THE RELATIONSHIP BETWEEN NATURAL PRODUCTS AND SYNTHETIC CHEMISTRY IN THE DISCOVERY PROCESS

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Abstract

Natural product drugs have been discovered from traditional medicines, by screening for functional activity and more recently from mechanistic screens. Often, these natural products are suitable for use as medicines as they are, and do not need synthetic chemistry. In any case, the natural products are often too complex to allow synthetic chemists to explore their structure activity relationships. Thus and it is almost a pre-requisite for any complex natural products drug that not only is it potent, but to it also has the desired metabolism-distribution, and low toxicity. In contrast, chemistry is most powerful when a synthetically accessible lead molecule is found. This becomes the basis for a chemistry program to convert the lead into a drug with the desired metabolism, distribution and toxicity profile. Analogues are made which progressively approach the desired *in-vivo* activity in the disease model based on learning cycle of *in-vivo* and *in-vitro* testing. This process needs considerable pharmacological knowledge, the synthesis of 1-2 thousand compounds and 60-120 person years of chemistry and biological effort. Predictive pharmacological models of disease are essential to the synthetic chemist, otherwise a diagnosis about whether an analogue is closer or further away from being a drug cannot be made. Therefore, the extraordinarily rapid development of the cyclo-oxygenase 2 inhibitors was enabled, by a long history of anti-inflammatory agents and predictive animal models of disease. This history stretches back to the natural product, salicin. The interesting fact is that nearly all the major areas of pharmacology, from cardiovascular to anti-depressants, trace their origins back to natural products. This process is still

occurring. In the new areas of pharmacology such as anti-cancer, immunomodulators and now diabetes, natural products are the pathfinder compounds. Therefore, rather than competing with synthetic chemistry, natural products are best used at the cutting edge of drug discovery where the new pharmacological insights they provide, will speed up the process of developing improved medicines using rational design.

Introduction

The pharmaceutical industry is under enormous pressure to decrease the 12-15 years currently needed to discover and develop new medicines. The technology needed to do this is seen to be in the combination genomics and high-throughput mechanistic based screening. The starting point for the discovery of new medicines is now at the gene level. This is the final movement of a technology which is perceived as moving the basis of drug discovery from functional activity in whole animals to cells, then proteins and now into genes. In this pursuit of technology, what has been forgotten is that the most useful mechanisms have been identified as a result of the understanding of the mechanism of action of certain pathfinder chemicals[1] which were originally discovered by their functional activity. Their functional activity provided the pharmacologist with a means to develop and test in-vivo animal models of disease, medicinal chemists with a baseline for improvement and allowed biochemists to determine the pathways and molecular basis of the physiological effect. Thus the original pathfinder drugs put in place the technology needed to development the next generation of drugs using a reverse of the process where the movement is from mechanism to functional activity.

When a functionally active chemical has been found which links a particular enzyme or receptor to a disease state, there are fast and inexpensive ways to find

1I am indebted to Dr. M. Legg (Zeneca Agriculture) for introducing me to the term 'Pathfinder' to succinctly describe the role of these biologically active chemicals in the development of new drugs and pesticides.

alternative low molecular weight chemicals which interfere with the in-vitro activity of the target enzyme or receptor protein. These 'hits' must then be moved from mechanist in-vitro activity to functional in-vivo activity in animal models of disease, i.e. be made functionally active. The challenge for the chemist is to move a chemical with *in-vitro* activity to one with *in-vivo* activity. The chemical must reach a sufficient concentration to cause the selective inhibition of the target protein *in-situ*. Physical diffusion barriers to chemical adsorption, active chemical defence mechanisms that metabolise and excrete 'foreign' chemicals and the micro-environment of the protein, all conspire against this goal being achieved. However, with considerable chemistry and biology effort, (costing in the region of $20 million) it is possible to achieve this goal with a high level of certainty. The cost of the effort required to do this medicinal chemistry program means that this activity, not the production of mechanistic hits, is currently the bottleneck in drug discovery (1).

