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Clinical Research - Past, Present & Future

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ABSTRACT

Clinical pharmacology/research has a very interesting history. It started in the 40's of the 20th century through the pioneering work of Harry Gold at Cornell University, New York. Clinical research is an integral part of drug development. Drug development can be hastened by a number of new techniques with reduction in cost. In addition reverse pharmacology approaches for drug discovery have come to occupy a special place. 85% of the neutral antagonists act as inverse agonists. Inverse agonists have a distinct effect on receptor regulation as opposed to neutral antagonists.

Orphan receptors constitute about 50% of the GPCRs. It is estimated that now there are nearly 175 orphan receptors after 125 having been deorphanised. Targeting these orphan receptors can lead to about the same number of ligands and antagonists thereof. Polymorphism of cytochrome P450 provides the basis for the use of predictive pharmacogenomics to yield drug therapies that are more efficient and safer. It is estimated that such personalized P450 gene-based treatment would be relevant for 10-20% of all drug therapy.

Key words : Clinical Pharmacology (a facet of clinical research) - Human experiments for safety and efficacy; G-protein coupled receptors (GPCRs) – some orphan receptors; Strategies for drug discovery – Deorphanisation of orphan receptors, allosteric receptor sites, in-silico drug screening.

Dr. O.D. Gulati, Date of Death : February 23, 2012.
NAMS GOLDEN JUBILEE LECTURE delivered at Nizam Institute of Medical Sciences, Hyderabad on July 01, 2011.

Prologue :

Experimental pharmacology in India dates back to Col. Sir Ram Nath Chopra* who was trained with Dixon in Cambridge where he imbibed the gospel of providing experimental proof of the claimed therapeutic efficacy of any new product. After a decade of return to India, he set up experimental pharmacology laboratory at the Tropical School of Medicine in Calcutta to give scientific basis for therapeutic claim made for many herbals in Ayurveda. He investigated a large number of plants experimentally and his researches culminated in several publications. In view of his pioneering work, he is rightly called the Father of Pharmacology in India (1).

His researches are the forerunner of subsequent experimental work in medical and pharmacy colleges and research institutes in India. This led to the training of a large manpower equipped to chair the departments of pharmacology.

Unfortunately, the country has not been able to contribute the introduction of new drugs in the therapeutic armamentarium of human disease. This is largely due to the fact that inputs involved in such endeavour in terms of time and money are mind boggling – the former about 10 years and the latter over 10 billion US dollars. Newer strategies involving pooling of resources by enterprising pharmaceutical companies may hopefully lead to realisation of this dream.

An additional focus in this paper is to use approaches that can yield new drugs with fraction of the cost. Thus, mining of the orphan receptor for drug discovery has already yielded a large number of new drugs (**Table 1**) with fraction of the cost. Similarly, computer simulation can also contribute new drugs with minimal financial inputs.

Of my bibliographic profile totalling about 138 publications, 23(from Medical College and SSG Hospital, Baroda) are in clinical research work done in collaboration with faculty of medicine (C.P.Munshi, P.M.Vaidya, B.T.Dave, K.M.Shah), faculty of pediatrics (Sanat Shah) faculty of dermatology, venereology and leprology (B.S.Verma, V.P.Vaishnav and B.G.Byakod) and faculty of obstetrics and gynecology. (L.V.Kelkar, V.V.Kelkar, T.V.Patel).

