drug King', and *The Historically Classified Materia Medica* (which recorded 1558 kinds of medicines in the Song Dynasty), etc. From the late Ming Dynasty (AD 1368–1644) to the Qing Dynasty (AD 1644–1911), during a long practising medical career of herbal collection, field investigation, experience verification and consultation of historical references, pharmacist Shizhen Li (Ming Dynasty) summarized his knowledge in a book entitled *Compendium of Materia Medica* (1578), with detailed descriptions of 1195 kinds of medicinal plants. The contents of this book were disseminated all over the world.

To date, approximately 12 807 kinds of medicinal materials from natural sources have been recorded in China. Among them, 11 146 are of plant origin, 1581 extracted from animals and 80 derived from minerals, including over 5000 clinically validated folk medicines. A majority of the more commonly used 400–500 kinds of traditional Chinese medicine (TCM) has been studied systematically and a variety of bioactive components discovered. Using the Shanghai Institute of Materia Medica as an example, more than 3000 single chemical entities with novel structures have been identified from TCM since the 1950s, and several of them have been developed into new drugs, including artemether, salvicine, huperzine A and depside salts, as described below.

3. SINGLE CHEMICAL ENTITIES: EAST MEETS WEST

In western countries too, herbal medicine has a long history of use. Hippocrates (460–377 BC), the father of ancient Greek medicine, paid great attention to the therapeutic value of diets and used *Fructus Hordei Alga*, *Codii Cylindrica* and *Radix et Rhizoma Veratri Nigri*, etc. to treat certain ailments. In the fourth century BC, Diocles Carystius of Greece, a student of Aristotle, put together a list of plants, along with their uses, titled *Rhizotomika*. Galen (approx. AD 129–200), the famous Roman physician and pharmacist, once composed a series of books describing various therapeutic methods and herbal medicines. He also classified many herbs based on botanical category and invented an opiate and a number of other pharmaceutical preparations. Indeed, many simple herbal extracts are still called 'Galenicals'. Later on, there appeared some well-known herbal works, including *Liber de Proprietatibus Rerum* written by an English monk, Bartholomew Glanvil, in the fifteenth century.

TCM and other historical or traditional approaches to therapy have employed mixtures of naturally occurring herbs and herbal extracts (Yuan & Lin 2000), and such mixtures are considered integral to the treatment. The foundation of various clinical efficacies observed in patients is believed to rely on numerous interacting combinations of natural products (Keith *et al.* 2005). However, bioactivities found in many of the natural product extracts later disappeared when the extracts were fractionated into individual chemical components (Foungbe *et al.* 1991; Turner 1996; Schuster 2001). On the other hand, clinical experience suggests that some medicinal plants *per se* show poor efficacy and severe side effects, while active constituents separated from crude materials often demonstrate the opposite, i.e. good efficacy and less toxicity. In fact, botanical medicine was transformed when the advancement of chemical techniques at the beginning of the nineteenth century made possible the isolation of chemical constituents from plants. In 1805, the German pharmacist, Serturmer, extracted morphine from opium and in the 1820s a French pharmacist isolated several alkaloids from plants, including quinine and caffeine. In 1893, aspirin was synthesized. In 1906, Paul Ehrlich developed his theory of 'magic bullets', the idea of a selective drug that would home in on its target while leaving the surrounding physical environment intact. Single chemical entities, which are more consistent and easier to quantify, have been judged more specific in their therapeutic focus than natural products. Thus, conventional methods that combine biology and chemistry, such as bioassay-guided isolation, structure determination and mechanism elucidation, etc., have since been widely adopted to identify and characterize individual constituents from extracts, leading to the eventual discovery of single compound-based therapeutics.

4. ARTEMISININ: A BENCH MARK

Modern drug screening (general screening and combination screening) uses laboratory animals as test subjects. It applies different kinds of techniques and

Figure 1. Artemisinin (I) and its *in vivo* metabolites. a, Endoperoxide bridge.

methods, such as mechanical equipment, electronic and optical instruments, behavioural observation, mathematical modelling, etc. Scientists are looking for lead compounds with specific structures and pharmacological effects often from natural sources (extracts, fractions or pure compounds). Based upon initial discoveries, further modification and optimization of lead structures via medicinal chemistry and biological screening are carried out in order to develop drug candidates with therapeutic value. This process has been constantly updated, improved and widely used for nearly a century. In fact, many drugs presently prescribed by physicians are either directly isolated from plants or artificially modified versions of natural products.

