From moulds to drugs

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The development of the cephalosporins in the last 40 years exemplifies many of the issues encountered during the complex process of drug development. The mould *Cephalosporium acremonium* that was the original starting point for the cephalosporin antibiotic series was discovered in 1948 in Sardinia, but it was not until 1953 that cephalosporin C was discovered, by Florey's group at Oxford, as a minor component of the mixture of antibiotics that this mould produces. Cephalosporin C has only modest antibacterial activity and was not made in quantity until 1960 when Morin et al. at the Lilly laboratories described a new procedure for obtaining 7-ACA. This development paved the way for the future development of large numbers of semisynthetic molecules.

The somewhat unprepossessing antibacterial potency of cephalosporin C is typical of activity that is often seen in novel molecules derived from microorganisms. The interest in novel, naturally occuring agents lies often in the 'scaffold' that the natural molecule provides. Such complex starting points would be very difficult and expensive to make by total synthesis yet can be made relatively easily by fermentation once strain selection and growth conditions are optimized for industrial-scale production. Cephalosporin C was not made by total synthesis until 1965, when Woodward described the synthesis in a Nobel lecture.

Interest in cephalosporin C in the early 1950s has to be placed in the context of the medical needs of that time. By 1952, 90% of staphylococci in London hospitals were already penicillin resistant and the newly available magic bullet, penicillin, looked under threat. Cephalosporin C was resistant to penicillinase and so offered promise. However, by 1961, penicillin itself had been modified to give new semisynthetic penicillins, cloxacillin and methicillin, both of which were more stable to penicillinase and active against *Staphylococcus aureus*. The role for cephalosporin C therefore looked uncertain but the basic 'scaffold' had two points where chemical modification could be made and this offered the possibility of increasing activity against Gram-negative organisms. The seven-position side chain had proved difficult to cleave but, once 7-ACA became available, numerous novel cephalosporins were prepared by re-acylation and the first cephalosporins, cephalothin and cephaloridine, reached the market in 1964. This process had taken more than 10 years with most of the time devoted to chemical synthesis and scale-up with a relatively brief clinical programme.

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DISCOVERY

In the last 50 years, new 'scaffolds' have been sought assiduously and many other antibiotic series such as the tetracyclines, aminoglycosides, oxazolidinones and streptogramins have emerged. Searching for soil or other environmental strains producing novel molecules results in high attrition rates and screening techniques need to be high throughput. Finding active leads is relatively easy but characterizing them can be a lengthy process. In the cephalosporin series, full characterization of the mode of action of the β-lactam series was not done until 1975 when Brian Spratt showed the relevance of penicillin binding proteins. This was more than 35 years after the discovery and clinical use of penicillin.

Nowadays, combinatorial chemistry techniques and genetic techniques should speed the process of preparing semisynthetic or fully synthetic molecules because a wide range of chemical options can be explored quickly and on a very small scale so that when larger scale chemistry begins, options have been defined more tightly.

Attrition rates for any drug development programme are high, typically one in 5000–10 000 at the early development stage, moving to one in 250 during phase III studies and one in five at postmarketing.

The original patents for cephaloridine and cephalothin date from 1954 and since that time at least another 25 000 patents have been filed, covering cephalosporins, intermediates and process patents. These underpin the market use of these agents and represent a very important sector of the intellectual property of the pharmaceutical industry.

SCALE -UP

In the story of mould to drug, the scale-up of production to an industrial scale capable of supplying many tonnes per annum is

a critical part of the endeavour. This part of the process, together with pharmaceutical formulation, is arguably the most important of the skills of the industry, yet it is often an unacknowledged part of the process.

