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Review Article

REVERSE PHARMACOLOGY: A NEW APPROACH TO DRUG DEVELOPMENT

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ABSTRACT

The pharmaceutical sector has traditionally been a vibrant, innovation-driven and highly successful component of global industry. A confluence of spectacular advances in chemistry, molecular biology, genomics and chemical technology and the cognate fields of spectroscopy, chromatography and crystallography led to the discovery and development of numerous novel therapeutic agents for the treatment of a wide spectrum of diseases. To facilitate this process, scientists launched a significant and noticeable effort aimed at improving the integration of discovery technologies, chemical sourcing for route selection/delivery of active pharmaceutical ingredients. However, recent trends indicate that this model may no longer ensure high growth rates. R & D expenses have risen enormously in last decade but surprisingly it has not led to a corresponding increase in the number and efficacy of new drugs. And so numbers of approved new chemical/molecular entities are declining. The extremely time consuming, complex and capital-intensive process makes companies 'target rich' but 'lead poor'.

Alternatively, reverse pharmacology also known as target base drug discovery (TDD) is now becoming a popular option in the field of drug discovery where a hypothesis is first made that modulation of the activity of a specific protein target will have beneficial therapeutic effects. Screening of chemical libraries of small molecules is then used to identify compounds that bind with high affinity to the target. The hits from these screens are then used as starting points for drug discovery. This method became popular after the sequencing of the human genome which allowed rapid cloning and synthesis of large quantities of purified proteins. This method is the most widely used in drug development today.

Keywords: Reverse pharmacology, strychnos alkaloids, physostigmine, curare.

INTRODUCTION

Traditional Medicine Knowledge which has evolved over several years of observations and experimentations is a valuable knowledge with the communities possessing this knowledge. The mass screening of plants in the search for new leads or drugs is not only expensive but also inefficient; alternatively traditional knowledge would offer better leads. About 60% of anticancer and 75% of anti-infective drugs approved from 1981-2002 could be traced to natural origins [1]. The use of plant remedies would be cheaper and more productive as described in ancient texts [2]. Many active compounds from traditional medicine sources could serve as good scaffolds for rational drug design. Most of these compounds are part of routinely used traditional medicines and hence their tolerance and safety are relatively better known than any other chemical entities that are new for human use [3]. Thus, traditional medicine based bio-prospecting offers unmatched structural variety as promising new leads. [4].

Traditional knowledge and clinical observations have provided a way for older molecules towards new applications. For instance, forskolin an alkaloid isolated by Hoechst and coleonol by Central Drug Research Institute (CDRI), CSIR, Lucknow a few decades ago from *Coleus forskholii* [5] and phytochemicals from *Stephania glabra*, are now being rediscovered as adenylate cyclase and nitric oxide activators, which may help in preventing conditions including obesity and atherosclerosis [6]. Small molecule drugs that can regulate TNF-α levels or activity may provide a cost-effective alternative to protein-based therapeutics [7]. Pancreatic lipase inhibitors, from natural products, are also the potential candidates which can be developed into new drugs for treatment of conditions like obesity [8]. A large number of promising leads for the development of newer anti-inflammatory drugs are also available in medicinal plants [9].

CURRENT SCENARIO OF DRUG DISCOVERY

The average cost and time of discovering, developing and launching a new drug by the pharmaceutical industry is increasing without an expected analogous increase in the number of newer, safer and better drugs. The number of approvals for new drugs has declined dramatically from 53 in the year 1996 to just 17 in 2007 [10]. The industry is facing a major challenge to sustain and grow, which is resulting in many mergers, acquisitions or closures [11]. The age of the blockbuster drug seems to be almost over. The increased cautious regulatory processes are adding more risk and years for the pharmaceutical companies. The United States Food and Drug Administration (US FDA)'s Drug Watch and Drug Advisory Committee briefings on new anticoagulant Ximelagatran of Astra Zeneca or COX II inhibitor Vioxx of Pfizer are very indicative of the impasse [12].

Drug discovery and development process involves a 10-15 years of investigation period and investments of the order of US $ 1 to 1.5 billion. This extremely complex, technology based and capital-intensive process has resulted in 'target rich lead poor' performance.

The figure below is depicting a process for safer and effective drug development which is faster and sustained.

The importance of experiential wisdom and holistic approach is rising to offer good base as an attractive discovery engine [3]. The World Health Organization's Commission on Intellectual Property and Innovation in Public Health also has recognized the promise and role of traditional medicine in drug development for affordable health solutions [13].