Past :

Gregory (1753-1821) has described the symptomatic heroic treatment of the time, consisting of blood letting, emesis and purgatives which were prescribed until the disappearance of morbid phenomena (i.e.symptoms and

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**** Col. Ram Nath Chopra was conferred Honorary Fellowship of the National Academy of Medical Sciences in 1961 : the other Honorary Fellowship awarded in 1961 (Foundation Year of the Academy) included Shri Jawahar Lal Nehru, Dr. B.C. Roy, Major Gen. S.L. Bhatia, Dr. H.M. Lazarus, Dr. Jivaraj N. Mehta, Dr. A. Lakshamanswami Mudaliar, Dr. N.A. Purandare, Major Gen. S.S. Sokey, and Dr. A.C. Ukil (Editor)***

signs). Very often there ensued rapidly a syncope, followed by death in such a way that the saying of this period, that the "patient is dead cured" was justifiably apt (1). Voltaire (1694-1776) was prompted to define therapeutics as the art of pouring drugs of which we know little into man of whom we know still less. In a similar vein Oliver Wendell Holmes (1809-1894) said "if all the materia medica (except, morphine) is sunk into the bottom of sea, it will be all the good for mankind and all the worse for fishes." These nihilistic dogmas of the time were pronounced by the prevalent despondency engendered by allopathy (a misnomer). Homeopathy originated as a reaction to this. In two recent articles published from UK, Homeopathy provides no more than placebo effect in the drugs prescribed by Homeopathic physicians (2,3). The scenario today is starkly opposite and fortunately we have new potent vaccines, antiinfectives, psychotherapeutics and several other drugs that have helped save million of lives (4).

After the experimental evaluation of drugs in animals, it is necessary to investigate drugs in human beings – Clinical Pharmacology. The credit for pioneering this must go to Harry Gold of Cornell University Medical School. Gold *et al* (5) were the first to bioassay digitalis in humans. I had the good fortune of meeting both Harry Gold and Walter Modell in September 1959. I was thrilled to watch Harry Gold performing his isolated tissue experiment. Walter Modell of the same institution continued the work initiated by Gold and a journal of Clinical Pharmacology was started.

U.K.Sheth is considered as the pioneer of clinical pharmacology in India.

Outstanding are the experiments of S.M.Smith in Salt Lake City (Smith *et al*, 1947) who received, while conscious and on artificial respiration, around three otherwise lethal doses of curare, to test whether it affected the brain; or J.B.S. Haldane, who, to make diving safer, had bends, oxygen convulsions and nitrogen narcosis; or the Professor E.A.Pask, who, to test a safety kit for the RAF which might be needed to protect an unconscious bailed-out pilot from drowning, had himself anaesthetized, curarized, and thrown wearing the kit into a swimming bath. These are all the outstanding pioneering examples of clinical research (6).

Present :

Clinical pharmacology, a facet of clinical research, is organized on human beings intended to provide adequate information on the drug use as a therapeutic agent for its safety and efficacy.

We need clinical trials for new drug discovery, new uses of existing drugs, new drug delivery systems and medical devices. Furthermore, clinical trials are also intended to get evidence to prove the efficacy of a given drug and safety in human beings. Only a well designed clinical study on a defined

population can give meaningful results-(positive or negative) about any therapeutic intervention (7).

Ethical Issues and Guidelines :

The concept of Ethics was developed in the 20th century with the growth of pharma industry. During 2nd world war, the Nazi physicians dehumanized Prisoners of War (POWs) during their clinical trials. The Nuremberg code and informed consent came as welcome steps. World Medical Association (WMA)-1964-guidance to physicians and other participants in medical research involving human subjects further streamlined the ethical requirements. Thalidomide disaster brought about an exponential growth in Pharmaceutical legislation

During 1950s proper testing of drugs on human beings was started. The concept of randomized controlled clinical trials was emphasized.

Several regulatory guidelines are available today. These include Drugs and Cosmetics Act 1947, Schedule-Y, Indian GCP Guidelines, ICMR Guidelines, WHO Guidelines and ICH-GCP Guidelines.

Other ethical issues and guidelines include those set forth by International Conference on Harmonization (ICH), Institutional Ethics Committee (IEC), Institutional Review Board (IRB)

Good Clinical Practice (GCP) :

It is the international standard for conducting clinical trials which lays down standard for design, conduct, monitoring, termination, analysis and documentation.

Informed consent is a written consent of a subject (in his own language) to participate in a trial.