One good example is the discovery of artemisinin. A series of pharmacological experiments demonstrated that the neutral fraction derived from the ethanol extract of *Artemisia annua* L. had a significant inhibitory effect on *Plasmodium falciparum* in both the Camp strain (chloroquine-sensitive) and the Smith strain (chloroquine-resistant). Toxicology studies proved that not only had the ethanol extract no major liabilities in animals, but a clinical trial involving 30 cases also yielded satisfactory results. Further research work revealed that the active compound is an endoperoxide sesquiterpene lactone, named artemisinin (structure I in figure 1), which was first purified in the 1970s. Its structure was determined by a group of Chinese scientists to be 3,6,9-trimethyl-octahydro-3,12-epoxy-pyranol[4,3-j]-1,2-benzodioxepin-10-one by X-ray diffraction analysis (Liu *et al.* 1979). It has a unique structure and lacks a nitrogen-containing

heterocyclic ring, which is common in most anti-malaria drugs (Guan *et al.* 1982). Through numerous studies of its chemistry, pharmacology, toxicology and in clinics, artemisinin was proven to be highly effective in treating malaria, including multi-drug resistant (MDR) strains. The isolation and characterization of artemisinin from *A. annua* L. was considered one of the most important discoveries in contemporary herbal research (Klayman 1985).

Although artemisinin has good efficacy against malaria, with properties such as low toxicity and rapid onset, there exist two major shortcomings: insolubility in water and oil, and poor oral bioavailability, resulting in difficulties with formulation. In order to overcome these shortcomings, *in vivo* metabolic and structural modification studies were carried out. All the metabolites isolated from urine had no anti-malaria activity due to the loss of an endoperoxide bridge. This suggests that the endoperoxide bridge is the active group responsible for its biological activity (figure 1). Thus, scientists synthesized a series of derivatives of artemisinin (Xu *et al.* 1983; 1984), which were tested in malarial animal models. More than 20 derivatives exhibited a better efficacy than artemisinin. For instance, deoxyartemisinin was successfully prepared from artemisinin in one step using NaBH_4 and $\text{BF}_3\text{Et}_2\text{O}$ in tetrahydrofuran (THF); it showed an eightfold increase in bioactivity *in vitro* against chloroquine-resistant malaria as compared with artemisinin. α -Artelinic acid is a potent, stable and water-soluble compound synthesized from artemisinin with blood schizonticidal activity against *P. falciparum*.

Artemether is a methyl ether derivative of artemisinin also found to be more active than artemisinin. This compound is reduced with sodium borohydride to produce dihydroartemisinin as a mixture of epimers. The mixture is treated with methanol and an acid catalyst to provide artemether that is effective in treating cerebral malaria by intramuscular injection. Artemether is the β -anomer of the ethyl ether of dihydroartemisinin. It was synthesized originally in China by a group of chemists working at the Shanghai Institute of Materia Medica (Li *et al.* 1981). Brossi *et al.* (1988) synthesized artemether by borohydride reduction of artemisinin to hydroartemisinin, etherification in boron trifluoride ethyrate, and finally separation of anomers. The compound has been formulated as a solution in sesame oil for intramuscular injection at a concentration of 160 mg ml^{-1} for preclinical and clinical studies. Artemether is virtually insoluble in water, but is soluble in 50–100% ethanol with water and a variety of oils. It has been proven to be stable in accelerated stability studies at 50°C for several months. It is very efficacious against drug-sensitive strains of *P. falciparum* *in vitro* as well as *in vivo* in different malarial animal models. Presently, artemether is registered in 27 countries, including France, Thailand, Pakistan, Sudan and Niger (Dhingra *et al.* 2000).

Schistosomiasis continues to rank second, after malaria, in the order of the world's parasitic diseases in terms of the extent of endemic areas and the number of infected people. More than two decades ago, Chinese scientists discovered that artemether was able to prevent schistosome infections (Xiao *et al.* 2002a). Detailed laboratory studies thereafter revealed that artemether

exhibited the highest activity against juvenile stages of the parasites, while adult worms were significantly less susceptible (Utzinger *et al.* 2001). The schistosomicidal effects of artemether were demonstrated by its ability to cause extensive ultrastructural damage to *Schistosoma mansoni*, thus confirming earlier findings with *Schistosoma japonicum* (Utzinger *et al.* 2000). Orally administered artemether at a dose of 6 mg kg⁻¹ (once every 2–3 weeks) resulted in no drug-related adverse events, and significantly reduced the incidence and intensity of schistosome infections in human subjects (Xiao *et al.* 2002b). Combined use of praziquantel and artemether was more efficacious than each drug administered alone (Utzinger *et al.* 2001). These observations led to the conclusion that artemether, when integrated with other control strategies, has considerable potential for reducing the current burden of schistosomiasis in different epidemiological settings.

