

## Case Histories of Drug Discovery

The Society for Medicines Research millennial Case Histories of Drug Discovery and Design meeting took place on 2 December 1999, presented to an audience of 170. One reason for the popularity of the event was the interest in hearing the views of the opening speaker, Dr James Niedel of Glaxo Wellcome on the subject of pharmaceutical R&D in the next millennium.

Three trends are to shape the pharmaceutical industry. These are improved performance, value of medicines and social responsibility. The first topic relates to the attrition rate in pharmaceutical development. Only one in 10 drugs that enter preclinical development are successfully registered, and this rate has to date not changed as a result of new technology in drug discovery. There is a great need to improve the selection processes of late discovery in order to limit the number of drugs to fail in development, since these failures are so costly. Candidate selection is a product of the ability to create chemical diversity and to choose molecules with optimal properties. The current standard approach is to use rapid parallel synthesis and combinatorial chemistry, followed by robotic screening. At Glaxo Wellcome, the Combinatorial Lead Optimisation Programme (CLOP) has recently been introduced to measure and predict selectivity, physicochemical properties, absorption, metabolism, tissue penetration and carcinogenicity.

If the analogy of drug discovery with finding needles in haystacks is correct, we require smaller haystacks with more needles. Glaxo Wellcome's approach to this is through intelligent library design, involving an understanding of the properties of drugs and the ability to predict activity. However, it is also important to improve target selection, a process that is increasingly dependent upon knowledge of human genetics.

A good example of the power of genetics is in the study of Single Nucleotide Polymorphisms (SNPs), which represent a small proportion of our DNA but are critically involved in generating our uniqueness. Mapping of the three million SNPs within the three billion base pairs of human genome is being used to identify genetic associations with a given disease. A consortium of 10 major pharmaceutical companies is financing the work, coordinated by the Wellcome Trust, and public availability of the data is guaranteed by publication on the Internet. Five academic institutions in the UK and US are generating the data. Susceptibility genes to a certain disease are determined by comparison of the SNP profile of a population without the disease to that of a matched population with the disease. For instance, in a piece of work that took four months to complete, three SNPs within a 10kb region on chromosome 12 show significant association with adult-onset diabetes. The expressed sequence tags (ESTs) and exons in the region have been predicted.

On the second subject, value of medicines, this is defined as perceived benefits less costs. For example, the costs of H<sub>2</sub> antagonists for gastrointestinal ulcers have been compared to the costs of hospitalisation for surgical treatment. This may result in a new approach to paying for drugs, in which patients are paying for quality of life. The pharmaceutical industry as a whole is devoting increasing effort to understanding value from the customer's perspective. This effort is driven by the increasing time, cost and effort required to bring significant new drugs to market, since R&D investment decisions are evermore long-term, and strategic in nature.

The issue of value is also associated with genetics, which can be applied to guide the prescription of the right drug for right patient. Genetic analysis can permit the correct diagnosis of a disease; and SNPs may also be used where side-effects of a drug are associated with genotype; for instance, with Glaxo Wellcome's lamotrigine for epilepsy, 3% of patients get rash (1% severe rash), thereby limiting overall prescription rates.

Finally, social responsibility may not be a term naturally connected with the pharmaceutical industry, but in Dr Niedel's view, it should become so. Glaxo Wellcome is supporting a substantial amount of basic research through partnership, for instance programmes for new vaccines at the Edward Jenner Institute, and into tuberculosis. Less well known is Glaxo Wellcome's donation of malorone for malaria in Africa. This is a particularly useful drug for strains resistant to existing anti-malarials.

Throughout the day there were three talks about new drugs for arthritis. The first of these concerned the development of Remicade™ (infliximab) from Dr. Tom Schaible (Centocor). Infliximab is a chimaeric antibody to the pro-inflammatory cytokine tumour necrosis factor (TNF)  $\alpha$ , which is produced by macrophages, endothelium and fibroblasts in inflammatory conditions. Significant efforts were made to maintain potency while reducing the immunogenicity of the antibody through humanisation. Infliximab is a 25% mouse (binding site region) and 75% human IgG<sub>1</sub>-type antibody, with very high K<sub>a</sub> of 10<sup>10</sup>M<sup>-1</sup>.