Regulatory Requirements :

For fulfilling regulatory requirements, inputs from the following may be needed depending on national norms:

- Drugs and Cosmetics Act, Schedule-Y,
- Drug Controller General of India (DCGI),
- United States Food and Drug Administration (USFDA) Act
- Medicinal and Health Care Products Regulatory Agency (MHRA)

Monitoring and Audit :

Monitoring is usually conducted by research associates and involves overseeing the progress of a clinical trial and ensuring that it is conducted according to the protocol, GCP, Standard Operating Procedure (SOPs) and the regulatory requirements. Audit is conducted during monitoring to systematically and independently

examine the trial related documents and activities. It also checks whether the trial is going on as per the protocol, GCP, SOP and whether it is recorded and reported accurately or not.

Bioavailability and Bioequivalence studies :

The former is done to understand how much of the drug is available for action; the latter is conducted for new formulations and compares new formulations of any established drug with the existing formulation.

Biostatistics :

Biostatistics is required during the protocol designing to calculate the number of patients to be included in the trial (sample size), randomization, to review the data and for the final analysis of results by applying suitable statistical methods.

Clinical Research 50 Years Ago and Now :

Clinical research 50 years ago was investigator driven informal activity. Clinical research now is multi-disciplinary, multinational, multibillion dollar global business governed by many complex and interrelated regulations and guidelines. The players in this multi-disciplinary approach include, pharma companies, biotech companies, Central Diagnostic Laboratories, Clinical Research Training Institutes, sponsors, investigators, monitors, auditors and quality control personnel, bio statisticians, data management groups, regulatory affairs, reporting and documentation – medical writing and business development groups.

Preclinical Studies :

Preclinical studies are conducted on experimental animals for safety and efficacy. Efficacy studies are done by in-vitro assays using only animal tissues or cells or enzyme systems and in-vivo assays by experiments using whole animals.

Toxicity studies are done in animals and include acute toxicity studies – LD50, subacute toxicity studies, chronic toxicity studies, special toxicity studies – carcinogenicity, teratogenecity, genotoxicity and effects on fertility and reproduction.

Clinical Trial Phases :

- Phase-I- Clinical pharmacology, safety of new drugs
- Phase-II-safety and efficacy of new drug in patients, exploratory trial.
- Phase-III- multicentric confirmatory trial.
- Phase-IV-post- marketing surveillance

Future:

Pharmacovigilance :

Pharmacovigilance is phase 4 clinical trial and is intended to unmask uncommon side effects and new serendipitous beneficial effects and has to be multicentric ongoing process. It is also necessary for coordination between different centers and this is a hallmark of the program.

Pharmacoepidemiology (Populational Pharmacology) :

Pharmacoepidemiology develops the following types of study:

i. Studies of drug effectiveness on large populations monitored during long-term periods

ii. Studies of adverse drug reactions with quantification of risk

iii. Drug utilization studies to asses the characteristics of drug prescription or consumption according to evidence based data or official guidelines.

iv. These studies also investigate differences in drug prescriptions according to national or regional habits, characteristics of the patients and physicians, among others.

v. Finally, pharmacoepidemiology asks the most important question with respect to drugs: what are we doing in clinical practice with the basic properties of drugs?

Dictionary of Pharmacoepidemiology gives detailed study of this topic (8).

Drug Discovery :

Role of Modern Techniques in Drug Discovery:

Modern day advances in medicinal chemistry (combinatorial library synthesis), molecular biology (recombinant DNA-technology, transgenic animal models, knock-out mice and human gene sequencing), information technology (chemi-informatics and bio-informatics), screening sciences (ultra high throughput and automation miniaturization, nuclear magnetic resonance imaging and positron emission tomography – PET) have greatly impacted the pharmacologist's approach to studying and identifying novel targets. They have led to strengthening of forward and reverse pharmacology **(Figure 1)**. Through these tools the pharmacolgist places potential drugs at the disposal of the clinical pharmacologist at a much rapid pace- **Table 1 (9)**.