It is known that cancer cells have a characteristically high iron influx, and artemisinin as well as its analogues could selectively kill cancer cells under conditions that increase intracellular iron concentrations. In the presence of ferrous iron, artemisinin becomes cytotoxic thereby exerting its anti-malaria effect. After incubation with holotransferrin, which increases the concentration of ferrous iron in cancer cells, dihydroartemisinin, an analogue of artemisinin, effectively killed one type of radiation-resistant human breast-cancer cell *in vitro*. The same treatment had considerably less impact on normal breast cells (Singh & Lai 2001). Further studies suggested that artemisinin derivatives mainly targeted the G1 phase of the cell cycle and were capable of inducing apoptosis (Li *et al.* 2001). When an analogue of artemisinin, artelinic acid, was linked to transferrin, the resultant conjugate was very potent and selective in killing human leukaemia cells (Moult-4; Lai *et al.* 2005). These findings indicate a novel therapeutic strategy in combating certain types of cancer.

5. CANCER THERAPY: EVER LASTING INTERSETS

Arsenic is a common, naturally occurring substance used in TCM practices for more than 2000 years. Apart from combating malaria and plague, this ancient remedy, containing 95% arsenic trioxide (As₂O₃), was once applied to cancer therapy (e.g. for treatment of chronic myelogenous leukaemia; CML). Such practice was abandoned in the early twentieth century due to its toxicity and with the advancement of modern medical sciences. In the early 1970s, physicians in China found that intravenous infusion of 1% arsenic trioxide led to complete remission (CR) in two-thirds of patients ill with acute promyelocytic leukaemia (APL; Zhang *et al.* 1995). This discovery was followed by a series of clinical studies that showed a CR induced by arsenic compounds in 85–90% of patients with both newly diagnosed and relapsed APL (Wang 2003). It was thus proposed that appropriate use of arsenic compounds in post-remission APL therapy could prevent recurrence and achieve a longer survival time. Further investigations suggest that arsenic trioxide exerts dose-dependent dual effects on APL cells: induction of apoptosis at high concentrations and initiation of

Structure II.

partial differentiation at low concentrations. These two actions are caused by rapid modulation and degradation of the promyelocytic-leukaemia retinoic acid receptor- α (PML-RAR α) oncoprotein (Zhou *et al.* 2005). A commercial version of arsenic product, trisenox, was approved in the USA, Europe and Japan to treat persistent and relapsed APL not long ago.

The TCM combination prescription *Radix Angelicae Sinensis* and *Aloe Pill* has been used to treat a variety of chronic ailments in China for some time. It includes medicinal plants, such as *Radix angelicae sinensis*, *Aloe*, *Radix gentianae*, *Fructus gardeniae*, *Radix scutellariae*, *Cortex phellodendri*, *Rhizoma coptidis*, *Folium isatidis*, *Radix Aucklandiae*, etc. Using the mouse L7212 leukaemia model, scientists found that the only active component contained in this prescription is *I. naturalis*. While its haemostatic, anti-pyretic, anti-inflammatory, sedative, anti-bacterial and anti-viral properties have been well documented, the associated side effects could not be ignored. With joint efforts made by medicinal chemists and pharmacologists, an active compound was later identified as indirubin (structure II), which showed different degrees of inhibitory effects on rat Walker tumour, mouse Lewis lung tumour, C615 breast cancer and L7212 leukaemia. It did not exhibit observable side effects and bone marrow suppression at a daily oral dose range between 100 and 500 mg kg⁻¹. The response rate in treating CML was 87.3%. The synthetic version of this compound was approved for marketing and may have implications for mitigating other myeloproliferative and acidophil-proliferative diseases (Tang 2000).

Studies exploring the mechanism of action suggest that indirubin is a potent inhibitor of cyclin-dependent kinases. It also interacts with other kinases, such as glycogen synthase kinase 3 β . These findings point to a potential of developing indirubin not only as a broad-spectrum anti-cancer drug but also as a novel therapeutic agent for certain central nervous system (CNS) diseases, including Alzheimer's disease (AD; Eisenbrand *et al.* 2004). In order to further improve the anti-cancer efficacy and reduce side effects, investigations on the structure–activity relationship (SAR) were carried out and more potent derivatives were identified.

Mylabris phalerata pallas has been used to treat goitre in TCM. Its principal active compound is cantharidin (structure III), which could inhibit experimental liver cancer and certain sarcoma in mice. Clinical data showed a good efficacy in treating primary liver cancer without affecting the peripheral blood white-cell counts (Wang 1980). Laboratory studies on hepatocellular carcinoma cells (Hep3B cells) revealed that cantharidin is an acute cytotoxic agent (Wang *et al.* 2000a,b). The major side effects of this compound relate to the urinary and digestive systems. Bioassay-guided structural

III

Structure III.

IV

Structure IV.

modification studies delivered two less toxic analogues, norcantharidin and *N*-hydroxycantharidin; both of them have been used in the treatment of primary liver cancer (McCluskey *et al.* 2000).