In the collagen-induced arthritis mouse model, the anti-TNF antibody stabilised progression of both swelling and histological damage. In cultured human synovial cells, infliximab was demonstrated to reduce IL-1 production.

Clinically, the measure that was used to establish infliximab's efficacy was the ACR 20% response, the number of patients reporting 20% fewer swollen and tender joints, and a 20% improvement in other criteria such as pain and a global assessment. Trials with 1, 3 and 10 mg/kg with or without methotrexate (MTX) showed a similar effect to MTX, but the durability of effect after dosing halted was better.

Subsequent trials used a dose of 3 or 10 mg/kg with four or eight-week infusion intervals on MTX-resistant patients. A clinical response was observed after 30 weeks, which could be quantified in terms of joint damage after 54 weeks' treatment. Results from extension of treatment to 102 weeks in terms of physical disability measurements are still due in 2000. Compared with MTX, infliximab at 3 mg/kg at eight-week infusion intervals was more effective (20 vs 50% improvement).

Despite the clinical success with infliximab, a major problem is its cost, currently \$10,000 per patient per year of dosing. This was one of the reasons for looking at another indication involving even greater patient suffering.

Crohn's Disease is a serious disorder for 500,000 patients in the US, approximately 70% of whom will eventually require surgery; 20–40% will develop fistulae (channels from gut to skin). It is neither medically nor surgically curable.

In a clinical trial of a single infusion of infliximab at 5mg/kg, a 70% decrease in Crohn's Disease Activity Index (CDAI), and a 70–80% response were observed. Clinical remission was observed in 39% of patients at two weeks and 48% at four weeks. Endoscopic examination showed reduced mucosal inflammation of the bowel. A second trial looked at healing of fistulae; infliximab produced complete closure of all fistulae in 40–50% of patients, and closure of at least half in 68% of patients. This represented the first drug to give such an effect in this horrible disease.

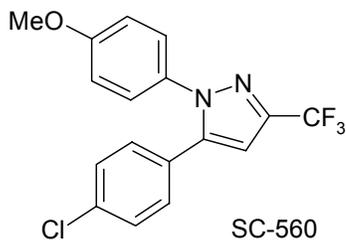
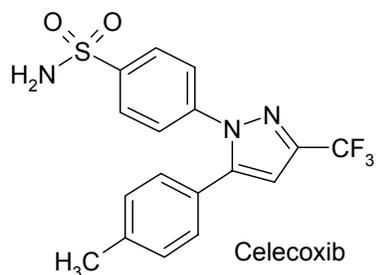
The second anti-arthritis was Celecoxib, a selective COX-2 inhibitor from Searle (Dr Timothy Maziasz). Non-steroidal anti-inflammatory agents (NSAIDs) have been a mainstay of anti-inflammatory treatment in arthritis for many years, and their mechanism of action has since 1971 been known to derive from inhibition of cyclooxygenase (COX), a key enzyme in the production of prostaglandins. However, some prostaglandins have beneficial effects such as on supporting renal and platelet function; and in protection of the gastroduodenum.

In 1989, the presence of two types of COX activity was demonstrated. One was constitutive (COX-1), present in the stomach, intestine, kidney and platelets; the other was inducible by inflammatory cytokines, and found at inflammatory sites in macrophages, synoviocytes and endothelial cells. Glucocorticoids block the mRNA expression of COX-2.

In 1991 the second COX gene was cloned and this led in 1992 to a research programme at Searle to find a selective COX-2 inhibitor. The *in-vitro* model that was deployed for the initial screening of new compounds used a human cloned enzyme. Drugs such as naproxen and other standard NSAIDs are non-selective for COX-1/COX-2 enzymes.