Drawbacks :

Although data can be generated at an extremely fast pace, the pace of analysis cannot cope up with the rate of generation of data.

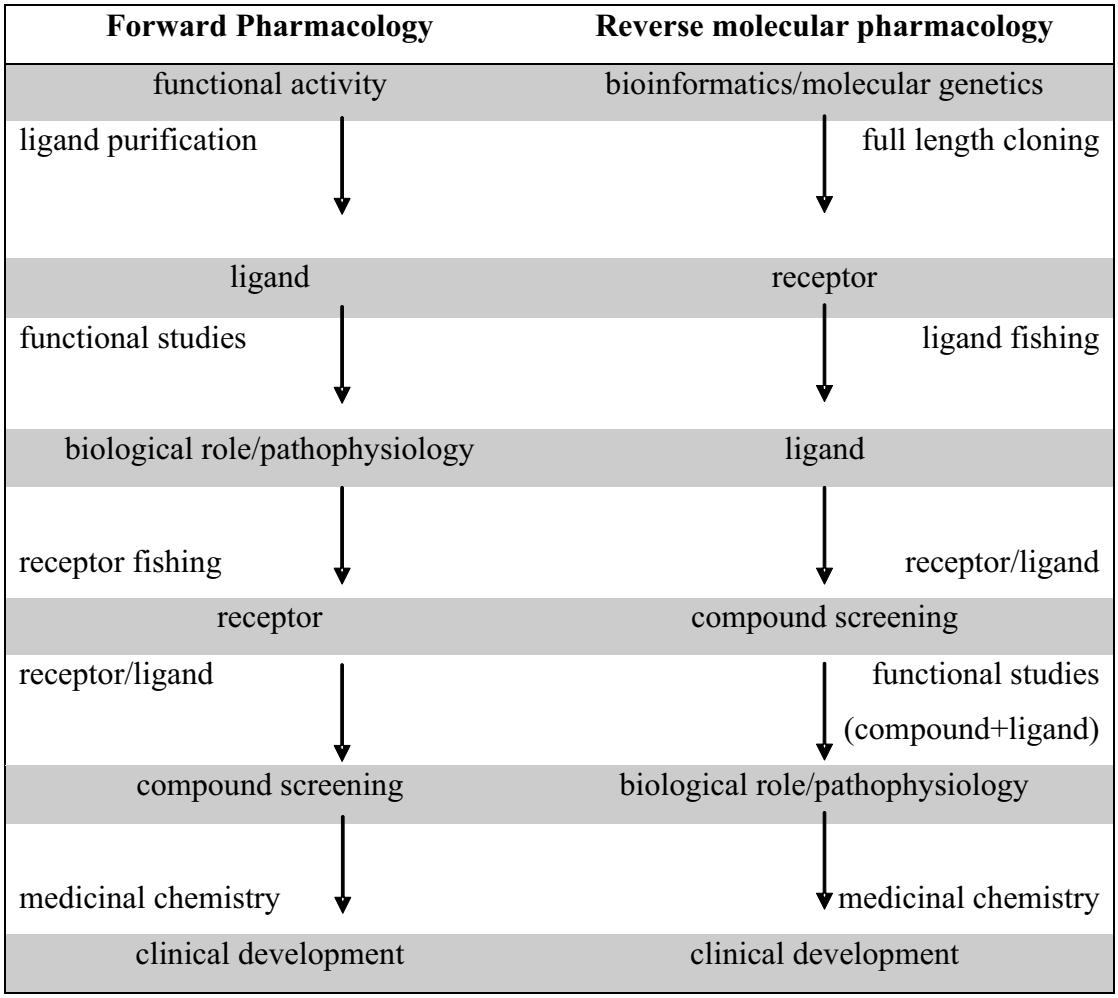

FIGURE 1 : Paradigm shift from forward pharmacology to reverse molecular pharmacological approaches for drug discovery.

