

пии уrolитиаза по сей день пользуются большим спросом и сохраняют свою актуальность.

Литература

1.Абу Али Ибн Сина. Канон врачебной науки. Кн. 5, 2-е издан. Ташкент: Фан, 1980. с.326

2.Иорданов Д., Николаев П., Бойчинов А. Фитотерапия. София: Медицина и физкультура, 1970. с.323.

3.Нуралиев Ю., Нодиров С. 110 рецептов Ибн Сины. С-Птр: Вести.,с.222

4.Соколов С.Я., Замотаев И.П. Справочник по лекарственным растениям. М.: Медицина, 1984.,с. 500.

THE ROLE OF THE ICBG PROGRAM IN BUILDING NEW PHARMACEUTICAL CAPABILITIES IN CENTRAL ASIA

Dushenkov¹ Vyacheslav, Akimaliev² Jamin, Buriev³ Khasan Ch., Lila⁴ Mary Ann, Nuraliev⁵ Yusuf, Pichkhadze⁶ Guram, Struwe¹ Lena, White¹ James F., Zylstra¹ Gerben J., Raskin¹ Ilya.

¹Rutgers University, New Brunswick, USA

²Kyrgyz Agricultural Research Institute, Bishkek, Kyrgyzstan

³Tashkent State Agrarian University, Tashkent, Uzbekistan

⁴University of Illinois, Urbana-Champaign, USA

⁵International Institute for Study of Avicenna Heritage and Pharmacology, Dushanbe, Tajikistan

⁶Kazakh National Medical University, Almaty, Kazakhstan

Introduction

The International Cooperative Biodiversity Groups (ICBG) Program was established in 1992 as a unique cooperation of the United States of America agencies to address the interdependent issues of drug discovery, biodiversity conservation, and sustainable economic growth. The program, administered by The Fogarty International Center of the National Institutes of Health, is cur-

rently funded through the National Institutes of Health (NIH), the National Science Foundation (NSF) the Department of Agriculture (USDA) and the Department of Energy (DOE). The ICBG program is focused on promoting human health improvement through drug discovery, creating of incentives for biodiversity conservation, and promoting scientific research and sustainable economic development. There are established ICBG programs in Papua New Guinea, Costa Rica, Panama, Fiji, Madagascar, Vietnam, Laos and Central Asia.

Substances of plant origin were for a long time the only source of medicinal drugs (Raskin and Ripoll, 2004). The isolation and exploration of alkaloids opened new ways to obtain chemically uniform single molecule therapeutics of natural origin (Mashkovskii, 1998). Discovery of penicillin effectively added microorganisms to the source of natural product-based therapeutics. Even in the era of combinatorial chemistry, more than 25% of new medicinal agents are related in some way to substances of plant origin (Newman et al., 2003). The rapidly growing application of preparations from sweet wormwood (*Artemisia annua* L.) in the struggle against malaria (Abdin et al., 2003) is a demonstrative example of the effectiveness of herbal medicines. Among 847 low-molecular-weight medicines introduced into the practice since 1981, 43 agents belong to natural compounds and 232 agents are derivatives of natural substances. Furthermore, in the remaining group of 572 preparations, an original link to natural compounds can be traced for 262 preparations (Newman and Cragg, 2007). Chemical defense is almost the only effective instrument in protection plants have against pathogenic organisms and multiple herbivorous animals. For effective defense against pathogens, plants have developed a complicated system comprising elements with different mechanisms of action (Lewis and Ausubel Frederick, 2006). Interactions of chemical components in the extracts play an important role in potentiation of plant preparations (Schmidt et al., 2008). For example, the alkaloid triptolide from

Tripterygium wilfordii Hook F., an effective rheumatoid arthritis therapy, is highly toxic to humans in a purified form, although the toxicity of non-purified triptolide in plant extracts is significantly reduced. Because of its high toxicity, purified triptolide has never been allowed for therapeutic usage, whereas the medicine based on *T. wilfordii* extracts is currently in the final stage of development (Ma et al., 2007). The effectiveness of non-purified plant preparations, as compared to that of purified ingredients, might involve other mechanisms such as protection of active substances from the attack of “foreign” enzymes, a faster transfer of the active substance across membranes, and circumvention of drug resistance of human organisms. Presently, there is a growing interest in mechanisms underlying the action of plant-derived substances on gene activities involved in disease development or vital functions of the organism (Evans et al., 2006). According to data of the Food and Agriculture Organization (FAO), more than 50000 plant species are used in traditional folk medicine throughout the world. The chemical diversity of the plant kingdom remains largely unexplored. Even less is known about biological activity of the chemical substances obtained from plants.

