Innovations

Chiral genetics
Chiroscience Group, plc

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In 1848, Louis Pasteur used tweezers to pick apart two types of tartaric acid crystals, and thus discovered the principle of chirality. Chiral compounds (from kheir, the Greek for hand) exist as one of two enantiomers — compounds with the same chemical formula but mirror-image structures.

Now, 150 years after Pasteur's discovery, the pharmaceutical industry is completing its conversion to single-enantiomer drugs. An estimated 78% of drugs will be single enantiomers by the year 2000. But a significant period of modern drug discovery has involved the generation of enantiomeric mixtures, or racemates, and a few companies are taking a hard look at those mixtures. Enantiomers of drugs often differ in their interactions with proteins, which after all are made of enantiomeric building blocks (L, not D, amino acids). If the enantiomers of carvone can elicit the distinctive smells of either caraway seeds or spearmint, it is not surprising that enantiomers of drugs differ in their effects.

Chiroscience Group, plc (Cambridge, UK) has identified a number of drugs as candidates for chiral switches — the selection of one enantiomer from a racemate drug based on its improved activity or reduced side effects relative to the mixture. One of those candidates — an enantiomer of bupivicaine — has just been approved for marketing as a local anesthetic. But in the face of competition, and in anticipation of the limited life of the chiral switch idea, Chiroscience has shifted its focus to unusual genetic diseases in the hope of identifying new drug targets.

Farming the old

Chiroscience began as Enzymatics, a company that used selective enzyme reactions to separate one enantiomer from a racemate. As it moved further into chiral chemistry, the company morphed into Chiroscience and identified several candidates for chiral switches. A couple of those drugs were licensed to other companies, leaving only levobupivicaine, an enantiomer of bupivicaine that substantially reduces the side effect of heart damage caused by the original drug.

Other companies, notably Sepracor Inc. (Marlborough, Massachusetts) and Celgene Corp. (Warren, New Jersey), have made similar discoveries. "Innovators are typically moving onto the next new entity, rather than investigating how to make the existing one better," says Steve Wald, vice president of chemistry research and development at Sepracor. "We're applying 90s technology to drugs developed in the 70s."

Chiroscience is either fast-moving and adaptable, or a company that can't settle on a vision.

Chiral switches are an attractive approach to drug discovery. The drug's synthesis and market are largely known, and a new patent can be issued with full protection of the enantiomer as a new chemical entity.

Sepracor's chiral switch candidates include the (*R*) enantiomer of the bronchodilator albuterol (better known as Ventolin or Proventil), which reduces the jitteriness and cardiac effects of albuterol, and no longer worsens asthma with repeated use. Sepracor is also investigating Eli Lilly's Prozac. The (*R*) enantiomer of Prozac has better pharmacokinetics than the racemate, and the (*S*) enantiomer shows activity against migraines that the racemate lacks.

Sepracor's Allegra illustrates another way to get around a side effect. In this case the side effect was cardiac arrhythmia when the allergy drug Seldane was combined with certain antibiotics or antifungals. (When Seldane was developed there were no models to detect this type of arrhythmia.) Allegra is an active metabolite of Seldane that no longer shows these interactions.

A chiral switch is not always the solution. The teratogenic effects of Celgene's thalidomide appear to be caused by the pharmacologically active enantiomer. In any case, administering a single enantiomer would be pointless, as the two enantiomers are interconverted in the body.

The new approach: chiral from the start

Chiral switches are a non-renewable resource, because the pharmaceutical industry is leaving racemates behind. In 1992, the Food and Drug Administration began demanding that single enantiomers be tested in trials, if at all possible. A racemate drug now requires three sets of clinical trials: with the racemate and each enantiomer. This has encouraged drug companies to develop single enantiomer drugs.

Improved chemistry has made that feasible. "Any drug that would be introduced today would be introduced as a single enantiomer," says Barry Trost (Stanford University, Palo Alto, California), although drugs whose enantiomers are interconverted in the body are one exception. Based on improvements in asymmetric chemistry, he says "there is no limit" to the number of possible chiral centers that can be incorporated into a drug. "What limits things is really the size of what can be a drug."

Thus there is a large demand for chiral synthesis and isolation of enantiomers. Chiroscience provides these services through its subsidiary Chirotech Technology Limited. In addition to providing contract chiral synthesis, Chirotech sells collections of chiral building blocks and catalysts.

Racemates can be resolved (separated into their constituent enantiomers) if one enantiomer preferentially crystallizes, reacts with

another chemical, or reacts with an enzyme. Chiroscience's founding technology of enzymic resolution remains the most common technique, but chemicals are more often used to racemize the undesirable enantiomer so that half of the starting material is not lost.