Table 1 (9) **: Using reverse pharmacology to identify novel receptor – ligand interactions with potential cardiovascular applications***

Orphan receptor	Cognate ligand	Putative cardiovascular actions	Potential indications
UT (GPR14,SENR)	Urotensin-II	Vasoconstriction, cardiac inotropy	Hypertension, heart failure
Mas	Angiotensin-(1-7)	Vasorelaxation,anti-diuretic	Hypertension, heart failure
GPR66(TGR1,FM 3)	Neuromedin U	Vasoconstriction,cardiac inotropy	Hypertension, heart failure

APJ	Apelin	Vasoconstriction,cardiac inotropy	Hypertension, heart failure
PTH_2	TIP-39	Vasodilatation (renal)	Hypertension, heart failure
GPR10(GR3,UHR -1)	Prolactin releasing peptide	BP regulation (SNP phenotype)	Hypertension
OXR (HFGAN72)	Orexin A,B	BP regulation (i.c.v. administration)	Hypertension
GPR103(HLWAR 77)	RF-amides**	BP regulation (i.c.v. administration)	Hypertension
TA	Trace amines(tyramine)	Vasoconstriction	Hypertension
GPR38	Motilin	Vasodilatation(indirect)	Hypertension
GHS-R	Ghrelin	Vasodilatation	Heart failure
LGR7,8	Relaxin	Cardiac inotrope, vasodilatation	Heart failure
$CRF_{1/2}$	Urocortin	Vasodilatation	Heart failure
edg-1(LP_{B1})	Sphingosine-1-phosphate	EC differentiation, PLC and MAPK activation	Athero-thrombosis, angiogenesis
edg-2,4,7(LP_{A1-3})	Lysophosphatidic acid	DNA synthesis, PLC and MAPK activation	Athero-thrombosis.
G2A	Lysophosphatidylc holine	Macrophage function	Athero-thrombosis
$P2Y_{12}$ (SP1999)	ADP	Platelet aggregation	Thrombosis
HM74/-A	Nicotinic acid	Lipid lowering, anti-lipolytic	Atherosclerosis,dysli pidemia
GPR40	Medium-chain fatty acids	Insulin regulation	Syndrome X
AdipoR1,R2	Adiponectin	Fatty acid metabolism	Syndrome X

* Abbreviations: BP, blood pressure; EC, endothelial cell; i.c.v. intracerebroventricular; MAPK, mitogen-activated protein kinase; PLC, phospholipase C; SNP, single nucleotide polymorphism.

** RF-amide, a family of neuropeptides that are characterized by the Arg-Phe-amide motif. The prototypic isotype is Phe-Met-Arg-Phe-amide (FMRF-amide).

G-Protein Coupled Receptors (GPCRs):

The receptor theory of drug action traces its origin to the work of Ehrlich (10), Langley (11), and Clark (12), who are credited with being the originators of receptor theory. In general classical theory evolved chronologically through concepts

further developed by Stephenson, Ariens, Furchgott, Gaddum, and Schild (13).

The past 25 years have witnessed a renaissance in receptor theory. GPCRs are now seen as interactive, information processing units beyond switches for G proteins **(Figure 2)** (14).

It is estimated that some 45-60% of the drug entities in any modern day pharmacopoeia exert their actions either directly or indirectly via GPCRs. GPCR (14) is now considered the jewel of pharmacology.

Agonism, Inverse Agonism and Neutral Antagonism :

Several mutations result in enhanced constitutive activity leading to inherited human diseases such as adreno-corticotropin resistance and hypocalciuric hypercalcemia. Significant activity of these receptors can be detected in the absence of agonists.