Located in the middle of Eurasia, stretched out between the Himalayas and the Russian steppes, and between the Caspian Sea and the highlands of Tibet, Central Asia is an attractive destination for the ICBG program due to its combination of biodiversity hotspots, historical use of plants for healing and health promotion, and a great need for biodiversity conservation and economic development. Unique steppe ecosystems stretch across central and western Kazakhstan. The lofty mountains of Tajikistan and Kyrgyzstan have earned them the title of “the roof of the world”. The region's two largest rivers - the Amu Darya and the Syr Darya - form in these highlands, falling westward and slicing through the lowlands of Turkmenistan, Uzbekistan, and Kazakhstan until they empty into the Aral Sea. Lake Balkhash in Kazakhstan and Lake Issyk-Kul in Kyrgyzstan are among the largest lakes of Eurasia.

Central Asia is the home of one of the most diverse and exceptional plant communities that reflects the overall biodiversity of the region. The vascular plants flora of the Central Asian region includes over 7,000 species, and is also characterized by a high degree of endemism.

The Central Asia ICBG program began in 2003 with the overall goal of building new pharmaceutical capabilities in Central Asia and linking natural products drug discovery and development to biodiversity conservation and economic development. Initially, from 2003-2005 the Central Asia ICBG Program operated in Kyrgyzstan and Uzbekistan. The Uzbekistan Associate Program was suspended in 2006 and in the spring of 2007 the Central Asia ICBG program was extended to Kazakhstan and Tajikistan.

Overview

The Central Asia ICBG program was structured to improve human health through drug discovery, promote scientific research and create incentives for conservation of biodiversity. A comprehensive functioning infrastructure was created and significant progress was made in knowledge creation, fundamental research, pharmaceutical development, and training. The program was structured into associate programs to address specific needs and manage activities in the participating countries. Specific associate programs included: Plants and Pharmacological, Structural and Analytical Chemistry, Fungal, Environmental Microbiology, Biodiversity, Conservation, and Training, Kazakhstan, Kyrgyzstan, Tajikistan, and Uzbekistan. National programs are structurally integrated into the US-based programs. The functioning network established by the Central Asia ICBG program included over 20 organizations in 5 countries and was supported by regular inter-institutional visits including four annual ICBG symposia held in Tashkent (Uzbekistan), New Brunswick (USA), Issyk-Kul (Kyrgyzstan) and Chicago (USA), respectively. There has been extensive training of Central Asian scientists by the US Associate Programs and, conversely, there has been extensive education of US

scientists by our Central Asian colleagues on the ecology and geography of their countries. Beginning 2003 over 45 expeditions were organized in Central Asia, many with the participations of US scientists. These expeditions resulted in the collecting of more than 300 soil samples for microbiological analysis, the building an extensive library of extracts from more than 1800 plant species, and the isolation of over 1500 endophytic fungal cultures. All the materials were collected and initially processed by the Central Asia Associate Program partners. Part of the animal testing was performed in Uzbekistan. The later part of the collected material was delivered to the US for further screening and evaluation. Over 50 screens to evaluate biological activity of plant extracts were deployed through the ICBG program partners. In addition, the following 5 animal models were used as pre-clinical follow-up: carageenan-induced rat paw edema model, cotton ball-induced granuloma formation, rat / mice forced swim test, mice high fat diet-induced diabetes model, and anti-hypoxic activity in rats.