REVERSE PHARMACOLOGY

Sir Peter B. Medawar rightly stated that "A synthetic discovery is always a first recognition of an event, a phenomenon, process, or a state of affairs not previously recognized or known. Most of the stirring and deeply influential discoveries of science come under this heading".

Reverse pharmacology is the science of integrating documented clinical/experimental hits, into leads by trans-disciplinary exploratory studies and further developing these into drug candidates by experimental and clinical research.

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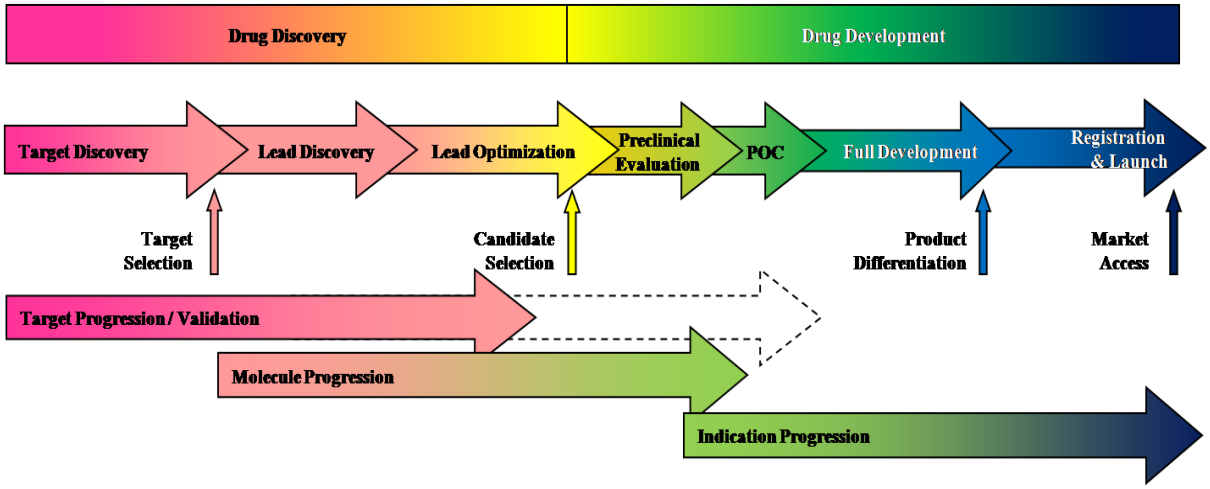

Fig. 1: Slow track pharmacology.

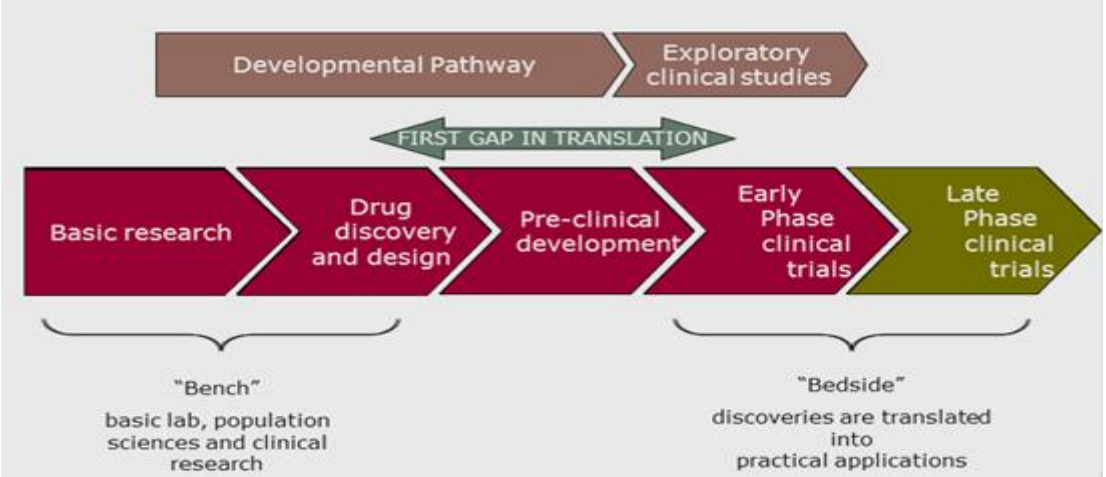

Fig. 2: Reverse pharmacology.

The scope of reverse pharmacology is to understand the mechanism of action at multiple levels of biological organization and to optimize safety, efficacy and acceptability of the leads in natural products, based on relevant science. Reverse pharmacology comprises of three stages—experiential, exploratory and experimental. Possessed with a mixed healthcare, India offers a goldmine for robust experiential documentation of clinical observations of bio-dynamic effects of standardised AYUSH or modern drugs.