Salvicine is a novel diterpenoid quinone (structure IV) obtained by structural modification of a natural product isolated from the TCM *Salvia prionitis* Hance (Labiatae; Zhang *et al.* 1999). Various *in vitro* and *in vivo* studies over the past decade demonstrated that salvicine has pharmacological properties such as inhibition of malignant tumour cells/xenograft growth and metastasis, suppression of MDR effects and blocking of angiogenesis (Qing *et al.* 1999; Zhang *et al.* 1999; Meng *et al.* 2001). Salvicine significantly inhibited the proliferation and growth of various solid tumours, including lung, gastric, liver, colonic, ovarian and cervical cancers, with better efficacy profiles than positive controls such as vincristine and etoposide (Miao *et al.* 2003). It also displayed some selectivity against lung and gastric cancers (Qing *et al.* 2001; Liu *et al.* 2004). The anti-angiogenic potential of salvicine was evidenced by its ability to suppress migration and tube formation in human microvascular endothelium cells and neovascularization of chick embryo chorioallantoic membrane. Another important observation is that salvicine treatment led to a marked inhibition of metastasis of orthotopic human breast tumour implants in nude mice; the drug sharply reduced the number of metastatic foci in the lungs at doses which did not decrease the tumour volume of the original tumour foci in the breasts (Zhang *et al.* 1999). This anti-cancer activity is associated with its ability to induce tumour cell apoptosis; salvicine treatment resulted in HL-60 cell apoptosis and downregulation of telomerase activity in a time- and concentration-dependent manner (Liu *et al.* 2002). One interesting and exciting finding is that salvicine was directly cytotoxic to various MDR cell lines (Miao *et al.* 2003). These cell lines were derived from different tissues and established with multiple conventional anti-cancer agents. Recent investigations discovered that salvicine is a novel non-intercalative topoisomerase II poison (Lu *et al.* 2005). It stimulates *c-fun* gene expression and inhibits *mdr-1* gene expression in MDR K562/A02 cells (Miao & Ding 2003), pointing to the mechanism by which salvicine

V

Structure V.

exhibits a broad spectrum of anti-MDR activities. The patent application for salvicine was granted in China and commercial development rights out-licensed. Clinical trials on this compound are currently being carried out at the Chinese Academy of Medical Sciences/Peking Union Medical College.

6. SCHIPERINE: BLOCKBUSTER IN PREPARATION

Huperzine A (structure V) is an alkaloid separated from *Huperzia serrata*, one of the most commonly used Chinese herbs for the treatment of contusion, strain and swelling, etc. Pharmacological studies indicated that it has a highly selective and long-lasting inhibitory effect on acetylcholinesterase (AChE) in the brain, and is capable of enhancing learning ability and memories in rat and mouse experimental models (Kozikauski *et al.* 1991). It is known that AChE inhibitors can be used to treat AD. Several clinical reports suggested that huperzine A facilitates cholinergic neurotransmission by increasing the concentration of acetylcholine in the CNS. Its activity is about 100 times greater than that of tetrahydroaminoacridine (THA, tacrine), a widely used drug against AD. Moreover, the toxicity is lower than several other AChE inhibitors (Campiani *et al.* 1993). Huperzine A is presently marketed in China as a therapeutic agent to treat AD. This discovery has attracted significant interest from AD researchers around the world.

Pure compounds isolated from plants are normally restricted by the available resources, or are at very low levels in the crude extracts. Hence, chemical synthesis becomes the usual way for large-scale production following structure determination. However, SAR studies are often required to overcome poor efficacy, side effects or toxicity and to circumvent the fact that some drugs are too complex to be produced by organic syntheses. Using the natural compound as a lead, active scaffolds and groups can be identified for chemical modification, thereby synthesizing a series of new analogues guided by SAR studies, with an ultimate goal of developing more new drugs. Following earlier success with huperzine A, a large number of analogues or derivatives were synthesized, including HA-1 (structure VI) and ZT-1 (structure VII), in order to find an AChE inhibitor with higher selectivity and lower toxicity. Extensive investigations *in vitro* and *in vivo* demonstrated that ZT-1 (schiperine) has a good selectivity between AChE and butyrylcholinesterase, higher bioavailability, lower toxicity and better restored effect to cognitive impairments compared with several currently prescribed drugs against AD, such as huperzine A, donepezil, tacrine and rivastigmine (Zhu 2004). This drug candidate is being developed worldwide and phase I clinical trials have been completed in

Figure 2. Synthetic route of gastrodin.

Structure VI.

Structure VII.