Plants and Pharmacological Associate Program

To identify anti-inflammatory leads the Plants and Pharmacological Associate Program employed cell-based gene expression assay (Dey et al., 2008). A mouse macrophage-based quantitative, reverse transcription polymerase chain reaction (qRT-PCR) system was optimized to screen plant extracts. The mRNA expression of cyclooxygenase-2, interleukin 1beta and inducible nitric oxide synthase genes in RAW mouse macrophages was determined quantitatively in response to treatment with plant extracts. Using 75% reduction in gene expression activity as a threshold value 7.5% percent of tested extracts were shown to have strong activity against at least one target gene. Eighteen extracts were prioritized for future study and development, based on their activity, relative novelty, and plant material availability. High-content PCR-array analyses measuring the expression of 84 key target genes selected from pathways involved in inflammation and immune signaling (purchased from SuperArray Inc., Frederick, MD)

was used to elucidate molecular mechanisms of action of the eleven extracts. This approach resulted in useful mechanistic data used for further lead prioritization. Eight selected extracts were tested in animal models to confirm that *in vitro* data can be validated *in vivo*. All tested extracts showed significant *in vivo* activity confirming the validity and high predictive value of the qRT-PCR screening. Ethnobotanical follow-up provided an additional route for identifying plants with anti-inflammatory properties. For example, a sesquiterpene lactone-enriched extract (STLEE) from the leaves of *Artemisia leucodes* (Asteraceae) showed strong anti-inflammatory activity *in vitro* and *in vivo* (Schmidt et al., 2008). In a carrageenan-induced paw edema studies performed in Uzbekistan, STLEE at 200 mg/kg was almost twice as effective compared to aspirin administered at the same dose.

Reduced brain-derived neurotrophic factor gene expression is associated with disorders ranging from major depression to Alzheimer's disease. The up-regulation of BDNF activity is considered to be a promising strategy for treating many mental disorders (Price et al., 2007). Therefore, over 400 plant extracts collected in Central Asia have been analyzed in a C6 glioma cell-based assay. Among these, 2.1% extracts exhibited more than a 2-fold up-regulation of BDNF mRNA in comparison to the untreated control. Some of the selected in this assay extracts demonstrated an effect comparable with common antidepressant medication desipramine in mice forced swim test.

Pennington Biomedical Research Center (PBRC) at Louisiana State University has been a major partner in the area of metabolic syndrome (diabetes and related diseases). A total of 20 different state-of-the-art *in vitro* assays have been used by PBRC to evaluate over 300 extracts from Central Asia. Fifteen species were prioritized as particularly active and promising leads for the future follow-up in the area of metabolic syndrome.

Fungal Associate Program

Fungal Associate Program concentrated its efforts on the search for therapeutic agents from endophytic species of fungi. These endophytes are widespread in grasses fungi from the family Clavicipitaceae (Hypocreales, Ascomycota). In these mutualistic associations, the endophyte grows systematically within its host, including the developing seeds and is entirely dependent on the survival and growth of the grass host plant for its growth. Studies of the range of nutrients on which Clavicipitaceae suggests that evolution of plant biotrophy and endophytism in the family was largely a phenomenon of reduction of enzymatic capabilities and increasing dependence on the host plant to provide nutrients for growth; concurrent with reduction was an apparent increase in the allocation of energy to production of particular secondary metabolites beneficial in the symbiosis (Torres et al., 2008). Plant families, plant parts, vegetation types, and elevation zones were assessed as sources of endophyte that produce bioactive metabolites in some bioassays designed to assess for specific targets (anti-inflammatory, anti-cancer, and screen for neurological disorders like measuring the expression of brain-derived neurotrophic factor). This preliminary study resulted in approximately 50 bioactivity hits using three assays. It was found that particular ecological habitats were more fruitful than others in the yield of bioactive endophytes. A trend was observed where particular plant organs tended to yield bioactive endophytes at a frequency that was slightly greater than that expected by chance.

Environmental Microbiology Associate Program

Many bacteria, particularly actinomycetes, are known to produce secondary metabolites synthesized by polyketide synthases (PKS). Bacterial polyketides are a particularly rich source of bioactive molecules, many of which are of potential pharmaceutical relevance. To directly access PKS gene diversity from soil, degenerate PCR primers for actinomycete type II KS α (ketosynthase) genes were developed (Wawrik et al., 2005). Bacterial communities were compared by terminal restriction fragment length poly-

morphism (TRFLP) analysis of PCR products generated using bacterial 16S rRNA gene primers (27F and 1525R) as well as an actinomycete-specific forward primer.

