THE IMPACT OF STEREOCHEMISTRY ON DRUG DEVELOPMENT AND USE

The Impact of Stereochemistry on Drug Development and Use

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Ṭo my wife Nagla and my sons Youssef, Faisal, and Basil,
who have filled my life with love, joy, and pride.

Hassaṇ Y. Aboul-Enein

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PREFACE

With the separation of the enantiomorphic crystals of ammonium sodium tartrate, Louis Pasteur uncovered one of the wonders of nature—the interrelationship of symmetry and asymmetry (1). His observations led him to formulate a proposal which is the foundation of molecular stereochemistry: "The optical activity of organic solutions is determined by molecular asymmetry, which produces nonsuperimposable mirror image structures" (2).

This proposition initiated an intense study of the physicochemical properties and theoretical nature of stereochemistry. During the remainder of the nineteenth century, the work of Pasteur, Van't Hoff, Le Bel, and Wislicenus expanded and clarified the concept of chiral molecules (3–5). During this period, the biological and pharmacological implications of stereochemistry were largely ignored, even though nature was the source of chiral chemicals. To some extent this was necessary since a chemical foundation was required to build a biological understanding.

The understanding and appreciation of the role stereochemistry plays in pharmacology also stems from the work of Pasteur. In 1858, Pasteur reported that the *dextro* form of ammonium tartrate was more rapidly destroyed by the mold *Penicillium glaucum* than the *levo* isomer (6). During the next 50 years, numerous similar examples were reported in diverse biological fields. Then, in 1908, Abderhalde and Müller reported the differential effects of (–)- and (+)-epinephrine on blood pressure (pressor effects) (7) and chirality entered mainstream pharmacological research. By the 1930s, Cushny (8) and Easson and Stedman (9) had laid the basis for the initial theoretical understanding of stereochemical differences in pharmacological activity.

As a result of the observations by Cushny, Easson, and Stedman, stereochemistry became an integral part of medicinal chemistry; in particular, a core element in the study of quantitative structure–activity relationships (QSAR). However, stereochemistry essentially remained buried and chiral drugs continued to be developed as racemates. Single isomer drugs were considered only if there were readily available single isomer starting materials (e.g., steroids, antibiotics) or inescapable pharmacological consequences associated with one of the isomers (e.g., *dextro*-methorphan was developed as an over-the-counter antitussive since *levo*-methorphan is a registered narcotic).

To a great extent, stereochemistry could be relegated to a secondary consideration because the pharmaceutical industry and the regulatory agencies lacked adequate analytical techniques. What you could not measure you could not require. This situation has dramatically changed over the past 15 years with the development of analytical methods capable of the rapid separation and accurate measurement of enantiomeric composition.

The breakthrough in this area came with the development of a commercially available HPLC chiral stationary phase (CSP) by W. H. Pirkle (10). This revolutionized the analytical and preparative separations of enantiomeric compounds and initiated a rapid increase in commercially available HPLC and GC CSPs. These technological advances have, in turn, produced an increased interest in the *in vivo* pharmacological fate of the separate enantiomers of chiral substances; particularly from the drug regulatory agencies. The response has been a sustained rise in the number of studies concerned with the pharmacokinetic and metabolic disposition of enantiomeric drugs. At the present time, these studies are routine procedures in the development and testing of new drugs, for both racemic and single-isomers formulations.

Perhaps the most cogent statement of the implications of stereochemical differences in pharmacokinetics and pharmacodynamics was presented by Ariëns (11):

> Often only one isomer is therapeutically active, but this does not mean that the other is really inactive. It may very well contribute to the side effects. The therapeutically nonactive isomer in a racemate should be regarded as an impurity (50% or more). It is emphasized how in clinical pharmacology, and particularly in pharmacokinetics, neglect of stereoselectivity in action leads to the performance of expensive, highly sophisticated scientific nonsense.

The phrase "highly sophisticated scientific nonsense" has been widely quoted and perhaps has been the single most important ideological statement in the current growth of stereochemical awareness in the pharmaceutical industry.

