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Review Article

Nuclear Magnetic Resonance Spectroscopy an Evolutionary Approach to Drug Design

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Abstract: Ever since Nuclear Magnetic Resonance (NMR) spectroscopy hit the analytical scene; its capabilities and applications continue to evolve. Originally designed as a way to verify the structure of relatively small compounds, the technology of NMR has exploded and become a valuable means for studying protein structure. NMR has proved to be a valuable tool in pharmaceutical research, as it has entered new arena of drug discovery and structural genomics. NMR can provide information on the three-dimensional structures of small molecules in solution, high-molecular-weight complexes, and the details of enzyme mechanisms that can be used to aid in drug design. In the present scenario, the availability of high magnetic fields; improved software, high resolution probes, and electronics; more versatile pulse programmers; and most importantly the development of 2D, 3D and 4D NMR, have revolutionized the field of drug discovery and development.

Keywords: NMR, HTS, NOE, Receptors.

Introduction

The process of preclinical drug discovery consists of two steps; finding of initial hits (binding ligands to a medicinal relevant target, usually a protein) and lead optimization.[1]

The general approach for hit finding and ligand optimization can be briefly summarized as follows: The target proteins of interest is over expressed and isotope labeled in bacteria. Subsequently, the three-dimensional structure (and often its molecular dynamics

as well) is determined via NMR spectroscopy. The next step is the identification of lead compounds in an NMR screening, preferably in a high-throughput manner. Several experiments have been developed for this purpose, saturation transfer difference spectroscopy (STD)[2-4], (reverse) nuclear overhauser effect (NOE) pumping[5-6] and transferred NOE[7-8], which can be used without isotope-labeled protein. During the lead evolution, the analysis of interactions between the initial hit and the protein leads to an optimized lead structure.

The process of drug development starts with a design or discovery phase. Discovery refers to the process of identifying a lead compound from a random screening program or from chance observation. Design implies a more directed and rational approach to the production of a lead compound. It can be "analogue-based" design or "receptor-based" design. Figure 1 shows the schematic illustration of the drug-development process, showing the contribution of NMR[9]. NMR approaches are classified into three classes:

- Small molecule NMR (studies of molecules of less than a few kilo daltons in molecular weight) – Contributes at the synthetic stage (as an analytical tool for verification of structure), and at the drug design stage, where it provides conformational information.
- Macromolecular NMR assists at design stage by providing structures of key protein targets.
- In-vivo NMR is valuable for investigating the effects of drugs on target organs.

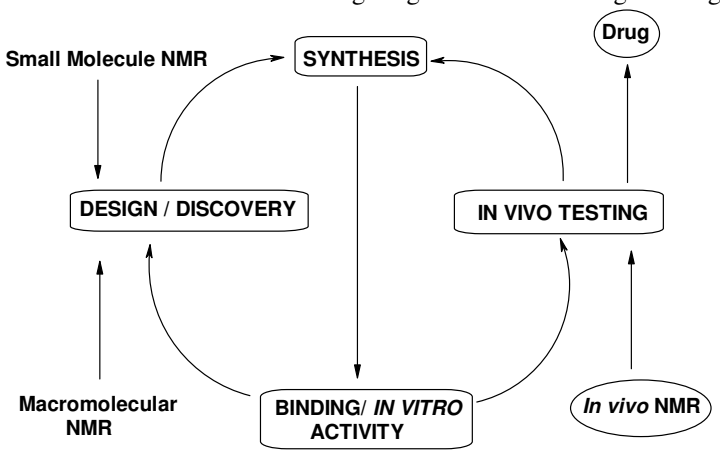

Figure 1. Schematic illustration of the drug development process, showing the contribution of NMR.

Structure based drug development

Structure based drug development is the use of atomic level three dimensional structural information about a demonstrated or putative macromolecular receptor to guide the design of a drug.[10] (Figure 2). After a lead compound is identified, by either serendipity, screening, or imputation based on related systems, and after the structure of its receptor is determined or inferred, a cyclic process can begin in which computer modeling, synthesis of new compounds, and biological testing can be used in some combination to improve binding to the receptor while addressing the concerns mentioned above relating to toxicity, bioavailability and metabolic stability[11-12].