Some drugs which had been considered as antagonists were subsequently found to reduce this activity. These drugs were assigned negative values of intrinsic activity – inverse

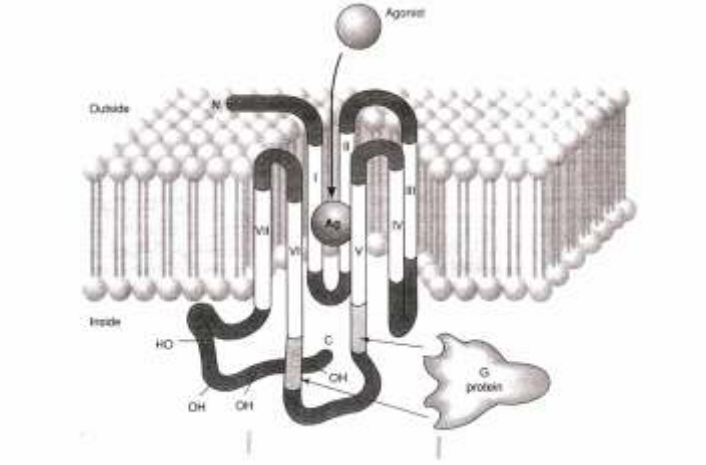

FIGURE 2 : T**ransmembrane topology of a typical serpentine receptor. :** *The receptor's amino (N) terminal is extra cellular (above the plane of the membrane), and its carboxyl (C) terminal intracellular. The terminals are connected by a polypeptide chain that traverses the plane of the membrane seven times. The hydrophobic transmembrane segments (light colour) are designated by Roman numerals (I-VII). The agonist (Ag) approaches the receptor from the extracellular fluid and binds to a site surrounded by the transmembrane regions of the receptor protein. G proteins (G) interact with cytoplasmic regions of the receptor, especially with portions of the third cytoplasmic loop between transmembrane regions V and VI. The receptor's cytoplasmic terminal tail contains numerous serine and threonine residues whose hydroxyl (-OH) groups can be phosphorylated. This phosphorylation may be associated with diminished receptor-G protein interaction.*

agonists. These drugs have different pharmacological potential from that of neutral antagonists. It is likely that inverse agonists will have a distinct effect on receptor regulation.

Full agonism and full inverse agonism constitute the extreme endpoints of a continuum that characterizes the functional properties of orthosteric ligands – conformational model. In this model the receptor constantly oscillates between active and inactive conformations. Agonists bind with high affinity to active conformation, which they stabilize whereas inverse agonists stabilize an inactive conformation. In fact 85% of competitive antagonists are inverse agonists.

Neutral antagonist is competing ligand that does not discriminate between active and inactive conformation. Thus the antagonists on their own do not favor any of the conformations of the receptor, but have the capacity to interfere with the other ligands.

Clearly, the use of inverse agonist that stabilizes the inactive conformation instead of neutral antagonist is preferable for the treatment of human diseases associated with GPCR – activating mutations. Several native receptors such as 5HT1A, 5HT2A and 5HT2C (5-hydroxytrectomy) receptors, melanocortin receptors and histamine H3 receptors show noticeable constitutive activities in physiological conditions suggesting that inverse agonists have great potential as inverse agonists

Orphan Receptors :

Of the 10000 – 30000 genes encoded by human genome, 600-1000 are estimated to be GPCRs, 30% are non sensory (olfactory and gustatory) 50% of this group are orphan receptors - presently the ligands are not defined for about 175 (15,16).

The pharmaceutical industry has recognized the power of genomics to provide it with new unique drug targets including over 100 GPCRs to a wide variety of human diseases and the historical sources of drugs that target GPCR –Table 1 (9). Thus, progress in fostering orphan GPCR by mining human genome has been impressive over the last two years and resulted in identification of numerous novel molecular targets **(Figure 3)** (17).