The direct connection between the advancement of enantioselective technology and the discovery, development, and marketing of chiral drugs has resulted in a rapid maturing of this field of research. It is safe to say that if a chiral substance exhibits pharmacological activity, the properties of the separate enantiomers of that substance will be defined, measured, and evaluated in relationship to the development of racemic or single-isomer therapeutic agent.

This is possible because adequate quantities of the separate isomers can be easily prepared and their pharmacological properties and fate determined alone and in the presence of the opposite enantiomer. These are accepted and, in fact, required experiments which are now routinely published in a variety of scientific journals and reported at international meetings. This was not always the case.

As with many new technologies, enantioselective analytical and pharmacological studies were often difficult to report in established journals. Indeed, new journals such as *Chirality* and *Tetrahedron Asymmetry* were created to give this new technology a voice. Chiral symposia, workshops, and meetings were formed to facilitate the dissemination of the scientific advancements and to educate colleagues. As the theory and practice of stereochemistry in pharmaceutical development grew, the symposia, workshops, and meetings spawned a number of books detailing these advancements.

These publications and advancements are the foundation for the present volume. This collection contains a series of articles that describe a mature field of work, one that has been reintegrated into the mainstream of the analytical and pharmacological sciences.

The initial chapters of this work address the pharmacological consequences of stereochemistry. The fact that enantiomers have different fates and effects is not surprising since living organisms contain numerous chiral biopolymers such as proteins, enzymes, cellular surfaces, etc. In fact, it is safe to say that any active pharmacological process has the possibility of being enantioselective or enantiospecific, and it probably is. Thus, protein binding, biotransformation, receptor binding, active transport into and out of cells, intracellular sequestration, DNA binding, etc. have been shown to discriminate between drug enantiomers. This situation has been generalized in "Pfeiffer's rule," which states that the more potent a drug, the more likely it is to show stereoselectivity due to a greater steric demand for tight receptor binding (12). It should be noted that since the physicochemical properties of enantiomers are equivalent, passive pharmacological processes, for example absorption, will be the same for both isomers.

Drug metabolism is a key aspect in the pharmacological fate and effect of a therapeutic agent. The enantioselectivities of the Phase I and Phase II microsomal transformations have been extensively studied and are a rich source of biological information. In addition, nonmicrosomal transformations have also shown stereochemical preferences. For examples, the aldoketo reductase mediated reduction of the prochiral drug metyrapone to the chiral metyrapol is not enantioselective in men, but has a pronounced enantioselectivity in women with (+)-metyrapol favored over the (–)-enantiomer by a factor of 1.6:1 (13).

However, these investigations have inherent problems stemming from the duality of enantiomers, which are at the same time chemically identical and spatially different molecules. This situation is illustrated by the results of a study of a metabolic enantiomeric interaction involving the competitive inhibition of (S)-warfarin-7-hydroxylase by (R)-warfarin (14). The authors of this study concluded that:

1. The kinetic parameters defining the interactions of two enantiomers of a racemic drug with the cytochrome P-450s or other macromolecular systems in the living organism can only be properly defined from experiments with the pure enantiomers;
2. an enantiomer of a racemic drug may contribute significantly to biological effect not by its inherent activity but by altering the pharmacokinetics of the eutomer;
3. enantiomeric interactions are not easily detected unless directly sought and may be relatively common.

Three chapters are devoted to the stereochemical aspects of drug metabolism and will give the reader an excellent overview of this area. Three additional chapters address particular aspects of chirality and drug activity. One presents the toxicological consequences of the stereoselectivity in the xenobiotic metabolism of alkenes and a second describes the synthesis, chromatographic resolution, and biological activity of chiral barbiturates. The remaining chapter presents the case of ethambitol, where the lack of a clear understanding of the stereochemical composition of the drug has hindered its effective therapeutic application.

