Science, art and drug discovery: a personal perspective

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ABSTRACT

The research programme that started in 1985 led to the approval of Sildenafil (Viagra®), in 1998, as the first oral treatment for male erectile dysfunction. The initial project objective was the design and synthesis of novel inhibitors of phosphodiesterase that would increase tissue levels of cGMP, and that could be beneficial for the treatment of cardiovascular conditions. Starting from zaprinast, a weak phosphodiesterase inhibitor, computer modelling guided rational medicinal chemistry to achieve significant increases in potency and selectivity for the 5-isoenzyme within a novel series of pyrazolopyrimidinones. Optimization of structure–activity relationships and pharmacokinetic properties led to sildenafil, which proved essentially devoid of cardiovascular activity in clinical trials. However, the emerging role of nitric oxide and cGMP in controlling blood flow in the penis suggested that sildenafil would have a beneficial effect on erectile function. This hypothesis was confirmed by extensive clinical trials in nearly 5000 patients and the Food and Drug Administration approved sildenafil in March 1998 for male erectile dysfunction. Sildenafil is now available in over 100 countries and more than 150 million tablets have been dispensed worldwide. The sildenafil research programme reflects a traditional approach to drug discovery, but pressures to improve productivity have prompted major investments in genome sciences and new technologies. The impact of these initiatives on the drug discovery paradigm will be discussed, particularly with respect to shortening time scales between identifying gene sequences and submitting innovative products for regulatory approval.

INTRODUCTION

The pharmaceutical industry invests between 10 and 20% of annual sales revenues in research and development, which is far higher than in other research-based sectors. However, transforming an idea into a marketed medicine can take between 10 and 20 years, and costs have risen to over $500 million, a 10-fold increase since 1976. Moreover, pharmaceutical research is associated with significant risk, since many drug candidates fail during safety and clinical evaluation, while only one in three marketed agents yields a return on investment. Consequently, the pharmaceutical industry is under intense pressure to increase productivity, and drug discovery is experiencing a paradigm shift, whereby the explosion in genome sciences and new technologies is being harnessed to produce innovative therapies within shorter time scales. While it is expected that new targets and screening hits will be identified at unprecedented rates, innovative science is still rate limiting in transforming leads into drug candidates. Moreover, since the structures of many receptors and enzymes are unknown, successful drug discovery teams must blend scientific data with experience and intuition to develop robust
hypotheses for drug–receptor interactions that guide rational design and synthesis. These challenges will be illustrated with a personal account of the research programme that led to sildenafil (Viagra®; for a previous account see [1]), followed by some thoughts on how drug discovery might evolve over the next decade.

THE ROAD TO SILDENAFIL

Atrial natriuretic factor (ANF), cGMP and phosphodiesterase (PDE)
The research programme that led to the discovery of sildenafil originated from our interest in ANF, an endogenous peptide with vasodilator and natriuretic properties. ANF exerts its physiological roles by stimulating guanylate cyclase to increase tissue levels of cGMP, although this second messenger is degraded rapidly by a specific PDE. In 1985, we suggested that compounds that blocked the destructive action of this PDE would preserve tissue levels of cGMP and hence would potentiate the vasodilator and natriuretic effects of ANF. We expected these PDE inhibitors to show potential for the treatment of hypertension and other cardiovascular indications [2].

Rational design of PDE5 inhibitors
In 1985, little was known about the physiological role of cGMP (Figure 1, structure 1) or the specific PDE (later characterized as PDE5) to support our theory, and there were no potent, selective PDE5 inhibitors available for laboratory studies. However, a search of the literature uncovered zaprinast (Figure 1, structure 2), a weak (IC_{50} 2.0 μM), but non-selective, inhibitor of the enzyme that showed vasodilator activity in vitro, and lowered blood pressure in the anaesthetized dog. However, the compound possessed additional pharmacological properties, and had been progressed to the clinic as an anti-allergy agent.