The response of soil bacterial communities to carbon source enrichment in small matrices was studied by means of terminal restriction fragment length polymorphism (TRFLP) analysis. The TRFLP analysis indicated that enrichment on structurally similar carbon sources selected for similar bacterial communities. The results also suggest that in order to maximize the diversity of bacteria recovered from the environment multiple enrichments should be performed using a chemically diverse set of carbon sources (Wawrik et al., 2005).

Terminal restriction fragment length polymorphism analysis using actinomycete 16S rRNA and type II PKS genes was employed to determine community profiles. The terminal fragment frequencies in soil samples had a lognormal distribution, indicating that the majority of actinomycete phylotypes and PKS pathways are present infrequently in the environment. Less than 1% of peaks were detected in more than 50% of samples and as many as 18% of the fragments were unique and detected in only one sample. Actinomycete 16S rRNA fingerprints clustered by country of origin, indicating that unique populations are present in North America and Central Asia (Wawrik et al., 2007). Sequence analysis of type II PKS gene fragments cloned from Uzbek soil revealed 35 novel sequence clades whose levels of identity to genes in the GenBank database ranged from 68 to 92%. The data indicate that actinomycetes are patchily distributed but that distinct populations are present in North America and Central Asia.

Three metagenome libraries were constructed as part of the first Central Asia ICBG project. Over one million PCR based screening assays were performed. Targets for the assays included genes encoding Actinomycete 16S rRNA, bacterial 16S rRNA, polyketide synthases (4 different PCR screens, 3 for Type I and 1 for Type 2), non-ribosomal peptide synthases (NRPS), chlorope-

oxidase, ribulose-1,5-bisphosphate carboxylase, alkane hydroxylase (AlkB family), alkane hydroxylase (P450 family), and Rieske-type dioxygenases.

Actinomycetes were isolated from a variety of soil samples. In particular, we targeted Actinomycete cultures producing antimicrobial compounds by a three step screening protocol. Strains showing the most promise (passing the pre-screen for antimicrobial activity and the secondary and tertiary screens to identify strains synthesizing known compounds) were used for further evaluation. Overall about 20,000 colonies were screened resulting in 94 pure cultures.

Structural and Analytical Chemistry Associate Program

The Structural and Analytical Chemistry Associate Program (SACAP) was based in the University of Illinois (Urbana-Champaign), USA. The SACAP successfully characterized unique biologically-active, potent constituents from both microbial and plant origin, which paves the way for commercialization of well defined and standardized preparations. Complex intractable proanthocyanidin moieties as well as phenylethanoid and cyanogenic glycosides have been characterized from indigenous species (Yousef et al., 2006; Grace et al., 2008). Phytoecdysteroids and diterpenoids including novel compounds have been isolated from endemic Uzbek *Ajuga*. Phytochemical constituents with anti-cancer activity and significant potential for development as biofuels have been isolated and characterized (Grace et al., 2007).

Biodiversity, Conservation, and Training Associate program

Biodiversity, Conservation, and Training Associate Program played a crucial role in the organization of training, database customization and maintenance, data submission and biodiversity conservation efforts. *NAPIS* (NATURAL Products Information System), a laboratory information management system (LIMS)-based database was used for data submission, storage and analysis. The *NAPIS* database was customized for the specific needs of the Cen-

tral Asia ICBG program. In the framework of the ICBG program several training courses on “Biodiversity documentation” were organized in Central Asia. To facilitate increased land protection in Central Asia with proper management of these resources for research, biodiscovery, and tourism a special workshop was organized in the United States. This workshop, called *Biodiversity Conservation, Science and Management of Protected Areas: The Yellowstone Experience*, was organized for ten Central Asia officials and ICBG scientists at Yellowstone National Park, 26 Aug – 3 Sep, 2007.

A Distinguished Lecture Series in Central Asia during 2005-2006 featured six speakers that gave seminars in Tashkent and Samarkand (Uzbekistan) and in Bishkek (Kyrgyzstan) on a variety of topics from Intellectual Property Rights, Human Health and Medicinal Plants, to Conservation and Ecotourism. In total, nearly 800 people attended these seminars.