Meticulous attention to minute clinical details, accompanied by an excellent record keeping would identify the clinical hits, for example, bleeding with aspirin or Parkinsonism with Rauwolfia serpentina.The exploratory studies would cover dose-activity in ambulant patients and selected *in-vitro* and *in-vivo* models to evaluate the key target. These exploratory leads are evaluated critically for resource allocation and state-of-the-art experimental studies like platelet aggregation with aspirin vis-à-vis bleeding and the role of dopamine depletion in extrapyramidal disorders vis-a-vis Rauwolfia. The experimental stage involving relevant basic and clinical science would be employed to study the plant or a molecule at different levels of biological organisation. This would define the safety, efficacy, preventive or therapeutic dimensions of the new or natural drug.

The reverse approach in pharmacology has been quite successfully applied in the past. The drawback was that the long time lags from the observational therapeutics to a new drug. For example, Raulwolfia serpentina was convincingly demonstrated to be an anti-hypertensive by

Gananath Sen and Kartik Bose in 1931. But a drug reserpine, emerged only after 20 years of work by Vakil, Bein, Mueller and Schlittler.

This happened because the path of reverse pharmacology was random and quite discontinuous. Currently, CSIR through the New Millennium Indian Leadership Initiative (NMITLI) has adopted the path of reverse pharmacology. The NMITLI team in the last four years has networked for R&D in a multi-institutional, multi-disciplinary endeavour in diabetes, arthritis and hepatitis. The results have been remarkable as to the hits and leads obtained. The paradigm of reverse pharmacology is actually a rediscovery of the path, which founded modern pharmacology. Table 1 lists the names of plants, clinical effects and experimental correlates. This list illustrates how novel clinical bio-dynamic effects can lead to the development of the basic disciplines in pharmacology and biology.

Table 1: Re-discovery of the paradigm of reverse pharmacology

Medicinal Plant	Clinical Effect	Experimental Correlate
Curare tomentosun	Paralysis and death	Neuromuscular block
Papaverum somniferum	Analgesia	Opioid receptors
Physostigma venenosum	Ordeal poison	Anticholinestrease
Cinchona	Fever cure	Antimalarial
Digitalis purpurea	Dropsy-relief	Na+ - K+ ATPase
Salix alba	Fever and pain relief	Prostaglandins
Strychnos nux-vomica	Stimulant and comulsant	Glycinergic receptors

Patwardhan Bhushan & Vaidya Ashok D. B,. , March 2010, Natural products drug discovery: Accelerating the clinical candidate development using reverse pharmacology approaches, Vol. 48 pp. 220-227.

R & D PATH FOR NATURAL PRODUCTS

These paths have been thoroughly explored for years and have yielded effective medicines. Although botanical medications continued to be produced in every country, their clinical efficacy was usually not evaluated and the composition of these complex mixtures was only crudely analysed. Investments in these methodologies remained scarce.

Main factors contributing to such slow-tracking include failure to distinguish folklore from traditionally established systems like Ayurveda and TCM, lack of proper identity and implementation of Good Laboratory Practices, improper experiential documentation, absence of Phase II dose optimizing studies, cultural prejudice for alien sciences, emphasis on the reductionist path, and lack of political and financial support.

An integrative and interdisciplinary approach is the key to understanding the breadth of the drug action resurrect natural products based drug discovery. The credit for stimulating interest of Indian chemists and pharmacologists in medicinal plants should rightfully go to Sir Ram Nath Chopra who has been acclaimed as the 'Father of Indian Pharmacology" [14].

Gananath Sen laid the foundation of Reverse Pharmacology of medicinal plants by pursuing clinically documented effects of Ayurvedic drugs [15]. *Rauwolfia serpentina* Benth, was a major discovery through this approach. Sen and Bose not only convincingly demonstrated the antihypertensive and tranquillizing effects of the plant but also observed unique side effects such as depression, extra pyramidal syndrome, gynecomastia and such [16]. It took decades to delineate mechanisms of these side effects. This was a watershed for new antidepressants, anti Parkinson's drugs and prolactin-reducing drugs [17].

In the west, pharmacology as a discipline grew rapidly when the plants toxic to humans such as strychnos alkaloids, physostigmine, and curare were studied for the mechanisms of action [18, 19]. Reverse pharmacology offers a major paradigm shift in drug discovery. Instead of serendipitous findings pursued randomly an organized path from clinical observations and success is established. The science has to integrate documented clinical and experiential hits into leads by interdisciplinary exploratory studies on defined targets *in vitro* and *in vivo* and conducting the gamut of developmental activities. Recently, India has amended the Drug Act to include a category of phytopharmaceuticals to be developed from medicinal plants by Reverse Pharmacology, with evidence of quality, safety and efficacy. These drugs will be distinct from traditional medicines like Ayurvedic, Unani or Siddha.