The latter chapter also reflects another by-product of the current preoccupation with drug stereochemistry, the "racemic switch." In the strategy, currently racemic drugs are reevaluated with the intention of developing a single-isomer formulation. The goal is a new chirally pure drug with a better therapeutic index than the racemate and, perhaps, new clinical applications. These possibilities are illustrated by the use of verapamil (VER) in the treatment of adriamycin-resistant tumors.

VER is a chiral calcium channel blocking drug widely used in the therapy of hypertension, supraventricular arrhythmias, and angina pectora (15). The enantiomers of VER have different pharmacodynamic and pharmacokinetic properties; for example, S-(–)-VER is 10 to 20 times more potent than R-(+)-VER [16]. In addition to its cardiovascular activities, VER has another possible clinical application in cancer chemotherapy as a modifier of multidrug resistance (MDR). Initial *in vitro* experiments have demonstrated that the presence of VER in the incubation media increased the cytotoxicity of vinca alkaloid and anthracycline derivatives in several resistant tumor cell lines; in particular adriamycin resistant cell lines (17). One proposed source of MDR is a decrease in the accumulation of intracellular concentrations of the antineoplastic agents due to increased expression of a glycoprotein which acts as an efflux pump for cyctostatic drugs (18). The effect of VER on MDR is due to the inhibition of this efflux pump (18).

Based upon the *in vitro* experiments, several clinical Phase I trials were carried out using VER in combination with adriamycin (19) and vinblastine (20).

However, these trials were not successful. Plasma levels which were comparable to the effective *in vitro* concentrations could not be achieved due to the cardiotoxicity of VER. The results of one study involving the treatment of MDR ovarian cancer patients with VER and adriamycin were summarized as follows:

> However, the high infusion rates of verapamil (9 μg/kg/min) required to achieve these plasma levels produced an unacceptable degree of cardiac toxicity. Two patients developed transient atropine-responsive complete heart block and four patients developed transient congestive heart failure with increases in pulmonary capillary wedge pressure... Future studies should use less cardiotoxic calcium channel blockers that can be safely administered to produce the plasma levels required for *in vitro* sensitization of drug resistant cells (19).

One less cardiotoxic compound is the "inactive" isomer of VER, (R)-(+)-VER. While this isomer of VER has on 1/10 to 1/20 of the negative dromotropic, inotropic, and vasodilating activity of (S)-(−)-VER, it has equivalent activity in the modification of MDR (17, 21). Clinical Phase I trials of (R)-(+)-VER are currently underway (21).

The next group of chapters in this volume addresses the preparation of chirally pure compounds and the analytical determination of stereochemical composition. The predominate theme is chromatographic enantioselective resolution on chiral stationary phases. This is the correct emphasis since, as stated above, the development of commercially available CSPs was the major technological advance which triggered the current chiral explosion. These chapters are presented by experts in the field and are recommended to the reader.

Chromatographic resolutions on CSPs are not the only approach to the determination of optical purity. Indirect determination based upon diastereomeric derivatizations is still a popular and effective strategy and is discussed in one of the chapters. In addition, the use of circular dichroism (CD) spectroscopy in stereochemical and analytical determinations is also discussed. Of further interest is the contribution describing the application of CD spectroscopy to the study of drug-protein binding. This is an interesting and powerful technique which often provides pharmacological information unattainable by other means.

The last section of the volume presents the current status of the regulatory–pharmaceutical industry debate concerning guidelines for the development and approval of stereoisomeric drugs. These chapters do not present the regulatory guidelines in their final form, especially since the international harmonization of drug regulations is an on-going process. However, they present the historical evolution of the debate as well as an outline of the final product.

No matter what form the final regulatory guidelines take, it is clear that drug development can no longer occur without consideration of drug stereochemistry. This is quite a change from the situation in the early 1980s. It is our hope that this volume will give the reader some sense of the magnitude of the stereochemical revolution that has occurred in the past 15 years and an understanding of the maturity of this area of research. We have in one sense come full circle and stand alongside Pasteur in amazement of nature's duality, symmetry, and dissymmetry, and its chemical and pharmacological consequences. It will be fascinating to see what the next 15 years will bring.

Montreal, Quebec, Canada
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