Training of a month’s length or longer included 128 people of all ranks from undergraduates to full professors. Most were trained in field collection or lab techniques for plants, fungi, or microbes, and/or NAPIS database entering and data management. In addition to this, in 2007 five scientists from Kazakhstan participated in a month-long biomedicine and screening training period at Rutgers University.

The manuscript for the book *Medicinal Plants of Central Asia: Kyrgyzstan and Uzbekistan* was submitted to Missouri Botanical Garden Press in mid-August 2007. This Russian-English book includes treatments of 208 medicinal plant species with information on morphology, distribution, traditional uses, chemistry, and common names in Russian, Uzbek, Kyrgyz, and English. The book will also include an English-Russian-English glossary to common terms used in medicine, plant morphology, and pharmacology. In total 54 publications resulted from ICBG program thus far. Twelve of those publications were coauthored by US and

Central Asia scientists. ICBG-related materials were presented at more than 70 conferences.

Conclusion

In five years the ICBG program has had tangible impact on the development of pharmacological capabilities, scientific research, ecological education and development in Central Asia. Initial contacts between US and Central Asia scientists established under the ICBG program are being extended to other programs and bilateral collaboration has strengthened considerably.

Acknowledgment

The project described was supported by Award Number U01TW006674 from the Fogarty International Center. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Fogarty International Center or the National Institutes of Health.

References

Abdin MZ, Israr M, U. RR, Jain SK (2003) Artemisinin, a Novel Antimalarial Drug: Biochemical and Molecular Approaches for Enhanced Production *Planta Med* **69**: 289-299

Dey M, Ripoll C, Pouleva R, Dorn R, Aranovich I, Zaurov D, Kurmukov A, Eliseyeva M, Belolipov I, Akimaliev A, Sodombekov I, Akimaliev D, Lila Mary A, Raskin I (2008) Plant extracts from central Asia showing antiinflammatory activities in gene expression assays. *Phytotherapy research* **22**: 929-934

Evans DA, Hirsch JB, Dushenkov S (2006) Phenolics, inflammation, and nutrigenomics. *Journal of the Science of Food and Agriculture* **86**: 2503-2509

Grace MH, Faraldos JA, Lila MA, Coates RM (2007) ent-Beyerane diterpenoids from the heartwood of *Excoecaria parvifolia*. *Phytochemistry (Elsevier)* **68**: 546-553

Grace MH, Yousef G, G., Raskin I, Lila Mary A (2008) Phytochemical characterization and antihypoxic efficacy of an

active extract of *Rhodiola heterodonta*. Phytochemical Analysis: in press

Lewis K, Ausubel Frederick M (2006) Prospects for plant-derived antibacterials. *Nature biotechnology* **24**: 1504-1507

Ma J, Dey M, Yang H, Poulev A, Pouleva R, Dorn R, Lipsky PE, Kennelly EJ, Raskin I (2007) Anti-inflammatory and immunosuppressive compounds from *Tripterygium wilfordii*. *Phytochemistry (Elsevier)* **68**: 1172-1178

Mashkovskii MD (1998) *Lekarstva XX veka (Medicines of XX Century)*. Novaya Volna, Moscow

Newman DJ, Cragg GM (2007) Natural products as sources of new drugs over the last 25 years. *Journal of natural products* **70**: 461-477

Newman DJ, Cragg GM, Snader KM (2003) Natural Products as Sources of New Drugs over the Period 1981-2002 *J. Nat. Prod.* **66**: 1022 -1037

Price RD, Milne SA, Sharkey J, Matsuoka N (2007) Advances in small molecules promoting neurotrophic function. *Pharmacology & therapeutics* **115**: 292-306

Raskin I, Ripoll C (2004) Can an apple a day keep the doctor away? *Current Pharmaceutical Design* **10**: 3419-3429

Schmidt B, Ribnicky DM, Poulev A, Logendra S, Cefalu WT, Raskin I (2008) A natural history of botanical therapeutics. *Metabolism, Clinical and Experimental* **57**: S3-S9