The synthetic programme produced over 2,500 compounds, of which over 75% were screened in *in vivo*. Over 280 compounds had an IC<sub>50</sub> less than 0.1 μM and over 340 were evaluated for oral activity. Seven compounds were taken into multi-species two-week toxicology studies, pharmacokinetics, process chemistry and formulation.

Celecoxib, the eventual development compound, was identified by random screening (from their agrochemical libraries). The enzyme inhibitory activity (Ki) was 15μM against COX-1 and 0.04μM against COX-2. Structurally, the sulfonamidophenyl group is a key feature, permitting time-dependent pseudo-irreversible block, by binding in a side-pocket of the enzyme's active site formed by conformational change. Interestingly, changing the sulfonamidophenyl group to a methoxy produces a COX-1 selective compound (COX-1: 0.009 μM; COX-2: 6.3 μM). This compound was used to prove the COX-2 hypothesis, since unlike the COX-2 selective compounds, SC-560 is neither anti-inflammatory nor analgesic.



There was an important issue with consistency of different *in-vitro* enzyme assays, which was overcome by using an *in vivo* model of selectivity following oral dosing. COX-2 enzyme activity was determined from lavage of a carrageenan-induced air pouch, and COX-1 from the gastric mucosa of the same rodent. Using this model, celecoxib exhibited an ED<sub>50</sub> of 0.2 mg/kg for COX-2 vs. over 200 mg/kg for COX-1.

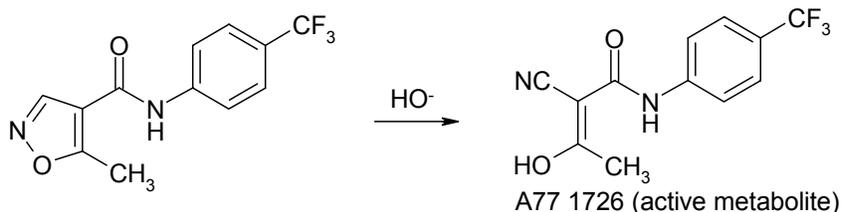
Other *in-vivo* models used to determine efficacy included carrageenan-induced paw swelling and adjuvant-induced chronic inflammation. In the latter, the ED<sub>50</sub> was 0.3 mg/kg, whereas acute gastrointestinal toxicity (a COX-1-related effect) was observed only above 2,000mg/kg in the rat. The benefit of COX-2 selectivity was marked in the dog, since this animal is particularly sensitive to conventional NSAIDs. Naproxen or ibuprofen, for instance, are only tolerated in the dog at one-tenth of the human therapeutic dose. Celecoxib, on the other hand was tolerated at six times the human therapeutic dose (of 6mg/kg/day) in the dog.

Celecoxib entered development 1994 and progressed rapidly. By May 1996, Phase-II efficacy had been carried out, Phase III was completed by December 1997, and the compound entered clinical use in January 1999. Beyond arthritis, there are a number of additional indications that are currently being targeted by COX-2 inhibitors. Trials are already under way in colon cancer and Alzheimer's disease. COX-2 also plays physiological roles in female reproduction, renal activity and neuronal plasticity.

The third and final new anti-arthritis that was discussed was leflunomide (Arava™), presented by Dr Uli Elben of Aventis.

The development story of leflunomide is unusual in a number of ways, the first being that it was discovered through an effect in an *in-vivo* rather than an *in-vitro* assay. In the adjuvant arthritis rat model, leflunomide had an ED<sub>50</sub> on paw volume of 1 mg/kg/day; it also reduced skeletal decay (by X-ray analysis). At the time of discovery, the mechanism of action was unknown, and it was not until 1995 that another company (Syntex) showed that leflunomide interfered with *de-novo* pyrimidine nucleotide biosynthesis. The biochemical basis for this effect was tracked to an inhibitory action on a mitochondrial enzyme, dihydroorotate dehydrogenase (DHODH), which is involved in the conversion of dihydroorotate to orotate in the uridine biosynthesis pathway. Activated lymphocytes are particularly dependent on pyrimidine synthesis as they lack an alternative pathway, and leflunomide therefore blocks T-cell clonal expansion between the G<sub>1</sub> and S phase.