Europe. The results showed that schiperine in all doses (1–3 mg) tested was able to significantly inhibit AchE activity and to restore the cognitive impairment induced by scopolamine. All of the physical examination indexes, including blood and urine routines, clinical biochemistry, blood pressure, heart rate, cardiograms and body temperature did not change significantly before and after schiperine administration; none of the trial subjects stopped dosing due to severe adverse events. As a result, phase II clinical trials have begun in 35 European hospitals under the direction of Prof. Bruno Vellas, head of the European Clinical Trial Centre for AD. Relevant patent applications were allowed in China and the USA (Zhu *et al.* 1999). Pending phases II and III clinical trial results, schiperine may have the potential of becoming a blockbuster drug developed independently by Chinese scientists to enter the mainstream international market in due course.

7. GASTRODIN: SYNTHESIS FROM NATURE

Gastrodia elata Blume is a well-known medicinal plant used in TCM to treat a variety of CNS and cardiovascular symptoms for more than 2000 years (Pharmacopoeia Commission of People's Republic of China 1995). Among the seven major constituents isolated, *p*-hydroxymethylphenyl-β-D-glucopyranoside (structure VIII) was identified for its sedative, anti-convulsion, anti-seizure and analgesic effects, and was

Structure VIII.

named gastrodin (Zhou *et al.* 1979). Using capillary electrophoresis, a rapid finger-printing technology was developed to monitor the quality of raw materials (Zhao *et al.* 1999). Due to the extremely low content (0.025%) in the rhizome, a total synthesis method was worked out (figure 2; Zhou *et al.* 1980) and subsequently applied to industrial production.

It was found that synthetic gastrodin is capable of protecting cultured neurons, reducing peripheral vascular resistance, increasing beating rates of myocardial cells, strengthening contractility and promoting energy metabolism (Lu *et al.* 2002). In addition, Hsieh and colleagues reported that gastrodin could selectively facilitate memory consolidation and retrieval after acute administration (Hsieh *et al.* 1997). No significant toxicity was discovered (Deng & Mo 1979).

Clinical trials in 349 subjects revealed that the effective rates of gastrodin in treating neurasthenia, neurasthenic syndrome and migraine headache were 89, 86 and 67%, respectively. It also showed some efficacy in mitigating patient complaints such as insomnia, fatigue, weakness, tinnitus, etc. (Lu *et al.* 2002). Due to the scarcity of *Gastrodia elata* Blume, gastrodin isolated from the natural plant was extremely expensive before its total synthesis. The cost of production was reduced by at least 100-fold thereafter, and since the 1980s, gastrodin has been manufactured and marketed by multiple pharmaceutical companies in China.

8. CARDIOVASCULAR INDICATIONS: AN EVOLVING STORY

Ginkgo biloba has long been used in herbal practices and is officially listed in the Chinese Pharmacopoeia. It has a worldwide acceptance in the treatment of cerebrovascular and cardiovascular disorders, neurosensory-related problems, disturbances in vigilance, short-term memory loss and other cognitive dysfunctions associated with ageing and senility. Ginkgolides, isolated from the root, bark and leaves of *G. biloba*, possess important pharmacological properties. Structural investigations on ginkgolides found that they are unique cage

IX:	R ₁ = OH	R ₂ = H	R ₃ = OH	R ₄ = H
X:	R ₁ = OH	R ₂ = OH	R ₃ = OH	R ₄ = H
XI:	R ₁ = OH	R ₂ = OH	R ₃ = OH	R ₄ = OH
XII:	R ₁ = H	R ₂ = OH	R ₃ = H	R ₄ = OH
XIII:	R ₁ = OH	R ₂ = H	R ₃ = OH	R ₄ = OH

Structures IX–XIII.

molecules, representing diterpene lactones incorporating a tertiary butyl group and six five-membered rings, including ginkgolides A (structure IX), B (structure X), C (structure XI), M (structure XII) and J (structure XIII; Maruyama *et al.* 1967*a–c*; Weinges *et al.* 1987). Ginkgolides specifically inhibit platelet-activating factor from binding to its receptor thereby preventing platelet aggregation. This group of natural compounds has a long history of use in humans, lacks toxicity and is totally resistant to metabolism. Among them, ginkgolide B is the most bioactive (Braquet 1985). In the late 1990s, comparative molecular field analysis (CoMFA), a three-dimensional quantitative SAR study, was conducted to understand the correlation between the physicochemical properties and the *in vitro* bioactivities of ginkgolide analogues. Based on the results of CoMFA analysis, scientists designed some new compounds, three of which demonstrated a two- to fourfold increase in potency compared with ginkgolides (Chen *et al.* 1998).