An effective chiral synthesis removes the need for any resolution or racemization. "Asymmetric catalysis is still in its infancy, so people are still using a lot of resolution," says Trost. "But as the chemistry gets further along, resolution will get shunted to the side. That will not happen overnight because there are significant cost issues in optimizing the reactions."

Chirotech has licensed Trost's methods for palladium-catalyzed asymmetric allylic substitutions, and DuPont's methods for transition-metal catalyzed asymmetric hydrogenation. Asymmetric epoxidation is also proving an important synthetic tool.

Thick bones and T cells

Sepracor emphasizes that there are over 500 drugs currently marketed as racemates, and the company's long list of developmental drugs suggests that they will be busy for some time. But for Chiroscience the list was dwindling by 1994, when John Padfield, a former executive at Glaxo, took over as CEO.

"We were well ahead of them," says Wald. "We launched the patent groundwork when they were still a company that did bioresolution."

Chiroscience added projects in phosphodiesterases and matrix metalloproteinases, but the real leap into the unknown was in 1996 when it acquired Darwin Molecular Corporation (Seattle, Washington). The gene-discovery arm of Darwin was absorbed into Chiroscience, while the pharmacogenomics side became a spin-off called Rapigene, Inc.

Darwin's genomics and genetics was a far cry from chiral synthesis. "Chiral chemistry was an excellent platform technology to launch a start-up, but ... gene discovery represents [a] much greater potential for generating innovative projects in the future," says Robert Jackson, the head of research and development at Chiroscience. Eventually, he says, the company aims to become an integrated gene-to-drug company.

For now Chiroscience is focusing on target discovery through genetics, but not just any genetics. "We are using mutation as a surrogate for drug action," says Jackson. One example is the gene for human sclerosteosis — a disease found in a small rural population in Southern Africa. Problems in bone turnover lead to bones that are up to twice as dense as normal, a cranium up to an inch thick, and eventually death from pressure on the brain if patients are not treated.

So where is this leading? "If we inhibit the gene product we should make bone more dense and have a potential osteoporosis drug," says Jackson. "Unlike cancer or atherosclerosis genes where you need to unravel what is the real cause, here the phenotype is self-validating. We know that if we inhibit this gene we will make bone denser — the phenotype tells us this." Jackson contrasts this approach with the brute-force isolation of multiple candidate genes by companies like Human Genome Sciences, Inc. (Rockville, Maryland), or the complex trait analysis by companies like Millennium Pharmaceuticals, Inc. (Cambridge, Massachusetts).

"There will probably be a limited number of these [human diseases] so we will supplement them with mouse genetics," says Jackson. "Mouse genetics is only just starting to gear up. This is a place where a small company can make a contribution."

The mouse mutants that have been analyzed, says Jackson, tend to be the ones with very obvious primary phenotypes. "There has been very little work where you have to look beyond things like coat color and test phenotypic functions like bone density and immune function."

Chiroscience's entry point is the Scurfy mouse, discovered in Oak Ridge, Tennessee, over 30 years ago. Affected mice have a spontaneous autoimmune disease, and die very young when their T cells mount a massive attack on all tissues. Chiroscience has isolated the relevant gene (although, as with the sclerosteosis gene, the identity of the gene has not been disclosed), and found that it controls a pathway that inhibits T cell proliferation. A drug that inhibits this pathway should act as an immunostimulant for those with HIV, especially as the CD4 population of T cells is specifically affected.

The pharmacogenomics offshoot

Rapigene's basic technology involves a series of oligonucleotides tagged with small molecules. The oligonucleotides stick to mRNA made by active genes, the tags are removed by shining light on the photocleavable linkers, and the tags are then detected by a mass spectrometer. With this process, Rapigene can detect the expression levels of up to 100 genes by analyzing a single well of a 96-well plate. "The beauty of the mass-spec tag is you can use a huge number of them — hundreds as compared to just a few fluorescent tags," says Jackson. "We see this as an alternative to Affymetrix-type chips."

The expression analysis should allow more accurate subdivisions of patient populations so that a positive outcome (or a side effect) in a particular minority can be detected. Rapigene's tags should be particularly useful in small trials, says Jackson, because "our technology can be reprogrammed at will."

Even as Chiroscience enters the brave new world of pharmacogenomics, Sepracor remains happy with its approach of updating imperfect drugs. "I don't think this is just a short-term strategy," says Wald. "In 2010 we are going to know something more about drugs and how to make them better."

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