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Classical vs reverse pharmacology in drug discovery

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Introduction: the development of drug discovery

The evolution of medical treatments has made remarkable strides thanks to advances in the technology used in drug discovery. In the early days of pharmacology, therapeutic drugs were created from derivatives of medicinal plants. This was the case for aspirin, which was derived from the bark of the willow tree. Some beneficial drugs have been discovered incidentally during scientific research. These desirable accidental discoveries are referred to as drug serendipity. In the 1960s, advances in pharmacology led to an elucidation of the function of cellular receptors, ion channels and enzymes. This information allowed the process of drug discovery to become more scientific and rational. It was during this period that many of the useful drugs that are currently still prescribed were discovered, including α-blockers, β-blockers, and calcium antagonists.

A genome is the complete set of genes in an organism. Genomics is of increasing interest to world leaders and was one of the topics discussed at the Summit of Industrialized Nations held in Okinawa in June 2000. The study of the human genome is certain to have an increasingly important role in the discovery of new therapeutic drugs, especially in light of the recent description of the entire human genome, which showed that humans possess almost twice as many genes as the fruit fly, *Drosophila melanogaster*. The advent of molecular cloning techniques has enabled many receptor and ion channel molecules to be classified, based on similarities in their amino-acid sequence and protein structure. One of the greatest challenges currently facing pharmaceutical companies is to assimilate the vast amount of information generated by genomic and proteomic research into drug discovery programs.

Classical pharmacology in drug discovery

The discovery of tamsulosin

In about 1975, in their small-scale clinical study, Caine *et al.* [1] reported that phenoxybenzamine, an α-adrenoceptor (αAR) antagonist, was effective in treating symptomatic BPH. At that time αARs were pharmacologically classified into two subtypes, α_1 and α_2. At about the same time it was discovered that the αAR that controls the contraction of the human prostatic smooth muscles was the α_1-subtype. Acting on these facts, Yamanouchi Research and Development began searching for a new type of α_1-blocker, with the hypothesis that a selective α_1-blocker could become a therapeutic agent for the voiding dysfunction associated with BPH.

As shown in Fig. 1, dichloroisoproterenol, the first β-adrenoceptor antagonist, was synthesized by chemically modifying the β-receptor agonist, isoproterenol [2]. To obtain a new α_1-antagonist, we used a similar approach in drug design by synthesizing and screening many derivatives of noradrenaline, an α_1-agonist. From among these derivatives a potent and selective α_1-antagonist, tamsulosin, was discovered. Tamsulosin is a new chemical class of α_1-blocker with a structure different from that of prazosin.

Investigation of tamsulosin's mechanism of action

Tamsulosin was investigated to discern the mechanism of its therapeutic action. An anaesthetized-dog model was used to investigate the α_1-blocking activity of tamsulosin in the lower urinary tract and blood vessels [3]. Phenylephrine, an α_1-agonist, was used to increase intraurethral pressure and blood pressure, and the inhibitory effect of tamsulosin measured. Tamsulosin shifted the dose–response curve for phenylephrine to the right in both the urethra and blood vessels. However, the inhibition of phenylephrine-induced pressure elevation by tamsulosin was more pronounced in the urethra than in blood vessels. This suggested that the α_1-blocking activity of tamsulosin is more selective for the urethra than for vascular endothelium.

To evaluate the α_1-blocking activity more quantitatively, the DR_{10} value was obtained; this is a