A New millennium for Technological Leadership Initiative (NMITLI) has resulted in hits, leads and effective formulations for diabetes, arthritis and hepatitis with novel mechanism of action and intellectual property [20]. Malaria, despite availability of a large number of chemotherapeutic agents, takes a severe toll in terms of mortality and morbidity. Common antimalarial drugs derived from plants include quinine and artmisinine. Recently, *Nyctanthes arbor-tristis* Linn has been shown to possess antimalarial activity. In a study in 120 patients, ninety-two (76.7%) showed complete parasite eradication and clinical cure within 7 days of treatment with the leaf paste of *N. arbor-tristis* [21]. The plant extracts are being standardized and studied phytochemically as exploratory studies have already shown antiplasmodial effects *in vitro* and disease modifying activity in patients [22]. This work has now been taken up by the ICMR Advanced Centre of Reverse Pharmacology in Traditional Medicine, in collaboration with the Centre of Molecular Parasitology at the Drexel University College of Medicine.

There is a need to develop an academic niche for Reverse Pharmacology in medical and pharmaceutical sciences colleges and drug R & D centres. Linkages must be established with Observational Therapeutics and Ayurvedic Pharmacoepidemiology to identify clinical hits. In India, major endeavours in this direction have been initiated already, both in the private and the public sectors of pharmaceutical R & D [23].

Artemisinin

The herb *Artemisia annua* has been used for many centuries in Chinese traditional medicine as a treatment in several thousand malaria patients in China, including those with both chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum*. Thus artimisinin and its derivatives offer promise as a totally new class of anti-malarial drugs [24]. This discovery of Artimisinin is a result of scientific work based knowledge from Traditional Chinese Medicine (TCM) and presents best case for reverse pharmacology approach.

Vaccine adjuvant

Despite centuries of vaccine use, still alum salts remain universal vaccine adjuvants licensed for human use. A project supported by Department of Science & Technology (DST), Govt. of India to develop herbal vaccine adjuvant was undertaken by the Interdisciplinary School of Health Sciences, University of Pune with Serum Institute of India as an industry partner. This project generally follows the reverse pharmacology approach based on Ayurvedic knowledge and previous scientific studies [25, 26]. Just within three years, the project has resulted in identification of few semi-pure leads that have considerable efficacy against polysaccharide, toxoid and recombinant group of vaccines. Also these leads were found to modulate T-helper cell immunity. Thus in a time span of three years a chemically characterized herbal fraction is ready to enter human clinical trial.

Osteoarthritis

for osteoarthritis herbal drug development NMITLI project involved a network of 16 national research institutions, modern medicine hospitals and pharmaceutical industries from India. The project used traditional knowledge guided platform where the base formulation was optimized with additional ingredients to obtain desired therapeutic activities. Thus, this project was completed in five years with expenditure of over US $ 2 million. This treatment may cost just US $ 25 a month for patients with much better therapeutic benefits including chondroprotection that no other modern drug offers. Currently, CSIR is in the process of identifying suitable industrial partner for further development, optimization, manufacturing, registrations and marketing.

Tanga project

In Tanzania, The Tanga AIDS Working Group (TAWG) has innovatively used indigenous knowledge (IK) to alleviate suffering from HIV/AIDS. The group has treated over 4000 AIDS patients with herbs prescribed by local healers. To facilitate this process, the Global Research Alliance and the World Bank have initiated a partnership between the Tanga AIDS Working Group and the US National Institutes

of Health to cooperate on the scientific validation of the efficacy of these herbal treatments.

Department of Ayurveda, Yoga, Unani, Siddha, Homeopathy (AYUSH) has recently established a research centre at the University of Mississippi Oxford to facilitate scientific investigations on Indian herbal drugs. A holistic approach based on systems biology seems much more suited to study therapeutic efficacy and pharmacodynamics of traditional medicine based drug development [27].

While we continue to use traditional knowledge and materials in the process of drug discovery and development, its intellectual rights protection has become an important issue.

Most of these compounds are part of routinely used traditional medicines and hence their tolerance and safety is assumed to be better than any other chemical entities that are totally new for human use.

Reverse pharmacology examples

1. Macuna pruriens for Pakinson's disease
2. Zingiber officinale for nausea/vomiting
3. Picrorhiza kuroa for hepatitis
4. Curcuma longa for Oral cancer
5. Panchvalkal for burns and wounds
6. Azadirachta indica for Malaria

Table 2: Drug Targets Identified By Reverse Pharmacology.