Despite such a broad profile, zaprinast provided a starting point for a rational medicinal chemistry programme aimed at increasing both potency and selectivity for PDE5. First, comparison of the electronic distribution in zaprinast and cGMP, the physiological substrate for PDE, showed that the dipole moments of the triazolo-pyrimidinone and guanine ring systems were similar in both magnitude and direction (9.5 and 7.4 respectively). This suggested that both heterocycles could be recognized in a similar manner within the PDE active site, and that modification of the parent triazolo-pyrimidinone could lead to enhanced enzyme affinity. Secondly, computational studies confirmed that cGMP preferred to adopt a syn conformation and that there was scope within the zaprinast skeleton to introduce isosteric replacements for the cyclic phosphate moiety. Finally, the X-ray structure of zaprinast showed an intramolecular hydrogen bond between the aryl oxygen and pyrimidinone hydrogen atoms that maintained coplanarity, and this part of the molecule was therefore left essentially unchanged.

These rationales were pursued by the chemistry team, who initially probed a series of heterocyclic replacements for the parent ring system in zaprinast. After studying several alternatives, a pyrazolopyrimidinone (Figure 1, structure 3) proved to be superior, with a 10-fold increase in potency against PDE5 (IC_{50} 0.3 μM), and reduced activity against other members of this extended enzyme family [3]. The next step was to exploit compound (3) as a template to probe the spatial region occupied by the cyclic phosphate moiety in the natural substrate. Thus it was proposed that extension of the 3-methyl group would be beneficial, as would introduction of a tetrahedral sulphonamide as an isosteric alternative for the natural cyclic phosphate (Figure 1, structure 1).

These ideas were brought together in 1989 with the synthesis of UK 92480 (sildenafil) (Figure 1, structure 4), which provided a 100-fold increase in PDE5 inhibitory activity (IC_{50} 3.5 nM) over zaprinast, together with unprecedented selectivity over other PDE isoenzymes [4].

Figure 1 Structures of cGMP (1), zaprinast (2), a pyrazolopyrimidinone template (3) and sildenafil (4)
Me, methyl; Et, ethyl; Pr, propyl. See the text for further details.
Sildenafil in the clinic: an initial setback
Despite this pharmacological profile, we were disappointed when the initial clinical performance of sildenafil in patients with coronary heart disease fell short of our expectations. However, rather than lose heart, it was decided to push the compound to its limit with a 10-day, multiple-dose study in volunteers, where it was expected that some clues to the clinical potential of sildenafil would emerge. In the event, a variety of effects were observed, including head and muscle ache, indigestion, and some increase in erectile function.

Nitric oxide (NO), PDE and sildenafil: a mechanistic rationale for erectile dysfunction (ED)
This final observation was most intriguing, and we decided to investigate the pharmacological basis for the effect of sildenafil on erectile function, and to determine the potential for use in patients suffering from ED (impotence). These clinical and laboratory studies with sildenafil coincided with a burgeoning interest in the physiological role of NO, an endogenous compound that was nominated 'Molecule of the Year' by Science in 1992. More recently, three of the pioneers of NO research were awarded a Nobel Prize for Physiology or Medicine in 1998. NO has been shown to produce a range of pharmacological effects through stimulation of guanylate cyclase but, by 1992, a strong case was emerging for a specific role in controlling erectile function. For example, NO mediates relaxation of human corpus cavernosum smooth muscle [6,7], which can be impaired in patients suffering from ED.

These observations, and in-house studies, were brought together within a unified hypothesis (Figure 2) whereby, on sexual stimulation, NO is released from non-adrenergic, non-cholinergic nerve endings in the penis, where it stimulates guanylate cyclase to increase cGMP levels in the corpus cavernosum. This second messenger then initiates the smooth muscle relaxation that increases blood flow to the penis and leads to an erection, which is subsequently terminated when cGMP is degraded by PDE5.

It was immediately realized that, by blocking the action of PDE5, sildenafil would potentiate the natural activity of NO, and would improve erectile function in conditions where NO release or smooth muscle relaxation are impaired in the penis. Importantly, sildenafil would only be effective in response to the neurogenic activity that accompanies sexual stimulation, since the compound has little or no direct relaxant effect on contracted human corpus cavernosum [8].

Sildenafil, a major medical advance for the treatment of ED
In parallel with our laboratory studies, project clinicians carefully examined the patient population suffering from ED, which is a common and serious condition [9] that may afflict up to 30 million men in the U.S.A. alone. In addition, it was apparent that there were no defined protocols for the study of an orally administered agent for this indication. Accordingly, the innovative step of developing the International Index of Erectile Function was taken to provide a detailed questionnaire that helps patients and partners record the extent of ED, and the impact of drug treatment [10]. Clearly, utility in patients would require a response after acute administration of sildenafil, rather than the multi-dose regimen used in the 10-day volunteer study.