Salviae miltiorrhizae, a TCM known as 'Dan Shen', has been routinely adopted clinically in China since its introduction in the 1960s, as an effective remedy for cerebrovascular disorders, angina pectoris and hypertension with minimal side effects. One of its active ingredients, tanshinone II-A, is a naturally occurring L-type Ca²⁺ channel blocker (Xu *et al.* 1996) and can cause coronary and peripheral vasodilation by reducing the influx of calcium into myocardial and smooth muscle cells (Zhou & Ruigrok 1990). Another active component is magnesium lithospermate B (MLB; structure XIV). Infusion of MLB into the post-ischaemic rabbit heart reduced damage to the myocardium (Fung *et al.* 1993) and intravenous injection of MLB (30 mg kg⁻¹) into rats resulted in a decrease in blood pressure with no change in the heart rate (Kamata *et al.* 1994). While the vasodilating and anti-hypertensive effects are attributed to an enhancement in the kallikrein-prostaglandin system (Yokozawa *et al.* 1992), its cardioprotective property may be directed against apoptosis, since both c-Jun N-terminal kinase 3 (JNK3) and stress-activated protein kinase activities were inhibited by MLB (Yeung *et al.* 2001; Yang *et al.* 2003).

Depside salts are an investigational drug containing MLB and its analogues for the treatment of chronic angina that afflicts more than 10 million people in China alone. MLB is used as the quality control standard for pharmaceutical preparation, which differentiates the

Structure XIV.

product from other remedies made from *Salvia miltiorrhizae*. Depside salts are formed of defined chemical components by a strict quality control procedure (i.e. fingerprinting diagram technique) from the herb, bulk material and formulation. Chemical processing involves a freeze-dry step to maintain stability of the polyphenolic substances. Relevant patent applications for this technique have been filed in China and the USA. Experimental data show that the drug could significantly reduce myocardial infarction size and attenuate ischaemic myocardial injury both *in vitro* and *in vivo*. Due to the absence of haemodynamic effects, deposite salts may have the potential to become an agent for combination therapy without concerns of hypotensive or bradycardiac side effects. In addition, it has inhibitory properties on platelet aggregation and thrombosis formation (Wang *et al.* 2000*a,b*; Wu *et al.* 2000; Tang *et al.* 2002). Human trials on this drug were completed recently in China and the results indicate that deposite salts: (i) was well-tolerated, (ii) improved the symptoms of exercise-induced myocardial ischemia, and (iii) lessened the severity of angina. Depside salts have been recently approved by the Chinese regulatory authorities as a new drug to treat coronary artery disease. Compared with existing pharmaceutical products made from *S. miltiorrhizae*, deposite salts are obviously superior in terms of quality, safety and efficacy, and would be a good model for TCM modernization.

Guanfu base A (GFA; structure XV) is a single chemical entity isolated from the tuber of the TCM *Aconitum coreanum* (Lévl.) Rapaics. A series of pharmacological studies have shown that GFA is capable of: (i) dose-dependently decreasing the heart rate elicited by the sinoatrial node via a direct mechanism and (ii) normalizing the heart rhythm in experimental arrhythmic models. This action may be related to its ability to reduce cardiac oxygen consumption and to improve coronary blood circulation without affecting myocardial contractility. It also inhibits both fast- and slow-response action potential of the myocardium. Electrophysiological investigations revealed that GFA blocks the fast Na⁺ channel current thereby stabilizing the myocardial cell membrane and prolonging the effective refractory period (Chen & Chen 1998). After intravenous injection into humans or rats, GFA is metabolized into GFI, GFA oxide, GFA glucuronide and sulphate conjugates, among others, leading to a reduction of efficacy due to bioconversion of GFA to metabolites of high polarity (A *et al.* 2002, 2003). Results obtained from clinical trials suggest that GFA is particularly efficacious in treating ventricular

Structure XV.

Structure XVI.

arrhythmia and paroxysmal supraventricular tachycardia with rapid onset and lower toxicity (Han *et al.* 2003). As a novel anti-arrhythmic and specific bradycardic agent, GFA possesses a bright market prospect.

Berberine (BBR) is a plant alkaloid with a long history of medicinal use in China, as a non-prescription drug to treat bacterial diarrhoea. It is present in the roots, rhizomes and stem bark of *Hydrastis canadensis*, *Coptis chinensis* (TCM), *Berberis aquifolium*, *Berberis vulgaris* and *Berberis aristata*. The chemical structure of BBR (structure XVI) was first identified in 1910 and the total synthesis accomplished in 1969. BBR extracts and decoctions were shown to have significant antimicrobial activities against a variety of organisms such as bacteria, viruses, fungi, protozoans, helminths and chlamydia. Currently, predominant clinical applications of BBR include bacterial diarrhoea, intestinal parasite infections and ocular trachoma infections (Birdsall & Kelly 1997). Various pharmacological properties have been recorded over the years relating to inhibition of: (i) metabolism in certain microorganisms (Ghosh *et al.* 1985), (ii) bacterial enterotoxin formation, intestinal fluid accumulation and ion secretion (Sack & Froelish 1982), (iii) inflammation (Fukuda *et al.* 1999), (iv) cyclooxygenase-2 (COX-2) transcription and *N*-acetyltransferase activity in colon and bladder cancer cell lines (Lin *et al.* 1999), and (v) the growth of mouse sarcoma cells in culture (Creasey 1979). During the course of decades of active research, some beneficial effects on the cardiovascular system and lipid metabolism were found, including inhibition of platelet aggregation and ventricular tachyarrhythmia, elevation of platelet counts in cases of primary and secondary thrombocytopenia, immunomodulation via increased blood flow to the spleen and macrophage activation, stimulation of bile and bilirubin secretion, and reduction of blood lipids (Marin-Neto *et al.* 1988; Ji *et al.* 1995; Birdsall & Kelly 1997).