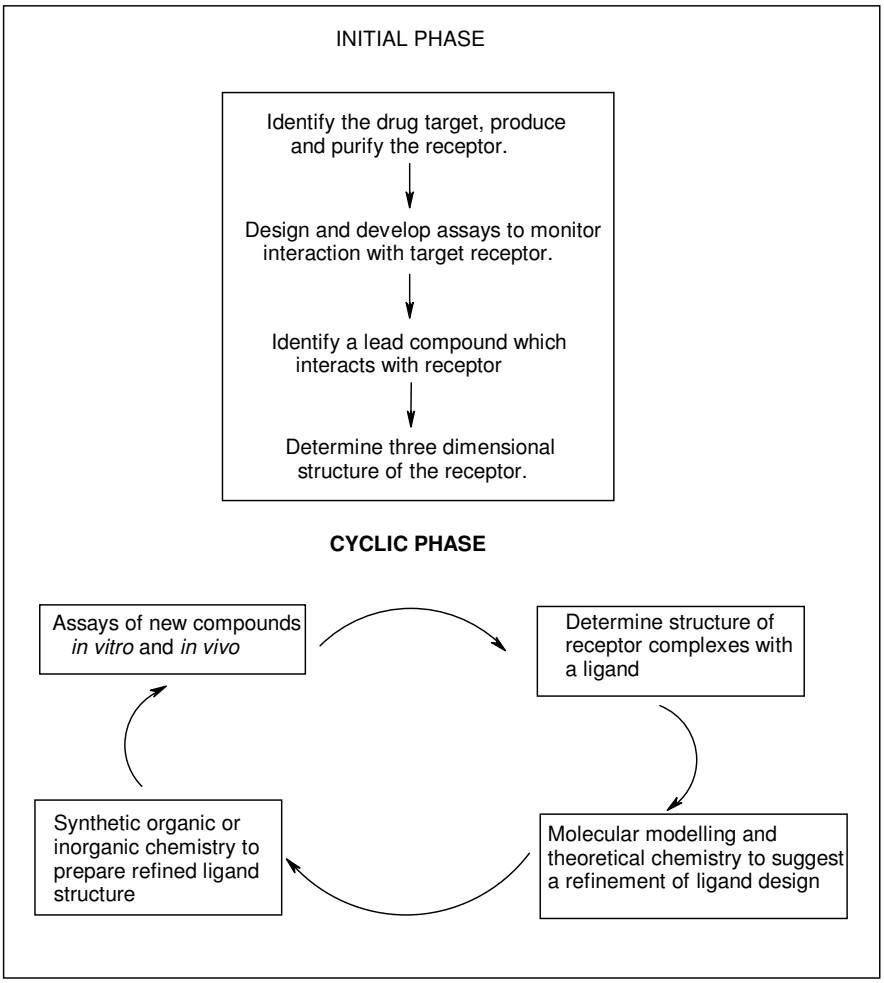

Figure 2. A representation of structure based drug design. An initial exploratory phase is followed by cycles of structure determination by X-ray crystallography, NMR or other methods, molecular modeling, synthetic chemistry, and biological evaluation.

Structures of drug-receptor complexes

A primary requirement for structure based drug development is knowledge about the three-dimensional nature of the drug receptor site. Various observations made by NMR can provide information about internuclear distances, dihedral angles, or the relative orientations of groups of nuclei. Each piece of information acts as a constraint on what the relative positions of some small set of atoms of the molecule under investigation can be; and these constraints enable the three-dimensional structure of the molecule to be defined; through computational methods and molecular modelling.

Utility of NMR for studying drug-receptor complexes

NMR can be used in titration experiments to demonstrate the stoichiometry of a ligand-receptor interaction and to provide a value for the equilibrium constant that characterizes

formation of the ligand receptor complex. NMR methods can also be used to determine the rate of dissociation of complexes. The basic suite of experiments used to define the three-dimensional structure of a receptor can also be applied to the determination of the structure (conformation) of a bound ligand[13].

Beyond three dimensional structural data, NMR experiments can potentially demonstrate the presence of multiple conformations of the ligand, the receptor, or the complex formed, as well as provides an estimate of the rate of interconversion of these conformations.