It is also possible to hypothesise a link between an enzyme or receptor and a disease state. The pharmacologist establishes a theoretical causal chain of logic between a target protein and the disease. However well researched and constructed the hypothesised link may be, the complexity of the biochemistry network often brings these linear chains of reason to no account. In my experience, only a small number of 'pure' mechanism based targets actually become the subject of major pre-clinical medicinal chemistry programs and even when they do, they have a much larger chance of failure than those which are following in the wake of a pathfinder chemical. The reasons for failure, the lack of chemists to deliver the right compounds, versus the pharmacologist chain of reasoning, are the subject of hot debate and considerable acrimony in the hallways of pharmaceutical research companies!

In this paper I will argue that the real importance of natural products is in their role as functionally active pathfinder chemicals in the drug discovery process. These

pathfinders validate the learning cycle between biologists and chemists, which is used to turn small molecular weight synthetic chemical leads into clinical trial candidates. I will argue that by putting them in direct competition with high-throughput mechanistic screens of synthetic chemical libraries we are devaluing natural products. Instead, I will suggest that natural products would be put to better use by pursuing their ability to show activity in low throughput functional screens in research programs directed at the cutting edge of pharmacology. In this way, their value as pathfinders into new areas of pharmacology can be fully exploited.

Natural Products and the Elucidation of Mechanism

With the enormous advances in biochemistry seen in the latter half of the twentieth century, it has been possible to pin down the mechanism of medicines to activity against a single enzyme or receptor. Once this was achieved, the way was opened to develop of improved or novel drugs. For example, the ability of certain snake venoms to cause a drop in blood pressure was shown to be caused by the inhibition of angiotensin converting enzyme (ACE). For patients suffering from high blood pressure, the modulation of this enzyme could be a useful therapy, and this pharmacological reasoning, combined with excellent medicinal chemistry lead to one of the great milestones in medicinal chemistry, the development of the ACE inhibitors. By understanding the mode of action of penicillin by its inhibition of a very specific protease unique to bacterial cell wall formation, other enzymes unique to an infectius organism became the targets of other anti-infective programs. Thus when a protease specific for AIDS replication was identified, chemists were confident of their ability to develop protease inhibitors and pharmacologists that this would be a good target for anti-viral drug development. Finally, the ability of medicinal chemists to develop chemicals which selectively inhibit sub-classes of the same receptor was spear-headed by the development of the anti-histamines, one class (H1) for motion sickness and the other (H2) for stomach ulcers. Thus when two forms of the target

enzyme for the aspirin based NSAIDS (Cyclooxygenase COX I & II) were discovered and it was recognised that one of the iso-enzymes (COXII) was absent from the gut, it was possible to rapidly development an 'aspirin' which did not cause bleeding in the stomach. In all of these programs, functionally active natural products acted as pathfinders in understanding the pharmacology and in developing the subsequent medicinal chemistry programs which yeilded greatly excellent synthetic chemical drugs(2).

I have previously speculated that natural products have functional activity not only because they are part of the natural chemical defences and biological signaling functions, but that they have also been pre-selected to survive the biological environment and are thus bio-available molecules (3). The combination of chemical defences, divergent evolution of chemical receptors, and general biological availability, make natural products an especially rich source of functionally active leads.

In order to understand the importance of functionally active leads in the discovery process, I would like to describe a model for drug development that I have used in my work as a consultant. It is based on the need to establish a learning cycle between biologists and chemists, which progressively moves a chemical lead into a drug that is a candidate for clinical trials. This process is really the one on which technical innovations should be focused, because it is the rate-limiting step in the discovery process.

Medicinal Chemistry-Biology Learning Cycle

The pre-clinical drug development process has as its end a drug candidate that is suitable for testing in human clinical trials. To achieve this, the candidate must pass pre-clinical toxicology testing, have its adsorption, distribution metabolism and

excretion understood in suitable animal models, and have good scientific evidence that it will provide medicinally beneficial effects. The latter normally requires showing efficacy in animal models of the disease. The early phase discovery process has usually produced a chemical lead that falls far short of these desired properties. The major cost and rate-limiting step is the process that changes an early lead into a chemical that is suitable for testing as a drug candidate. In this process, cycles of chemical modification based on the results of biological testing are used to move the lead chemical closer to the desired biological properties. The medicinal chemist takes a lead role in deciding which chemical modifications will be attempted in order to move the chemical towards a drug candidate. This is a complex judgement based on prior knowledge derived from biological data from testing earlier analogues in the lead series, on the results of other chemists efforts to improve the properties of the lead, on personal experience from other projects and general knowledge and inputs from theoretical and practical chemistry and biology. The process is distinct from random screening because it has an actual drug as its end for which it uses low throughput *in-vivo* tests which require a highly skilled diagnosis to judge the quality of the test chemical.