Figure 2  Mechanistic pathways for NO and sildenafil in erectile function
With a clear understanding of the mechanism of action, and with clinical methodology in place, Phase 2 trials with sildenafil in patients suffering from ED started in 1994. Towards the end of 1997, a database on nearly 5000 patients was submitted to the Food and Drugs Administration (FDA), and approval for use in ED was granted on March 27, 1998, after fast-track review. These extensive clinical studies showed that sildenafil produced a dose-related effect in patients, with improved erectile function of 65, 79 and 88% reported after single administration of 10, 25 and 50 mg respectively. Moreover, the response to sildenafil was similar whether the underlying cause of ED was diagnosed as organic (68%), psychogenic (84%) or mixed (77%). Overall, toleration is excellent, side effects such as headache and indigestion tend to be mild [11], and the low discontinuation rate (2.5%) is essentially the same as for placebo (2.3%). At the 1999 meeting of the American Urology Society, it was reported that 93% of patients who participated in a Viagra* study remained satisfied with the drug after 2 years. A small number of patients (3%) experienced some transient visual disturbance that probably resulted from weak interaction of sildenafil (IC_{50} 34–38 nM) with PDE6, the predominant PDE isomorph in the retina [4].

Sildenafil can be used for ED patients with a range of co-existing conditions, such as diabetes, hypertension, spinal cord injury and following radical prostatectomy, and can be co-administered with a variety of common medications. When used as directed, the incidence of cardiovascular effects has been similar for patients taking sildenafil and for a population with the same age and risk profile, both during the clinical development phase and in more extensive post-approval surveillance [12]. However, the use of sildenafil is contra-indicated in patients who use regular, or intermittent, nitrates in any form [13].

Sildenafil is a major medical and scientific advance that provides the first effective treatment for ED that can be administered orally, and has a highly favourable safety profile. The product is now available in over 100 countries, some 200000 prescriptions are written each week and more than 150 million tablets have been dispensed worldwide. Millions of patients suffering from ED have benefitted from effective treatment with sildenafil, and this distressing condition is now much better understood, and is discussed more openly, than previously. However, it is sobering to recall that the sildenafil discovery programme started in 1985, and that the innovative and dedicated efforts of some 1500 Pfizer staff, and many external colleagues, were required over 13 years to bring the product to approval.

**DRUG DISCOVERY IN THE 21st CENTURY**

The research programme that led to sildenafil provides an example of rational compound design, sequential medicinal chemistry and expert pharmacological evaluation that has characterized pharmaceutical research for the past decades, and may continue to serve as a model for the future. However, time scales for such a classical approach are lengthy, failure rates are high, and the escalating costs of drug discovery and development may become unsustainable. Even so, most major pharmaceutical companies have set ambitious targets of doubling, or trebling, productivity during this decade, and are making major investments, internally and externally, in genome sciences and new technologies. These initiatives are expected to revolutionize the drug discovery paradigm (Figure 3) by shortening time scales between identifying a gene sequence of interest and submitting a new product for regulatory approval.

Current bottlenecks are assigning function to novel genes and proteins, and choosing the most relevant biological targets for in-depth pursuit. Moreover, while high-throughput screening initiatives often identify structures with binding affinities in the 10 μM region, significant chemistry effort is required to transform these weak hits into lead series that confirm that the desired pharmacological profile is attainable. When all of the target biological properties are expressed in a single molecule, a candidate is nominated for pre-clinical development, although the chances of reaching approval are only around 1 in 12. Clearly, there are many opportunities for improving productivity throughout this drug discovery paradigm (Figure 3), but there is considerable debate over which intervention points will provide the greatest advantage.

There have been significant investments in positional cloning, where specific gene defects have been correlated with some diseases, but few tractable drug targets have emerged. Searching for genes associated with disease may be more fruitful, since single-nucleotide polymorphism analysis has been claimed to locate regions of DNA associated with migraine, Type II diabetes and psoriasis. However, alternative estimates of the number of single-nucleotide polymorphisms required to find genes related to common diseases have risen to between 600 000 and 1 million, which has significant cost and time implications.