NMR Parameters

NMR spectra arise because of the absorption or emission of energy by nuclear spins. The parameters that characterize an NMR spectral result for a single conformation or chemical species include chemical shifts, spin-coupling constant and spin relaxation rates[14-16]. For studies of tertiary structure by NMR, change in shielding parameters for the spins of the molecule as a result of change in environment (resulting from binding of molecules to a protein or nucleic acid receptor) is utilized. In the absence of detectable changes in shielding parameter, binding may still be detectable through NMR experiments that measure nuclear spin relaxation times. Scalar coupling produces fine structure in an NMR spectrum and arises because the NMR behavior of a given spin can be influenced by the presence of other nuclear spins. Significance for studies of three-dimensional structures by NMR is the variation of three-bond coupling constants with the dihedral angle between them. Three bond carbon-carbon, carbon-proton, proton-proton, and nitrogen-proton coupling constants can convey dihedral angle information. Determination of three-bond coupling constants can be a powerful means of defining the conformation of a ligand in the bound and unbound state. Spin-Lattice Relaxation (T_1), Transverse Relaxation (R_2) are the parameters related to spin of the nuclei and relaxation mechanism of spins in NMR.

Determination of dissociation rates for drug-receptor complexes

NMR methods for estimating ligand binding or dissociation rates, require the presence of a chromophoric group within the ligand or receptor that is responsive to the binding process. The chromophore is a distinct and assignable NMR signal from the free ligand or receptor or the ligand receptor complex. The rate constants for dissociation of the complex (K_{BF}), can be defined; if there is a change in one or more of the NMR parameters

$$K_{BF} = K_{FB} \quad K_D$$

Where K_D is the dissociation constant of the complex.

K_{FB} = Diffusion limit ($K_{FB} \sim 10^{10}\ M^{-1}S^{-1}$).

The rates characteristics of a ligand receptor interaction are measured by saturation transfer, inversion transfer, two-dimensional exchange spectroscopy, line shape methods and spin echo methods.

Recent developments in the field of NMR

In the past few years, applying innovative approaches, much progress has been made for improving NMR as a powerful tool for industrial drug research. Different software packages have been developed for complete spectral assignment of all peaks. The introduction of pre-amplifier and radio frequency coils of the probe heads to about 20 K, enhances high resolution NMR and increases the signal to noise ratio about four fold. New hardware setups for data acquisition enable a “Just-in-time” preparation of the samples, including the transfer to the magnet, locking and shimming. Numerous new pulse sequences, improve previous technology in terms of sensitivity, resolution, selectivity, speed

and efficiency. Several "new" NMR parameters such as residual dipolar coupling (RDC)[17] Cross-correlated relaxation, "unusual" Non-Karplus-type coupling constants, or scalar couplings across hydrogen bonds provide more and more information about the interacting molecules.

Pulsed Fourier Transform NMR

Important applications include:

- Two dimensional NMR (2D-NMR) data are collected as a function of two independent time domains, followed by double fourier transformation.
- Insensitive Nucleus Enhancement by Polarization Transfer (INEPT) – especially applicable for sensitivity enhancement in the study of relatively insensitive multinuclei resonance such as ^{15}N, ^{17}O *etc*.
- Incredible Natural Abundance Double Quantum Transition Experiment (INADEQUATE). This is a unique value in providing detailed C-C bonding information by observing the natural abundance ^{13}C-^{13}C scalar (spin-spin) couplings.
- Multiple Quantum Technique (MQ-NMR) – For study of the orientation of molecules in space when coupling between proton spins produces a large number of lines in the NMR spectrum.

NOE Experiments

Several forms of NOE like steady state NOE, NOE build up, transient NOE, two-dimensional NOE, rotating frame NOES, transferred NOES have greatly improvised the study of ligand-receptor complexes[18]. Transferred NOES, in particular provide information about intranuclear distances within the drug-receptor complex by observing signals that largely correspond to those of the free drug.