Structure XVII.

In an attempt at screening novel cholesterol-lowering agents, BBR was randomly discovered to elevate low-density lipoprotein receptor (LDLR) expression in HepG2 cells. Oral administration of BBR for three months in 32 hypercholesterolemic patients reduced serum cholesterol by 29%, triglycerides by 35% and LDL-cholesterol by 25%. Treatment of hyperlipidemic hamsters with BBR decreased serum cholesterol by 40% and LDL-cholesterol by 42%, with a 3.5-fold increase in hepatic LDLR mRNA and 2.6-fold increase in hepatic LDLR protein. Using human hepatoma cells, it was found that BBR upregulates LDLR expression by stabilizing LDLR mRNA through the 5' proximal section of the LDLR mRNA 3'UTR (untranslated region), which is independent of sterol regulatory-element binding proteins, but dependent upon extracellular signal-regulated kinase activation, suggesting a mechanism of action distinct from that of statins (Kong *et al.* 2004). These findings point to a potential use of BBR as a monotherapy to treat hypercholesterolemia or it may be explored in combination therapy with statins, currently prescribed worldwide.

9. ANTI-INFECTIVES: ADDRESSING UNMET NEEDS

Hepatitis is a major public health concern affecting almost 10% of the Chinese population. Based on the early success of bifendate (biphenyl dimethoxy dicarboxylate; structure XVII), a compound isolated from a commonly used TCM, *Fructus schisandrae*, Liu and his colleagues discovered an analogue through SAR studies—bicyclol, which has a better hepatoprotective profile than its parent compound (Liu 2002). Pharmacological studies showed that bicyclol (structure XVIII) was able to significantly reduce hepatic injuries caused by a variety of toxic agents leading to decreases in serum alanine/aspartate transaminase levels and pathological alterations in the liver. In addition, it could inhibit human and duck hepatitis virus DNA replication and secretion of hepatitis B surface/e' antigens (HBsAg/HBeAg) by viral infected cells (Liu 2001). No obvious toxicity was noted (Liu *et al.* 2005).

Investigations on the mechanisms of action revealed that the hepatoprotective effect of bicyclol is mediated via elimination of free radicals, thereby stabilizing the hepatocyte membrane and nuclear DNA (Liu 2001). In human liver-cancer cell lines (HepG2 and Bel7402), bicyclol is capable of inducing apoptosis and enhancing

Structure XVIII.

α -fetal protein expression (Li 2002). Latest observations suggest that bicyclol neutralizes concanavalin A-related liver damage through suppression of hepatic *Fas/FasL* expression and tumour necrosis factor- α (TNF- α) release (Li & Liu 2004).

Multi-centre, double-blind and randomized clinical trials in patients ill with chronic hepatitis B and C indicate that bicyclol was efficacious in improving both symptoms and liver enzymes: two-thirds of the subjects remained effective three months after the last oral dose, suggesting a very low rate of relapses; HBeAg-negative and HBeAb-positive conversions were seen in some patients especially those with more severe manifestations; no significant adverse effect was recorded at any dose tested (Li 2002; Yao *et al.* 2002). With intellectual property rights protected in 15 countries or regions, bicyclol was approved for marketing in 2001 and shows great promise in treating viral hepatitis.

10. HIGH-THROUGHPUT TECHNOLOGIES: THE MARCH OF SCIENCE

The above illustrates natural product-based drug innovation in China over several decades and was largely dependent upon historical precedents (e.g. experiences in TCM) and/or classical pharmacology. Although random compound screening in animal models is still a useful approach to discover new drugs, the disadvantages are obvious. It requires a large amount of compound, its sensitivity is low and it is extremely laborious. Since the amount of active constituents present in natural products is usually very small, it is impractical, in most cases, to supply sufficient quantities of pure natural compounds for animal experimentation. A note of caution is that promising hits might be prematurely rejected owing to toxicities discovered in cell-based screens, while their detoxification in the liver may have revealed a safety profile in the animal body (Liska 1998).