Scale-up may involve fermentation, as in the case of the cephalosporins where typically cephalosporin C is prepared in 100 000 liter batches and converted to 7-ACA in a very rapid process. Semi-synthetic molecules then require chemical modification via a series of steps. In some cases, particular processes may be needed to generate a form of a molecule that is particularly suitable for formulation or activity. Examples include chiral syntheses and the production of particular forms to ensure stability as in the case of the pentahydrate crystalline form of ceftazidime. The drug form can be critically important in determining how an agent is used clinically and there can be key differences between generic agents and the innovator forms that derive from different methods of manufacture. It is usual for any change in process to be registered and where any change in impurities or form are shown, a manufacturer may need to demonstrate additional toxicological or bioequivalence data as a minimum, in addition to routine chemical and pharmaceutical stability data. All these nuances of production and formulation are a major part of the work of the industry. Getting these aspects optimized contributes to overall development time and costs. At every stage specific 'know how' is often involved and so process patenting is a key part of the total intellectual property associated with any drug.

Process chemistry must also be conducted under conditions that are safe and environmentally appropriate, so methods must take account of these needs. Operators at both primary and secondary level processing may need to be protected from daily exposure to a drug that might be used only for short periods in patients. There may also be specific issues of hypersensitizing potential or other risks that need to be minimized via environmental design or in exceptional circumstances protective clothing for operators.

FORMULATION

Pharmaceutical formulation involves ensuring that the drug can be delivered appropriately to the requisite site of action in man. In addition, the drug must be presented in suitable packaging to guarantee a stable and effective product throughout a stated shelf-life of an adequate duration, often several years.

Drug form and formulation can profoundly affect bioavailability in man. Cephalosporins were available initially only by injection and with a relatively short half-life, meaning that multiple daily doses were needed. Cephalothin and ceftriaxone represent extremes in the cephalosporin series because cephalothin is readily de-acetylated by esterase activity in man to the less active des-acetyl form, resulting in an elimination half-life of just less than 1 hour. Ceftriaxone in contrast, by modification of the 3-position side chain, can be given once or twice daily because there is no instability and the elimination half-life is 6–8 h.

Among the orally absorbed cephalosporins, the earliest agent cephalexin and its closely related analogs, cephradine, cefaclor and cefprozil, are all actively absorbed because they are recognized by di-amino-acid uptake pathways in the intestinal mucosa. This results in almost complete oral absorption, with blood levels similar to those achieved by injection of the same agents. In contrast, the later generations of cephalosporins have proved more difficult to formulate for oral use and, in many cases, prodrugs have been made, often using bulky esters to make the molecules more lipophilic and hence able to cross the intestinal mucosa after removal of the ester group by esterase activity. Cefuroxime axetil is an example of this type of agent, and is absorbed following hydrolysis by a specific esterase. Its formulation for pediatric use proved unusual because the molecule, like many β-lactams, has a bitter taste as a powder. To overcome this, the cefuroxime axetil is wax coated before being formulated in a fruit-flavoured suspension.

Issues of solubility and stability are a particular concern for all cephalosporins and indeed most β-lactam antibiotics because these compounds tend to be relatively unstable in the presence of moisture. This means that packaging needs to be moisture proof and moisture content often needs to be controlled during production processes.

SAFETY STUDIES

The cephalosporin series has been remarkably free from major toxicity issues, due in part to the high degree of selectivity of the mode of action. Man has no counterpart to the penicillin-binding proteins of the bacterial cell wall and so the β-lactams are highly selective. This has meant that most cephalosporins have been used in patients of all ages, including, in many cases, neonates. They are suggested for use in most therapeutic and prophylactic indications. Use includes meningitis and severe infections in neutropenics where cephalosporins alone and in combination have been part of 'gold-standard' treatment regimes for the last 15–20 years. The earliest compounds, cephalothin and cephaloridine, raised some concerns because they were eliminated by tubular secretion, and in very high doses nephrotoxicity was a concern. However, later compounds overcame this issue. The cephalosporins penetrate tissues and tissue fluids effectively, including bone, and they cross inflamed meninges making them an important option in meningitis.