Leflunomide is much more potent in rodents than in humans (for example, IC<sub>50</sub> to rat DHODH is 16 nM, but 657 nM to the human form), a complicating factor during development since the levels required to produce human effects were toxic in animals. Another complication was that the compound has an active metabolite (A77 1726).



Clinical studies showed a long half-life of 14–16 days (due to entero-hepatic recirculation), which was reduced with co-administration of cholestyramine. The long half-life led to a two-part dosing regime in the Phase-III studies of 100mg over three days (loading), then 20mg/day. This was part of a 12-month study in patients with rheumatoid

arthritis for at least six months in comparison with methotrexate (MTX). There was little statistical difference in efficacy between the drugs, but quality of life studies do show significant improvement with leflunomide over MTX. In a further study in comparison with sulfasalazine, extended to 24 months, leflunomide showed significantly superior physiological function, ACR response rates and patient global assessment.

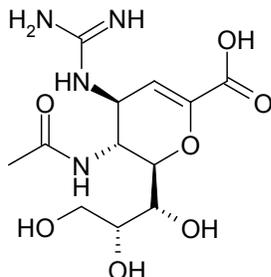
Moving away from arthritis to infective diseases, Dr Rob Fenton (Glaxo Wellcome) presented the story of the discovery of Relenza™ (zanamavir) for influenza infection of the upper respiratory tract.

Influenza virus is transmitted in droplets as people sneeze, cough or talk, particularly in close contact. Sufferers are highly infectious from a few days before to 5–7 days after the appearance of symptoms. After an incubation period of 1–5 days, there is a rapid onset of fever, chills, cough, myalgia, malaise and anorexia. Fever is most prominent within 24–48 hours (up to 41°C), and can lead to viral pneumonia. Recovery normally takes 7–10 days, but the seriousness of the disease is attested by statistics of 50,000–300,000 hospitalisations annually in the US, substantial absenteeism from school and workplace, together with the associated burden on families.

Biologically this is a negative-strand RNA virus, coated with haemagglutinin and neuraminidase. Viral replication occurs internally to the host cell, but disaggregation of viral particles requires the action of the enzyme neuraminidase.

Research into neuraminidase inhibitors started in the 1960s with compounds structurally related to sialic acid.

Meindl and Tuppy (1969) developed a non-selective analogue but it was rapidly cleared by the kidney and produced no activity in animal models. In 1978 Graeme Laeve prepared crystals of neuraminidase and in 1983 Peter Colman and José Varghese produce 3D images by x-ray. This led in 1993 to the rational design and synthesis by Mark van Itzstein of potent inhibitors. These compounds were licensed by Glaxo Wellcome from Biota, Australia.



Zanamavir binds to neuraminidase three times stronger than the natural substrate sialic acid. The compound inhibits all standard influenza A and B viruses ( $IC_{50}$  0.004–0.014  $\mu\text{mol/l}$  on A; 0.005  $\mu\text{mol/l}$  on B). In addition there is a good profile to the development of resistance. A ferret animal model was developed, in which zanamavir suppressed the pyrexia response. Zanamavir is delivered using a Rotadisk® dry-powder inhaler which permits good deep-lung distribution and very rapid action. Given at a dose of 10mg b.i.d. for five days, the compound was effective in the clinic in reducing symptoms by approximately 1–2.5 days. There was low systemic exposure and no increase in adverse events compared to a placebo.

Zanamavir is currently the topic of a great deal of debate about reimbursement. In Britain, there is concern about the lack of clinical data in high-risk populations. This group includes those with chronic respiratory or cardiovascular disease, the elderly (>65) and those who are compromised immunologically. There is an improvement in these patients but because only low numbers have been tested ( $n = 89$ ) this improvement (2.5 day shortening of symptoms, 29% decrease in complications; 30% decrease in antibiotic use) does not yet reach statistical significance.