Dramatic improvements in DNA sequencing techniques have brought completion of the human genome in sight, and there are expectations that this will usher in a new era of biological understanding and drug discovery opportunities. Although deposition of the complete
human genome sequence in public databases will be an outstanding scientific achievement, and emotional landmark, these data have little intrinsic value without functional and biological correlates. A rough analogy might be to compare collecting the names of everyone living in Framingham, without documenting their roles and relationships, with the richness of the database from the Framingham Heart Study.

Delineating the function of genes and their encoded proteins is a significant challenge, and there are major initiatives in bioinformatics to attempt to keep pace with data mountains that seem to increase exponentially. The dream of translating a gene sequence into a three-dimensional protein structure in silico is still some way off, and IBM has announced a $100 million investment to build a petaflop computer that will be 500 times faster than today’s machines to tackle the challenge of protein folding. In parallel, structural genomics initiatives aim to determine three-dimensional protein structures on an industrial scale and to assign function by shape comparison with known biological mediators, since structure is better conserved than sequence during evolution. Various technologies are available to knock-out gene function in vitro and in vivo and to study resultant changes in phenotype, but none appears to have broad-scale application, particularly for genes of unknown function. For example, these are few more emotive or high-profile genes than BRCA1 and BRCA2, which have been implicated in breast cancer, yet, after years of research, a link with DNA repair is only just emerging. Transcript profiling using DNA microarrays is being widely used to generate thousands of similar compounds, but limited synthetic options have not allowed access to the rich molecular architectures often required for interaction with biological targets. In response, emphasis has now shifted to building compound files with maximum structural richness and diversity, since designed libraries should have higher hit rates.

Once a lead has been identified, automated high-speed parallel synthesis may be appropriate, since wide variation of substituents around the pharmacophore template is often required to optimize potency/selectivity and in vivo performance. An important paradigm shift has been to focus on ‘drug like’ lead series with physicochemical and pharmacokinetic properties consistent with the rigorous demands of subsequent development. In silico predictions of preferred molecular properties are being developed, and lead series selection can be further enhanced by high-speed docking of virtual compound libraries with target proteins on the computer screen. Even so, transforming a hit from a high-throughput screen into a drug candidate is still the rate-limiting step, since innovative medicinal chemistry and astute pharmacological evaluation have not yet been automated!

Significant gains will be realized through new methods for predictive toxicology, although it is unlikely that regulatory authorities will dispense with animal safety studies in the short term. However, transcript profiling is being used to demonstrate changes in gene/protein expression associated with toxic events, so that multiple lead series can be prioritized, and unnecessary safety evaluation eliminated.

Only 1 in 12 development candidates reaches the market, and attrition is roughly 50% at both the preclinical and clinical stages. It is sobering to realize that these traditional failure rates and productivity losses reflect an era when a significant proportion of development candidates were improved versions of drugs with established clinical efficacy, and that these figures may deteriorate as the focus turns to unproven mechanisms. Clearly, continued efforts to improve target selection and to reduce compound-related failure will be essential. In addition, it will be important to correlate pharmacogenetic and surrogate markers with drug response during Phase 2 trials, so that the significant cost and time scales for Phase 3 can be reduced through more appropriate
patient selection. For example, it has recently been shown that DNA microarrays can be used to display differences in gene expression in various cancers, and, for diffuse large B-cell lymphoma, patients could be stratified with respect to survival, and presumably treatment regimens. Moreover, if these techniques can identify those patients that may be subject to adverse events, this could also facilitate clinical evaluation and avoid the idiosyncratic reactions that can lead to drug withdrawal.

POSTSCRIPT

The scientific programme that led to sildenafil provides many examples of the innovation, multi-disciplinary teamwork and clear decision making that are essential for success in any research project. However, these well-established principles may be challenged as drug discovery undergoes a major paradigm shift to harness advances in genome sciences and new technologies in order to reduce time scales and improve productivity. No doubt these ambitions will be realized, but an over-reliance on automation, robotics and massive data sets is unlikely to stimulate the high level of creativity that is fundamental for the discovery of innovative new medicines. Therefore it will be important to integrate new scientific advances into an environment that builds on traditional skills, fosters multidisciplinary interactions between teams and individuals, and is primed to exploit Pasteur’s Dictum that ‘chance favours the prepared mind’.

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REFERENCES