Schmidt BM, Poulev A, Dey M, Gries M, Kurmukov AG, Zakirov SK, Lyapina NR, Belolipov I, Lila MA, Raskin I (2008) An extract from *Artemisia leucodes* reduces acute and chronic inflammation in rats. *J. Ethnopharmacology* **in press**

Torres MS, Singh AP, Vorsa N, White JF, Jr. (2008) An analysis of ergot alkaloids in the clavicipitaceae (hypocreales, ascomycota) and ecological implications. *Symbiosis* **46**: 11-19

Wawrik B, Kerkhof L, Kukor J, Zylstra G (2005) Effect of different carbon sources on community composition of bacterial

enrichments from soil. *Applied and Environmental Microbiology* **71**: 6776-6783

Wawrik B, Kerkhof L, Zylstra GJ, Kukor JJ (2005) Identification of unique type II polyketide synthase genes in soil. *Applied and Environmental Microbiology* **71**: 2232-2238

Wawrik B, Kutliev D, Abdivasievna UA, Kukor JJ, Zylstra GJ, Kerkhof L (2007) Biogeography of actinomycete communities and type II polyketide synthase genes in soils collected in New Jersey and Central Asia. *Applied and Environmental Microbiology* **73**: 2982-2989

Yousef GG, Grace MH, Cheng DM, Belolipov IV, Raskin I, Lila MA (2006) Comparative phytochemical characterization of three *Rhodiola* species. *Phytochemistry (Elsevier)* **67**: 2380-2391

РОЛЬ ПРОГРАММЫ ICBG В РАЗВИТИИ ФАРМАЦЕВТИЧЕСКИХ ВОЗМОЖНОСТЕЙ В ЦЕНТРАЛЬНОЙ АЗИИ

Душенков¹ Вячеслав, Акималиев² Джамин, Буриев³ Хасан Ч., Лайла⁴ Мари Анн, Нуралиев⁵ Юсуф, Пичхадзе⁶ Гурам, Струве¹ Лена, Вайт¹ Джеймс Ф., Зулстра¹ Гербен Дж., Раскин¹ Илья.

Программа «Международные группы по сотрудничеству в области биологического разнообразия – ICBG» была основана в 1992 г. в результате сотрудничества различных агентства правительства США. Программа ведет активную работу в Папуа Новая Гвинея, Коста-Рике, Панаме, Фиджи, Мадагаскаре, Вьетнаме, Лаосе и Центральной Азии. Центрально-Азиатская программа ICBG включает сотрудничество между Казахстаном, Киргизстаном, Таджикистаном, Узбекистаном и США. Центрально-Азиатская программа ICBG направлена на поиск новых биологически активных веществ из растений и микроорганизмов и сохранение биологического разнообразия региона. С начала функционирования программы в 2003 году ученые из стран региона совместно с коллегами из США участвовали в экспедициях, оценке фармакологических свойств продуктов растительного и микробного происхождения, раз-

работке мер охраны уникального биологического разнообразия Центральной Азии. Программа внесла весомый вклад в развитие науки и фармацевтических возможностей стран-участниц. По материалам программы опубликовано более 50 статей, из которых 12 написаны совместно с учеными Центрально-Азиатского региона и США.

TRADITIONAL CHINESE MEDICINE AND CONTEMPORARY WESTERN

Javokhirlal Muzaffari

Beijing Uni-Con International Medical Center

[Dr. Mae-Wan Ho](#) *discovers out how traditional Chinese medicine is at the heart of indigenous Chinese culture, and suggests how it could be understood in terms of contemporary Western science.*

[Sources and references](#) for this article are posted on ISIS Members' website. [Details here](#) .

The first book of Chinese medicine to have been preserved for posterity is *Neijing*, or *Classic of Internal Medicine*, by Huangdi, the Yellow Emperor, which appeared during the 'Spring and Autumn and Warring States Period (770-221 BC), and sums up medical achievements that were made before.

Accompanying *Neijing* is *Nanjing*, or *Classic of Difficult Medical Problems*, and other medical texts written before the Han Dynasty (206 BC to 220 AD). These laid the theoretical foundations of traditional Chinese medical practices that must have gone back to the dawn of indigenous Chinese culture.

During the Han Dynasty, other important texts were added, in particular, Han Zhongjing's *Treatise on Febrile and Miscellaneous*