Receptor	Ligand	Year	Major function
Adenosine A_1, A_2 (RDC 7, RDC 8)	Adenosine	1990-1991	Platelet function, anxiety
ORL-1	Nociceptin/Orphanin FQ	1995	Stress, Pain
Orexin-1 and 2	Orexins/Hypocretins	1998	Food intake, sleep-wakefulness
GPR10	Prolactin-releasing peptide	1998	Sleep, absence seizure
APJ	Apelin	1998	Unknown
GHS-R	Ghrelin	1999	Food intake, GH secretion
SLC-1 (MHC 1)	MCH	1999	Food intake
GPR 14	Urotensin II	1999	Vasoconstriction
Histamine H3 (GPCR97)	Histamine	1999	Central nervous system-obesity, psychiatry
FM-3/4	Neuromedin U	2000	Unknown
Histamine H4 (GPRv53)	Histamine	2000	Inflammation, eosinophilia, chemotaxis
GPR54	Mestatin	2001	Cell proliferation, development
GPR73 a/b	Prokineticin1/2	2002	Angiogenesis, circadian rhythm
GPR7 and GPR8	NPB and NPW	2002	Food intake, unknown
GPCR135 and GPCR142	Relaxin	2003	Unknown
GPR91	Succinate	2004	Increases BP

Reverse pharmacology was only sporadically applied to new drug development. It is the need of the time to document unknown, unintended and desirable novel prophylactic and therapeutic effects in observational therapeutics. Several new classes of drugs have accidentally emerged by this path. For example, oral sulphonylureas emerged due to hypoglycaemic reactions to certain sulphonamides Thalidomide optical isomer emerged to be useful to control erythema nodoium and for multiple myceloma. Bromocriptine was first shown clinically in India to regress pituitary prolactinoma. Then, the new micro-prolactoma therapy emerged. Recently, an excellent review was written by Takeshi Sakurai on reverse pharmacology of orexin.

Recently, ICMR has established an advanced Centre for Reverse Pharmacology at Bhavan's Swami Prakashananda Ayurveda Research Centre, where the research focus is on diabetes, musculo-skeletal health, malaria, cancer and neuronal plasticity.

FUTURE PERSPECTIVES

Single drugs may not be an ideal way to treat a patient, with so many characteristics that are so individual, associated with the challenges of genetic diversity. Genome-wide functional screening against disease targets may be the practical approach. Combining Ayurveda and functional genomics in a systems biology scenario may reveal the pathway analysis of crude and active components [28]. Pharmacogenomics is now significantly influencing drug discovery and genotyping is recommended for drugs that are metabolized by enzymes whose genes have inactivating polymorphisms [29]. Efforts to correlate genotype and phenotype-based traditional methodology of classifying humans into three major Prakriti types or constitutions described in Ayurveda have opened an exciting scientific chapter and will help the progress of individualized medicine approaches [30, 31].

Innovative approaches inspired by traditional knowledge will remain important to accelerate the discovery process and add new life especially in the existing global economic environment.

However, despite an immense potential and possibilities very few success stories are available as of now. National efforts like NMITLI will remain important. The Government of India's golden triangle project integrating biomedicine, modern sciences and traditional medicine is indicative of trend where traditional sciences like Ayurveda are aggressively embracing scientific evidence base and integrated research [32, 33]. Best of public and private sector partners comprising academia and industry should come together to reap significant benefits from these seemingly low fashionable but highly gifted explorations based on traditional knowledge. This highly reductionist approach relies on the isolation of an identified molecular target, often an enzyme, and pursuit of a small molecule to modulate the target's known or predicted function. Thus, reverse pharmacology provides the new platform for drug development.

CONCLUSION

The modern drug discovery processes have started revisiting traditional knowledge and ethnopharmacology to reduce the innovation deficit faced today that would help reaching to the top in Sciences especially for developing counties like India [34]. A recent analysis article on curcumin published by Cell is good indication of the growing interest in mainstream high impact journals [35]. Traditional knowledge and experiential database can reduce time, money and toxicity - the three main hurdles in the drug development, to provide new functional leads. Systems biology and Reverse Pharmacology approaches need to be developed further and optimized as novel means for fast track drug discovery and development where the newer, safer and effective drugs will remain just a spin off and research continues keeping our hope for blockbusters alive. With Ayurveda, the normal drug discovery course of 'Laboratory to Clinics' actually becomes from 'Clinics to Laboratories'— a true Reverse Pharmacology Approach.

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