Advantages of NMR

The unique opportunity to investigate molecules in different environments and of directly studying intermolecular interactions, as well as molecular dynamics, make NMR a unique method for structural study of drugs methods use non-crystalline samples, typically aqueous solutions. The solution conditions (pH, temperature, nature and concentration of buffers, concentration of the macro molecule) can be varied. NMR methods are unique in being able to detect the presence of significant amounts of two or more conformations [19-21] and to obtain quantitative information on dynamic processes, including the rates of conformational interconversion, rates of exchange of liable protons, particularly amide NH protons, and rates of segmental backbone and side-chain motions. The resolution of NMR structure depends on the number and distribution within the molecule of the constraints that led to the definition of the structure. NMR spectroscopy is currently, the only way to determine 3D structures in solution[22]. This makes NMR specially relevant for biological systems, in particular for determining structures of drugs[23] or other biological substrates like peptides, and structures of receptors and drug-receptor complexes.

Limitations of NMR

The conformation of a drug in solution is often highly dependent on its environment and is not necessarily the same as the bioactive conformation. In fact, for most molecules, several

conformations with only very small differences exist in solution and in these cases, very little can be learned from conformational studies to aid in the design of new molecules[24].

- Rapidly equilibrating structures cause averaged NMR parameters, which do not correspond to "mean" conformations.
- NMR Studies are currently restrained to very small receptors or receptor-substrate complexes. The upper limit of molecular size accessible to NMR spectroscopy is in the order of 30 Kda[25].
- NMR requires dissolved molecules that are tumbling fast and mainly isotropically in solution. This limitation, as well as that of size, is especially serious in studies of membrane bound complexes such as G-protein-coupled receptors[26].

Applications of NMR advanced techniques in drug designing and development

Following are a few examples of the use of NMR in drug designing:

- Structure-function studies of proteins, measuring changes in chemical shifts and hydrogen exchange rates serves to elucidate, the dynamics of ligand binding and determine critical residues for ligand-protein interactions[27].
- ^{15}N – and ^{1}H-2-D-Heteronuclear Single Quantum Correlation (2-D HSQC) NMR is used to test the binding of drugs to ^{15}N-labelled proteins.
- Using SAR by NMR, novel lead compounds are constructed that do not exist in corporate libraries and can not be found using conventional methods[28]. Also this technique avoids the cost and time associated with synthesizing large numbers of complex molecules.
- NMR approach in Enhancing High Throughout Screening (HTS) assays The simplistic nature of SAR by NMR makes it ideal for implementing as an assay-requiring only the target-protein and the test compound and the assay detects differences in the ligand-binding sites of the target protein.
- The Abbott group used NMR-based HTS assays to find novel, weak inhibitors to the Erm family of methyl transferases, which are ultimately responsible for the erythromycin resistance in bacteria.
- NMR approach has been used to design conformationally constrained opioid peptides[29-30] and peptide inhibitors of cholate uptake by hepatocytes.
- Conformation of ACTINOMYCIN-D in solution has been determined by COSY-NMR[31].
- Sulindac and sulindac sulfide with albumin, Fluorine NMR provides a means of exploring the kinetics of drug binding, as well as competition and cooperation in the interaction of other ligands with these sites[32].
- Other examples are: Hirudin-derived peptides bound to thrombin[33], calcheamicin with duplex DNA, immuno-suppressant – immunophilin complexes via structure determination, predicting the biochemical function and structure of novel gene products will become possible.

Genomic sequence information provides a means for identifying gene products involved in human disease or unique to specific pathogens. The next step of the genome project is to determine the structures and function of the corresponding proteins and there comes in the scenario NMR spectroscopy.

The Next Step: Structural Genomics

Proton NMR will eventually evolve to be at the forefront of structural genomics and drug discovery. By developing technology that connects gene sequence to protein function *viz.* structure determination. The development of new, pulse and multiple pulse NMR techniques (high resolution NMR) constitutes a major role in the study of molecular structure and conformation, kinetics and biochemical studies.

Recent developments in NMR

The development of new, pulse, and multiple pulse NMR techniques (high resolution NMR) constitutes a major role in the study of molecular structure and conformation, kinetics, peptide sequences and biochemical studies.

Conclusions

The unique abilities of NMR methods to provide information about the structures and conformations of ligand molecules, receptors and ligand receptor complexes, as well as the dynamic aspects of these species, expanding as technological advances make possible experiments at magnetic fields of 1000 MHZ or beyond. The use of *in vivo* NMR in the evaluation of drug performance and in elucidation of the basic mechanisms of drug action, still rather limited in its scope, offers a potentially important extra dimension for drug development efforts.

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