The quality of the biological data is paramount in the decision about whether a given chemical is closer or further away from the desired properties. This high level diagnosis is made from data derived from numerous sources. The confidence level is very much determined by the extent to which the chemistry and mechanism of action of the drug has been previously described. Since a disease model is only proven when it has been shown to predict a chemical activity in the human, it is only validated retrospectively. A novel therapy will require untested animal models of disease and so the confidence in the data derived from the model is substantially reduced. Similarly new chemical entities have unknown metabolites and side effects are much more problematic in the prediction of adverse side effects. Therefore a chemist is conservative and more likely to work with classes of compounds which have

previously been shown to be safe.

Although the chemistry is far from deterministic, the process is rationally guided by high level diagnosis and judgement where experience guides the hand of chance to evolve chemicals into the desired property space by a series of incremental improvements. On top of this is the competition from other pharmaceutical companies, patents to be avoided or circumvented. This tends to over-shadow the technical challenges in producing new chemicals with the required structure and purity in sufficient scale for testing in animals. By synthesising in the region of 1,000-2,000 chemicals and testing a large number in advanced models of disease and toxicity, the process has a one in three chance of producing a chemical with the required safety and efficacy to test in humans. The medicinal chemistry program is complete and the proof of the pharmacology which originally drove the program now in the hands of the clinicians. Only one in three of the programs that reach this stage will succeed in producing a medicine for human use!

Mechanistic Lead Discovery and Natural Products.

Chemists have a number of avenues to derive the lead that is to become part of the medicinal chemistry program. The most favoured is one which has been designed by the chemist using a rationale based on the known substrate or hormone which is the substrate of an enzyme or effector of a receptor. Protease inhibitors are particular favourites for this approach where the substrate is specific for the enzyme. By substituting non-hydrolysable bonds, it is relatively easy to produce a specific enzyme inhibitor. Computational chemistry can also be used to assist in the design of the molecule, but it is often more efficient to simple change substituents on the lead to empirically find how to make the binding more specific and tight. With protease as target, selective inhibitors active at nM concentration can be rapidly produced. Combinatorial chemistry (4) can often be used to great effect where in-vitro tests of

efficacy are available. However, the issue of metabolism, distribution and toxicity, still means that many hundreds of analogues will need to be made in order to hone in on the correct pharmacological profile. One draw back with this rational approach is that the knowledge of the targets substrate is known to competitors, therefore a rational approach is likely to lead to the same classes of compounds being developed. As a result, there is a high probability of patent infringement, as a number of chemistry groups from pharmaceutical companies all pursue the same chemistry. Rational people all arrive at the same end point!

In many cases, the substrate or effector may be of a more complex nature making chemistry time consuming and expensive. For example, carbohydrates and lipids are particularly difficult to work with. In this case, random screening becomes a powerful tool to finding new leads. It is also a way of breaking free from the rational approach into areas of chemistry which would not normally be pursued. For screening to be successful, it is necessary to collect a large and diverse collection of chemicals, and to have a rapid method for looking at activity. Given a library of 1,000,000 or so diverse chemicals, it is relatively easy to find several classes of chemicals that are potential leads for a chemistry program and show selective sub micromolar inhibition of small molecule-protein interactions. It is likely that a component of the chemical collection used for random screening will be natural products. These are normally screened as extracts, in which case purification is needed in order to identify the structure of the chemical. The use of hplc and mass spec has greatly improved the ability to identify the actual chemical entity responsible for the hit. The other approach is to screen pre-purified natural products. If natural product extracts are made to go in a head-to-head race with a chemical library, then their structure activity relationships emerge several months after the screens are completed. There is also the high probability that it will be difficult to obtain materials for more advanced testing, and that the chemical structure will be not be easily accessible for making analogues. In those cases where rational approach is also being

used, natural products tend to come in 3rd place after synthetic chemistry hits in the race for chemistry support.