Methods to target orphan receptors : (18)

1. Mining of the human genome.
2. Advances in quantification of tissue and disease – specific gene expression
3. Receptor – ligand pairing to find cognate ligand for an orphan receptor is identified by screening panels of known GPCR ligands from tissue extracts

This is a potential area that can yield about 175 agonists & 175 antagonists (15,19) with about 80% as inverse

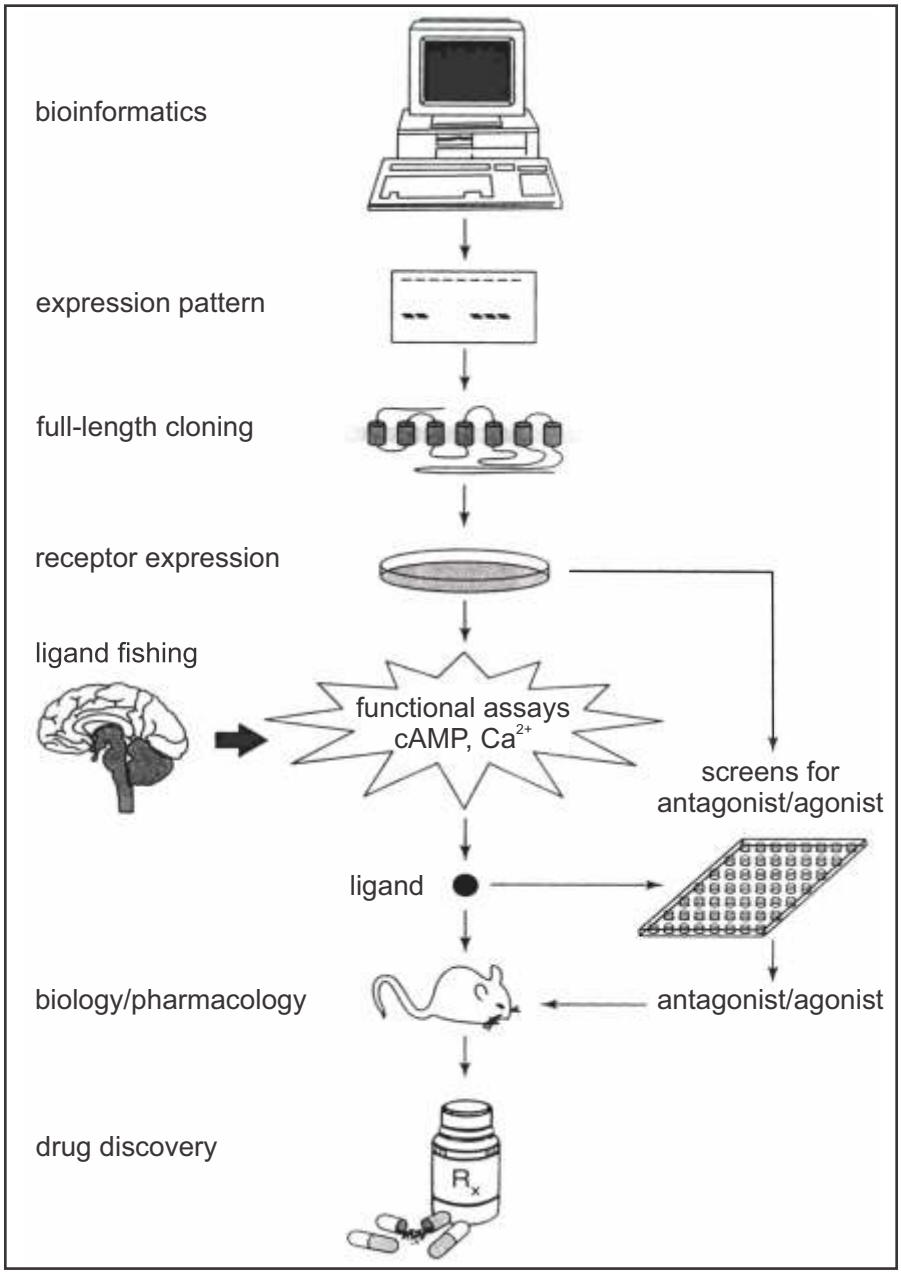

FIGURE 3 : Strategy for using orphan G protein-coupled receptors as targets for drug discovery.