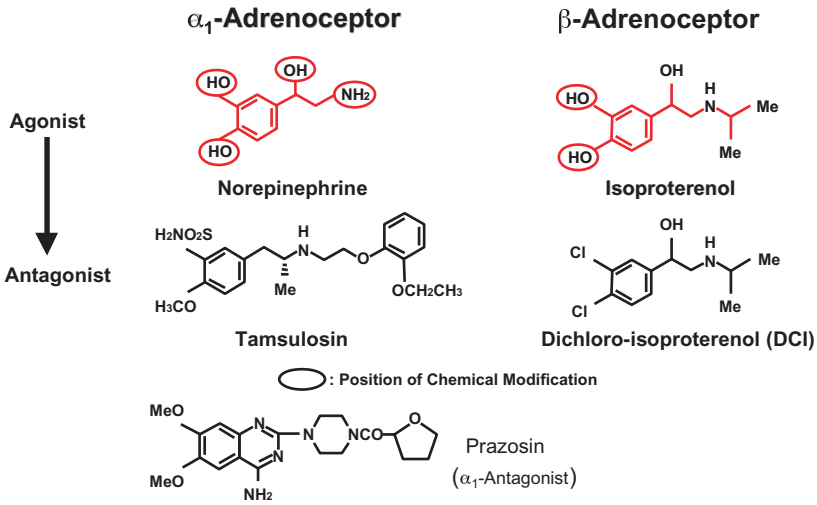

Fig. 1. Development of the α_{1a}AR antagonist tamsulosin. Adapted from [2].

pharmacological index of the dose of an antagonist required to cause a 10-fold shift to the right in the dose–response curve for the agonist. The ratio of the indices for each biological site represents the relative tissue selectivity. For tamsulosin, the DR_{10} was 1.8 for the urethra and 24.1 for the blood vessels, with a urethral selectivity value of 13.3. This was higher than that of prazosin, which was 5.1.

In addition to these *in vivo* pharmacological studies, receptor-binding assays were conducted to measure the selectivity for the prostate. Again, tamsulosin showed higher selectivity for the prostate than for blood vessels. Moreover, in placebo-controlled clinical studies, tamsulosin was found to be extremely effective for treating symptomatic BPH without affecting blood pressure. Although a few cases of orthostatic hypotension were reported, the difference between placebo and tamsulosin (0.4 mg) was not statistically significant [4]. In these pharmacological and clinical studies conducted from 1975 to 1993, it was determined that tamsulosin showed a functional selectivity for the prostate. However, it was still not clear why tamsulosin was so tissue-specific.

In the 1990s, receptor research began to be conducted at the gene level; it became possible to clone receptor-subtype genes. Dr Schwinn's research group at Duke University published the first genetic data on αARs [5]. The α_1ARs were further classified into the subtypes α_{1a}, α_{1b} and α_{1d}. It became clear that α_{1a}ARs were involved in prostatic contractions and α_{1b}ARs, in vascular contractions.

Table 1 [6] shows the affinity of various α_1-blockers in cloned α_1AR subtypes. Tamsulosin showed an affinity for α_{1a}ARs that was 30 times higher than that for α_{1b}ARs, i.e. it showed a higher selectivity for prostate tissue than for blood vessels. Meanwhile, conventional α_1-blockers, e.g. prazosin and terazosin, showed no difference in affinity for the two α_1AR subtypes. Therefore it was concluded that tamsulosin's prostate selectivity was derived from its high affinity for α_{1a}ARs.

Table 1 Affinity of α_1AR antagonists for cloned α_1AR subtypes

	Affinity Ki (nmol/L)		
Antagonist	*α_{1a} (prostate)*	*α_{1b} (blood vessel)*	*Prostate selectivity (for α_{1a} receptor)*
Tamsulosin	0.03	0.87	29.0
Prazosin	0.29	0.19	0.66
Terazosin	8.1	1.9	0.23
Doxazosin	2.1	0.9	0.43
Alfuzosin	5.5	2.1	0.38

Data summarized from information reported in [6].

Reverse pharmacology in drug discovery

In the process of reverse pharmacology, protein targets are first identified that may be critical intervention points in a disease process. Bioinformatics is an emerging science that can harness the power of the computer to store vast DNA-sequence databases and compare three-dimensional protein structures according to probable function. This can enable the identification of novel distant homologues not limited to sequence similarities, a process termed 'target mining'. Using the continuing flow of new sequence information supplied by genomics research, bioinformatics can identify high-quality protein targets for subsequent full-length cloning.