The tremendous progress made in life sciences has resulted in the definition of many pathological processes and mechanisms of drug action. This advancement has led to the establishment of various molecular and cellular bioassays in conjunction with HTS methods (Kell 1999). HTS decreases the amount of testing compound required such that only microgram quantities are needed. This is advantageous for certain natural products that are difficult to isolate and purify, and permits compounds that are difficult to synthesize to be assayed. Coupled with

Structure XIX.

this progress is the development of combinatorial chemistry, where large and structurally diverse chemical libraries can be generated at an unprecedented rate using different techniques including parallel synthesis. Innovations in computer applications, automation technologies, microfluid management and software design have made it possible to screen hundreds of thousands of compounds within a short period of time, thereby expediting the pace of identifying active molecules or 'hits' that can be further developed to potential drug 'leads' with desired therapeutic activity (Sittampalam *et al.* 1997). For instance, the first natural protein tyrosine phosphatase 1B (PTP1B) inhibitor was discovered recently from a commonly used TCM, *Broussonetia papyrifera*, by a group of Chinese scientists following HTS (Chen *et al.* 2002; structure XIX). Since PTP1B is regarded as a key factor involved in the pathogenesis of type 2 diabetes, the finding will certainly aid in the current pursuit of novel therapeutics for this debilitating disease.

The application of HTS methods and establishment of large-scale sample libraries have accelerated the development of sample preparation techniques, e.g. rapid extraction and isolation methods from natural products, combinatorial biosynthesis, combinatorial chemistry for focused libraries, etc. These advances have widened the scope of drug screening and range of materials to be assayed (Houston & Banks 1997). In the past decade, tremendous progress has been made in HTS technology. For example, the number of compounds assayed has increased from 100 000 per year to 100 000 per day, or to even higher numbers in industrial organizations. This implies an enormous demand for structurally diversified chemical compounds (Sundberg 2000). Combinatorial chemistry is an effective method for solving this problem. It is a general term for the approach to synthesize compounds in parallel rather than sequentially. Various techniques have been developed, and some of them are capable of generating vast numbers of different compounds very rapidly. These methods tend to be based on peptides or oligonucleotides. Therefore, although biological activity could be found in HTS, the active compound is unlikely to have the physicochemical properties of a drug. In contrast, natural products are expected to show the necessary chemical diversity and 'drug-like' properties (i.e. they can be absorbed and metabolized *in vivo*). Bioactive natural

products often appear as part of a family of related molecules, so that it is possible to isolate a number of homologues and obtain SAR information. Of course, lead compounds discovered from screening natural products can be optimized by conventional medicinal chemistry or by application of combinatorial approaches. Since only a small fraction of the plant diversity in the world has been tested for biological activities, it can be assumed that natural products will continue to offer novel leads for drug discovery if they are available for screening.

However, natural products are unattractive to many pharmaceutical companies owing to perceived difficulties related to complexities of phytochemistry and to their continued access and supply (Harvey 1999). The technical difficulties concerning isolation and structural elucidation of bioactive natural products are being solved with contributions by chemists worldwide. For instance, extracts can be processed before use via bioassays in order to remove many of the reactive compounds that are likely to cause false-positive results. Fractionation of extracts that are active in screening can be performed rapidly by using high-performance liquid chromatography (HPLC), and subsequent fraction analysis by liquid chromatography/mass spectrometry (LC/MS) or even nuclear magnetic resonance (NMR) spectroscopy. By comparing MS data with those from libraries of known compounds, novel molecules in the extract can be distinguished from previously identified compounds. With automated sample injection and fraction collection, the HPLC system can readily and rapidly be used to isolate tens of milligrams of pure compounds, whose structure is usually resolved by NMR spectroscopy. The entire procedure from a crude extract to a defined molecule can be a matter of days rather than the several months that was routine a few years ago (Hughes 1998; Harvey 1999; Lawrence 1999). Such an approach is presently employed in many pharmaceutical research institutions in conjunction with HTS technologies aiming at collection of active effluents, isolation of active constituents and identification of pharmacophores from natural products. Structural modification will follow to develop drug candidates.

In conclusion, through years of unremitting effort, China's drug innovation system, consisting of contemporary technology platforms and historical experience, has reaped its first fruits. A number of novel therapeutics developed from medicinal plants, with defined mechanisms of action and worldwide intellectual property rights, have been or will soon be introduced to both domestic and international markets. In order to strengthen the overall competitiveness, China's indigenous pharmaceutical industry is undergoing an unprecedented transformation from imitation to innovation. With sustained economic progress and continued advancement in science and technology, drug discovery from natural products will continue to be a focal point of the nation's drive for TCM modernization.

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