agonists. Some of the competitive antagonists could also be inverse agonists

Drug Discovery through Receptor Conformation Theory:

The past decade has witnessed a dramatic increase in the identification of allosteric modulators of G-protein-coupled receptor (GPCR) activity. Concomitantly, several new perspectives and hypotheses regarding the way ligands regulate GPCR signaling have also emerged. The concepts of collateral efficacy and permissive agonism-antagonism intersect the field of allosteric GPCR modulation. Despite the challenges associated with detecting and quantifying the myriad of possible allosteric effects on GPCR activity, it is proposed that allosteric ligands offer the exciting prospect of engendering stimulus-bias in orthosteric ligand signaling, thus paving the way for not only receptor-selective but also signaling-pathway-selective therapies. Examples: Beta-blockers, Metabotropic glutamate receptor activation (20).

Personalized Therapy :

The field of cytochrome P450 pharmacogenetics has progressed rapidly during the past 25 years. All the major human drug-metabolizing P450 enzymes have been identified and cloned, and the major gene variants that cause inter-individual variability in drug response and are related to adverse drug reactions have been identified. This information now provides the basis for the use of predictive pharmacogenetics to yield drug therapies that are more efficient and safer. Today, we understand which drugs warrant dosing based on pharmacogenetics to improve drug treatment and increasing the efficacy of drugs and health in general. It is estimated that such personalized P450 gene-based treatment would be relevant for 10-20% of all drug therapy (21).

Computational Neuropharmacology :

A promising and innovative discipline named as computational neuropharmacology as summarized below has emerged. This discipline which combines state-of-the-art computational techniques established in basic neurosciences with traditional neuropharmacological methods could open new vistas in drug discovery that might help the development of new therapies for neurological and psychological disorder. The kinetic analysis of modulatory effect on the interaction of gamma amino butyric acid (GABA) transmitter and GABAA receptor which alters septo-hippocample rhythmic activity to screen and design putative subunit dependent selective anxiolytics (drugs to relieve anxiety). Secondly, the dynamic sub-cellular and network activity in epilepsy are described in the context of finding new targets and methods for seizure control (22).

Summary :

Clinical research has a very interesting history. It started about 50 years ago. Clinical research is an integral part of drug development.

Clinical pharmacology started in the 40's of the 20th century through the pioneering work of Harry Gold at Cornell University New York. Clinical research is an integral part of drug development. Unlike the past, today the process has gained a unique position due to the regulatory requirements and ethical guidelines available globally. Designing, conducting, monitoring, appropriate quality assurance and data management determine the success of the clinical research. Pharmacovigilance and pharmacoepidemiology are now gaining importance since without these no clinical pharmacology work can be complete.

Drug discovery can be hastened by a number of new techniques with reduction in cost. In addition reverse pharmacology approaches for drug discovery have come to occupy a special place. There is only one drawback i.e. the pace of analysis of data cannot cope up with the rate of generation of new data. 85% of the neutral antagonists act as inverse agonists. Inverse agonists have a distinct effect on receptor regulation as opposed to neutral antagonists.

Orphan receptors constitute about 50% of the GPCRs. It is estimated that now there are nearly 175 orphan receptors after 125 having been deorphanised. Targeting these orphan receptors can lead to the same number of ligands and antagonists thereof. Polymorphism of cytochrome P450 provides the basis for the use of predictive pharmacogenomics to yield drug therapies that are more efficient and safer. Such personalized P450 gene-based treatment would be relevant for 10-20% of all drug therapy. Allosteric conformation of receptors and in-silico technology could provide other inputs for drug discovery.

Orphan receptors, conformation of receptors, polymorphism of P450 and insilico discovery are new challenges that can yield new inputs for clinical research.

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My wife Saroj willingly let me attend to the write up of manuscript of this paper despite her not too good health.

This paper is dedicated to our two sons Deepak and Ashok who are no more with us.

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