When competing with a successful rational design program, any chemical or natural product screening hits are going to need some special characteristics in order to have chemistry effort diverted to pursuing the hit. One of the most successful ways of making a random screening hit stand out above the rest is to show some activity in a functional test using an animal model or cell based assay. In order to do this; it is useful to have at least mg amounts on hand. Resynthesis of chemical hits takes time and effort, and chemical support for non-validated hits is limiting. Most chemists are working on rate limiting and resource intensive medicinal chemistry programs. Therefore the reality is that where competition exists for the application of rational design, hits from a random screen will be in second place for chemistry support, and their only chance for redemption is to show significantly improved functional activity and toxicity against that currently achieved by the rationally designed lead. Natural products do have a particularly useful role in this area because they often have the ability to show functional activity without further chemistry. Therefore, one approach is to only isolate and determine the structure of natural products when they have show functional activity in animal models of disease.

An increasing number of targets are emerging from genomics and proteonics (5). Thus the effort will be to develop ultra-high throughput screens able to keep up with the numbers of emerging targets. In these screens, the extra time needed to work with natural products makes them even less competitive. Because medicinal chemistry is rate limiting, it is argued that these screening hits will be used to test the validity of the target in a specific disease process. This requires a functionally active chemical to be found directly by screening but this can only be recognised if expensive and low-throughput models of disease are available. What is more likely to happen is that leads which do not have a detailed pharmacology to support their role in disease

processes will not be followed-up. Therefore, I believe that proteonics and ultra-highthoughput screens will do little to address the need to reduce the cost and timelines of drug development.

The Requisite use of Natural Products

Against these rather depressing trends, what is the use for natural products? Natural products have made a massive input with over 50% of prescription drugs being based on them and are continuing to contribute to the development of new drugs with 44% of New Chemical Entities registered by the FDA being natural products or natural product derived (6). Despite a massive technology focus on mechanism, natural products are still at the cutting edge of pharmacology producing functionally active molecules for cancer therapy, (e.g. taxol), immuno-suppressants (cyclosporin) and more recently in the enormously technically demanding production of small molecule mimics of insulin (7). This past and recent history says that instead of using natural products in we should ensure that they are being used as pathfinders in functional screens directed at advancing pharmacology.

Natural products also have an important role in random screening, but should only be pursued if they demonstrate functional activity. Only by becoming drugs, or advanced drug leads can they bypass the expensive synthetic chemistry development route used for chemical leads. This requires the advanced disease models and the commitment to produce sufficient material to allow functional testing. I would reserve my precious natural product resource to screens that demonstrate this capability. In the absence of functional activity, natural products will not compete with rational design or synthetic chemicals emerging from random screenings and their pursuit will be a waste of effort. With so many potential mechanistic targets for each disease, it is also a waste to test a functionally active natural product against only one target. Thus extracts which have been selected based on ethnobotanical

information need to go into functional but not mechanism based screens. Great care needs to be set up to make sure that functional screens do not rediscovering the same activity, but the rationale that 'novel chemistry means novel mechanism of action' - a role served admirably by natural products- is the key to finding new leads by this means. Finally, natural products should be used in cutting edge of pharmacology research where the chemistry is not available, e.g. in the search for small molecular weight mimetics of proteins.

Conclusion

After considering the current needs of drug discovery and the contribution made by natural products, I believe that natural products should be returned to their proven role as pathfinders for pharmacologist and medicinal chemists. They do this by showing in vivo functional activity in models of disease. When they are treated the as 'just another chemical in our vast libraries used for mechanism based screening' not only are they non-competitive against synthetic chemicals and rational design, but they are also being seriously undervalued. As pathfinders, they address the current medicinal chemistry bottleneck in drug discovery, which is not finding leads, but is the enormous cost of progressing leads through medicinal chemistry programs which turn them into candidates for clinical trials.

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