It is important to recognise that zanamavir is potentially useful not just for flu sufferers, but also prophylactically, for instance in the family of a sufferer. There is a substantial expense associated with zanamavir, but then flu is a disease which is, in a variety of ways, costly to society.

The next case history concerns a second use for the 5-HT<sub>3</sub> antagonist class of drugs — the first use was for cancer therapy-induced emesis. Professor Pat Humphrey (Glaxo Wellcome), who has pioneered research in the serotonin field and already been integral to the discovery of two new agents in this area (imigran and ondansetron) presented the story of the development of alosetron for irritable bowel disease.

The 5-HT<sub>3</sub> receptor is unusual in the serotonin family of receptors in that it is a cation channel rather than a G-protein-coupled receptor (GPCR). It was thought initially to be important in migraine, but the first clinical use derived from the finding that ondansetron, with a pK<sub>b</sub> 8.6 for 5-HT<sub>3</sub> receptors, was anti-emetic in the ferret.

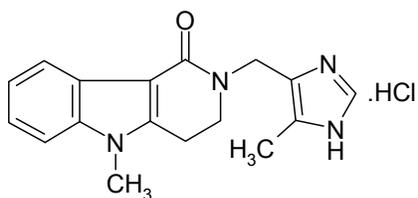
The therapeutic importance of serotonergic agents in gastrointestinal conditions has not been given substantial attention, despite the presence of the 5-HT<sub>3</sub> receptor on sensory nerves in the gut, and the fact that very substantial amounts of 5-HT are present in, and released from enterochromaffin cells, via vagal nerve transmission through 5-HT<sub>3</sub> receptors.

The presence of 5-HT<sub>3</sub> receptors in the brain, mostly in the area postrema and also in the cortex and amygdala, suggested, among others, a role in anxiety. Despite an effect of 1mg/kg alosetron in the mouse light/dark box test comparable to 1mg/kg diazepam, anxiolysis is not seen in man.

Irritable bowel syndrome is one of most common GI-related disorders, occurring in 15% of the population at one time or another. It is more often seen in females, which account for 70–75% of all cases, and characterised by abdominal pain or discomfort and altered bowel function. There are different kinds of IBS, but in the diarrhoea-predominant type, there is a postprandial increase in plasma 5-HT.

Glaxo Wellcome used a rat model in which a latex balloon was inserted in the colorectum of fasted males and inflated to cause rectal distension, leading to a hypotensive response. This was thought to be nociceptive reflex as it was blocked by opiates. The 5-HT<sub>3</sub> antagonists, such as ondansetron (ID<sub>50</sub> of 18µg/kg), were also inhibitors.

Clinically, it has been found that alosetron affects not only bowel function, but also the pain severity and urgency in IBS. The pain component is thought to be a visceral allodynia. Alosetron has no effect on normal small intestine transit, but reverses egg albumin-induced increase in transit, a model of the diarrhoea in IBS.



Finally, Zyprexa® (olanzapine) is the winner of the 1999 SMR Award for drug discovery, and a presentation was made to the three recipients, Drs David Tupper, Terrence Hotten and N. Moore from Lilly, UK.

Schizophrenia one of most severe of mental illnesses and affects 1% of population before age of 45 (50 million worldwide). Schizophrenia is characterised by three groups of symptoms:

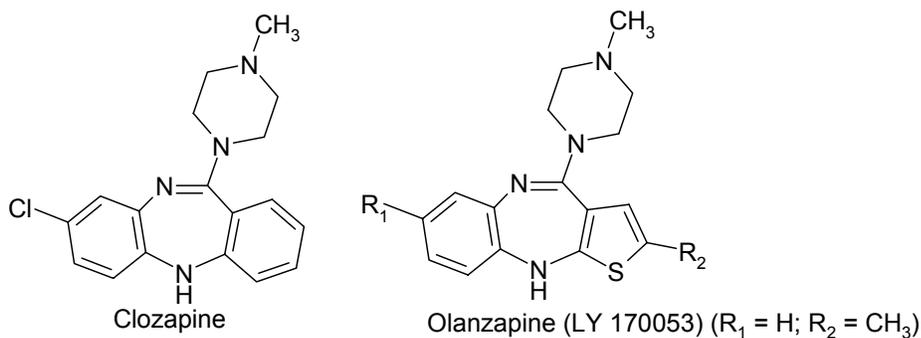
- positive: delusions, hallucinations, bizarre behaviour and impairment of communication;
- negative: social withdrawal, slowness of thinking, emotional blunting and lack of drive; and
- cognitive dysfunction: affecting memory, reasoning, etc.

The consequences of schizophrenia can be severe: incidence of suicide in sufferers is 10–12 times normal.

Typical antipsychotic agents such as haloperidol and chlorpromazine are dopamine antagonists, effectively treating positive symptoms but ineffective against negative or cognitive dysfunction. All have unpleasant mechanism-related side-effects such as extrapyramidal symptoms (EPS) and tardive dyskinesias (TD).

Market introduction in 1970 of the first atypical antipsychotic, clozapine, produced for the first time effective control of positive symptoms with a low incidence of EPS and TD and an effect on negative symptoms. However, agranulocytosis (depletion of white cells) in some patients led to its withdrawal in 1975. It was re-introduced in 1989 as second-line therapy, but is an expensive option due to the need for blood monitoring.

Clozapine one of a series of diarylepinines. Lilly's chemical approach included investigation of a series of thiophene isosteres for either of the phenyl rings in clozapine. Of the various isomeric possibilities, only the (1, 5) system gave activity in models of antipsychotic activity, of which (2, 3-b) had the best overall pharmacological profiles.



Four members of the series went into development. Three were terminated: one due to granulocytopenia in dog (LY 120062;  $R_1 = Et$ ,  $R_2 = F$ ), another due to hepatotoxicity in man (flumezapine, LY 120363;  $R_1 = Me$ ,  $R_2 = F$ ), and a third due to increased cholesterol in the dog (LY 120363;  $R_1 = Et$ ,  $R_2 = H$ ). The fourth was olanzapine (LY 120363;  $R_1 = Et$ ,  $R_2 = F$ ) which progressed without the toxicological problems associated with the others. The structural chemical reasons for these problems remain unexplained.

*In-vitro* binding studies were unavailable for early studies, so behavioural studies were used instead. These included looking for a block of the conditioned avoidance response as an index of antipsychotic activity, and an induction of catalepsy as an index of EPS liability. The ratio between these two effects needed to be high. Based on this assessment, olanzapine was as selective as clozapine (whereas haloperidol was non-selective), but five times as potent. However, when analysed by binding studies alone, olanzapine had mixed effects against a number of dopamine and 5-HT receptors, and some muscarinic activity too. Had binding studies been a primary method of selecting compounds, it is unlikely olanzapine would have been developed.

Clinical development initially involved five small pilot studies; these were sufficiently encouraging to undertake overlapping pivotal Phase-II trials. In four multinational trials over 2,500 patients were treated, some in excess of one year. The results of these studies showed that olanzapine was superior to haloperidol in treatment of positive symptoms, effective with negative symptoms and produced a low incidence of EPS side-effects, leading to superior long-term compliance. The drug was also effective in clozapine-resistant and intolerant patients. EU and US registrations were submitted simultaneously in September 1995, with approval a year later. By the end of Q3 1999, 3.5 million patients worldwide had received Zyprexa, and sales had exceeded \$3.5 billion). It is now approved in 87 countries and marketed in 78.

The SMR Case Histories Meeting is probably unique in the calendar, focusing in detail on the stories behind the research successes that led to new therapeutics. This year's programme featured talks on a number of very difficult-to-treat diseases, and the value of these case histories is the greater for that. In many ways the symposium is an educational resource, and the opportunity for it to reach a substantial number of people internationally through the Webcast is heartening for many in drug discovery.