CLINICAL STUDIES

The cephalosporins have been extensively studied in clinical trials worldwide. The regulatory environment has become more demanding in the last 40 years since the introduction of cephalothin. For antibiotics today, there are some particular issues that regulators and industry alike are wrestling with. These relate to problems of demonstrating efficacy of new agents against emerging and resistant strains. In the last 20 years, we have seen the emergence of several new pathogens such as *Legionella* spp. or *Helicobacter pylori*, whilst immunocompromized patients may become infected with organisms hitherto considered to be of limited pathogenic potential. In these clinical scenarios, the relationship of the pathogen and pathology is not necessarily as obvious as in some classical infections of the past. In the case of resistant strains such as penicillin-resistant pneumococci (PRP) or methicillin-resistant *S. aureus* (MRSA), the pathological significance is not always straightforward. Additionally in the constrained environment of clinical studies, new resistances may be poorly represented among patients who are recruited to trials, and consequently efficacy cannot be demonstrated against resistant isolates at levels that are statistically meaningful. So new algorithms are needed for trial designs that assess efficacy against resistant pathogens. Increasingly, pharmacokinetic together with pharmacodynamic parameters are being used to support efficacy against new resistance markers. Unless these types of data are used as a surrogate, drug development to answer the needs of bacterial resistance will not be possible and by virtue of their superior generation turnover, resistant bacteria will continue to gain ascendancy.

CEPHALOSPORINS IN THE MARKETPLACE

The cephalosporin series of antibiotics has been very successful in global use and the β-lactam agents are still a major group. This is probably due to the specificity of the mode of action of these agents, which is still a distinguishing feature and real strength of the series. The cephalosporins have contributed to both hospital and community medicine for the last 45 years and their development has mirrored medical needs during that time. The series has shown itself able to encompass new resistances, particularly β-lactamases. In the 1970s the discovery of the oxime moiety contributed particularly in this regard and this has been used in most cephalosporins since the discovery of cefuroxime. The addition of the aminothiazolyl ring at the seven position in cefotaxime by Roussel-Uclaf, marked another key milestone and a 10-fold improvement in potency against Gram-negative isolates. This side chain has been extensively used in almost all third-generation oral and injectable molecules. Efficacy against *Pseudomonas aeruginosa* had always seemed elusive in the cephalosporin series until the late 1970s, when cefsulodin, the narrow spectrum agent, and ceftazidime, a carboxyalkyloxime, were described. The latter molecule has been widely used and continues to set the standard for β-lactams in terms of antipseudomonal activity. Two methoximes with novel quaternary ammonium 3-substituents, cefpirome and cefepime, have also been marketed subsequently and show clinical efficacy equivalent to that of ceftazidime. More recently, attempts have been made to improve antipseudomonal activity by using catechol mimics or catecholic groups at the seven or three position in order to achieve active uptake of the cephalosporins via iron-transport mechanisms. The concept was shown to be valid by several groups within the pharmaceutical industry but no development candidate emerged, perhaps because of issues of toxicity. In the last year several cephalosporins with enhanced activity against MRSA have been described. These agents, exemplified by a Roche compound, RO63–9141, have considerably improved activity against MRSA compared with other cephalosporins and it remains to be seen whether this important group of organisms can be encompassed in the cephalosporin spectrum.

CEPHALOSPORINS FOR THE FUTURE

Can the cephalosporin series continue to meet medical needs? Currently the most pressing medical issues in infections in critical care include vancomycin-resistant enterococci (VRE), particularly *Enterococcus faecium*. These strains are particularly intractable and are often multiresistant. The marketed cephalosporins have never included enterococci in the spectrum and this may be a gap that the series cannot fill. If the spectre of vancomycin-resistant staphylococci becomes a reality on a large scale, the cephalosporins may not meet this challenge either. Currently, agents like cefotaxime and ceftriaxone continue to be first-line therapy in meningitis, but PRP are becoming increasingly common and these agents may not meet this challenge in the longer term, even with higher doses. The cephalosporin series may now be nearing the end of its exploitable variations but the β-lactam family may still be able to meet the new bacterial challenges. Novel carbapenems and trinems both offer prospects of broader spectra with enhanced anti-Gram-positive cover, which should be able to include current problems of PRP, VRE and MRSA.