Likely receptors are then exposed to candidate ligands in binding assays, a process termed 'ligand fishing'. A high-throughput screening system for receptor/ligand interaction is constructed and millions of compounds

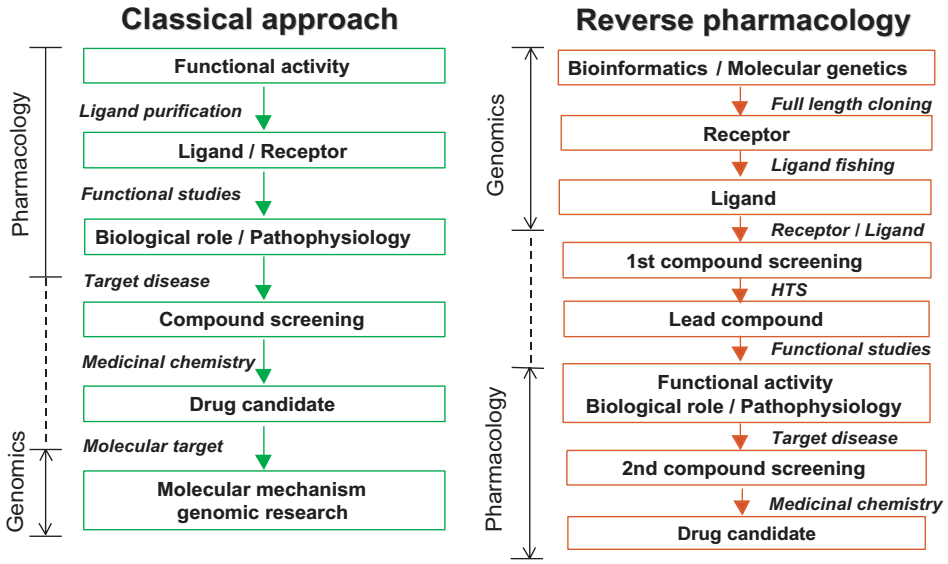

Fig. 2. Comparison of classical and reverse pharmacology approaches in drug discovery.

screened for selective affinity. Promising candidates are modified chemically and subjected to functional studies that use the desired biological response as the index. This process should result in a drug that can be tested first in an experimental and then in a clinical situation. In this process, drug discovery is driven by the mechanistic basis of the disease.

Comparison of classical and reverse pharmacology approaches in drug discovery

As previously mentioned, the discovery of tamsulosin was a process that moved from functional activity, through repeated screenings, towards an understanding of molecular structure. First, the function of the receptor was analysed by using a specific pharmacological technique. Tamsulosin was developed through a targeted drug-design programme, and tamsulosin's higher affinity for α_{1a}ARs later elucidated through genomic study; this process took over 5 years. Pharmacological reaction was slow, and the drug screening using the previously described pharmacological method was time-consuming and inefficient. Using current technology and genomics, scientists can now move in the opposite direction to that used in classical and traditional pharmacological research for new drug agents. This technique, referred to as reverse pharmacology, has many benefits. The drug discovery process should begin with genomic study, proceed through screenings, and end with functional studies.

Figure 2 compares the classical and reverse pharmacological approaches in the drug discovery process. At first sight the reverse pharmacology approach has many steps that differ from those of the classical approach. From our experience, the reverse pharmacology approach takes about 2 years to obtain a new drug candidate. Hence, it is much faster and more efficient than the classical approach, which takes ≈ 5 years.

Conclusions

Sequencing of the human genome was completed in 2000; in future we expect to see advances in research pertaining to gene function, with the result that drug discovery based on genomics will make advances that are even more remarkable. New techniques and technology may well speed the development of innovative drugs for many currently untreatable diseases. Thus, the 21st century will be a golden era for drug discovery, thanks to the use of information provided by genomic research.

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