product; and (iii) in the case of drugs bound by plasma proteins, the fraction of drug in plasma that is bioavailable for transport into brain. Owing to enhanced dissociation mechanisms that operate *in vivo* within the brain capillary compartment, the brain bioavailable fraction might be much greater than the free fraction measured *in vitro* [4]. The pharmaceutical industry uses methods such as equilibrium dialysis or ultrafiltration to measure the fraction of drug that is free in plasma. If a drug is >95% plasma-protein bound, then frequently the drug is no longer considered a lead candidate because it is erroneously assumed that the drug is precluded from entering the brain by restrictive plasma-protein binding. For many years, the most profitable drug in the world was diazepam. This lipophilic amine is >95% plasma-protein bound and >95% is transported into brain on a single circulatory pass. The bioavailable fraction of diazepam in the brain capillary is much higher than the fraction of drug that is free (unbound) *in vitro*. This finding of a high-bioavailable drug fraction *in vivo* in the brain capillary, compared with the free fraction *in vitro*, is the rule, not the exception [4].

The measurement of the BBB PS product can be performed either *in vitro* using a tissue culture model of the BBB, or *in vivo* using quantitative animal models. The animal models are labor intensive and many in industry seek to measure PS products with *in vitro* BBB models. However, brain capillary endothelial monolayers in cell culture form a leaky barrier with an electrical resistance that is more than tenfold lower than that found at the BBB *in vivo*. Moreover, the gene expression of most BBB transporters is downregulated 100-fold in culture. Consequently, lipid-mediated transport is overestimated by up to 100-fold, and carrier-mediated transport is underestimated by up to 100-fold with *in vitro* BBB models [5]. For example, 3,4-dihydroxy-L-phenylalanine (L-DOPA) is pharmacologically active in the brain because this drug is transported into brain via the BBB large neutral amino acid transporter 1 (LAT1). However, owing to the marked suppression of LAT1 gene expression in cell culture, the carrier-mediated transport of neutral amino acid drugs such as L-DOPA would not be detected by screening drug candidates with an *in vitro* BBB model.

Unfortunately, there is no short cut to the accurate measurement of BBB PS products. What works now is what has worked for 50 years – the quantification of BBB PS products with one of several defined *in vivo* physiological animal models. These include either carotid arterial injection and/or infusion methods, or quantitative intravenous injection methods that are measured over brief time periods with simultaneous estimates of the plasma area under the concentration curve.

Log(BB) must be replaced by the *in vivo* BBB PS product as the standard in the field. Otherwise, *in silico* models of brain penetration will continue to be of only limited benefit to industry.

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Safety pharmacology in pharmaceutical development and approval

Shayne C. Gad, CRC Press, US$149.95, 189 pages (hbk), ISBN 0-8493-1380-5

During the past decade, the emergence of non-clinical safety pharmacology as a distinct discipline in the drug discovery process has been fuelled by drug failures and advances in science and technology. Regulatory guidelines on safety pharmacology have been subject to harmonization through the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) process (http://www.ich.org). Safety pharmacology studies investigate the potential undesirable pharmacodynamic effects of a compound on physiological functions in relation to exposure to the drug in and above the therapeutic range. Furthermore, the specific objectives of safety pharmacology studies are: (i) to identify the undesirable

pharmacodynamic properties of a substance that might have relevance to its human safety; (ii) to evaluate adverse pharmacodynamic and/or pathophysiological effects of a substance that are observed in toxicology and/or clinical studies; and (iii) to investigate the mechanism of observed and/or suspected adverse pharmacodynamic effects (http://www.emea.eu.int/pdfs/human/ich/053900en.pdf). For situations that are typically encountered during the conventional drug development process, safety pharmacology includes the assessment of the effects of a drug on vital functions (the so-called 'core battery') such as the cardiovascular, central nervous and respiratory systems; these effects should be evaluated before exposing humans to the drug (http://www.emea.eu.int/pdfs/human/ich/028695en.pdf).

In an industrial practice setting, there are several approaches to non-clinical safety pharmacology evaluations. The approaches covered in this book are screening during the early discovery phases, safety assessment before first exposure to humans and safety assessment during clinical development. The progress made in developing high-throughput screening techniques, such as those for unwanted ion channel activity [e.g. human ether-a-go-go related gene (hERG) blockage], has contributed to the establishment of the current practices for early screening campaigns in conventional drug discovery. Compounds that have the lowest safety liabilities are being carried forward for further evaluations. This book provides a useful practical introduction to the studies that are required for conventional drug development and dedicates separate chapters to the application of this approach to drug development for each of the core systems and the renal, gastrointestinal and immune systems. Gad has also included a chapter that introduces the concepts and principles of drug screening. In preparation for the investigational new drug (IND) phase of drug development, safety pharmacology is a mandatory regulatory discipline and studies should be conducted to good laboratory practices (GLPs). However, the book lacks a discussion of how to tackle the case-by-case approaches that could be considered for products where the existing paradigms for safety evaluation would not be appropriate or relevant (e.g. drugs under development for indications in life-threatening disease), which would perhaps have been beneficial for the reader.

Chapter ten (entitled Integration of evaluations of safety pharmacology endpoints into existing study designs) describes the integration of safety pharmacology endpoints into (regulatory) toxicology studies. In addition, this useful and relevant chapter underlines the importance of cross-functional dialogue between scientists in designing, conducting and reporting research programmes. It is clear that Gad has drawn on his wealth of experience in the field of non-clinical safety assessment in the writing of this book. However, with respect to the coverage of the regulatory issues, the book is slightly biased towards experiences from drug development programmes in the US. One possible explanation for this might be that sponsors usually talk to the FDA about the non-clinical study requirements during the IND procedures and during the conduct of early clinical trials, whereas this has not always been the case in Europe.

During the approval procedure, regulatory review typically includes an assessment of how non-clinical safety pharmacology findings have been addressed in clinical studies and how findings could potentially affect the benefit–risk balance for a proposed clinical use of the drug. At this stage, most non-clinical safety pharmacology findings are outweighed by clinical experience. However, some safety pharmacology aspects, for example, the assessment of arrhythmogenic risk, are generally integrated with the assessment of toxicology, metabolism, kinetics and clinical studies to arrive at a conclusion concerning the safe use of the drug in humans. Although suggested in the title, the book does not cover several specific aspects of approval, including the common technical document, regulatory interpretation of findings and product labelling (e.g. a summary of product characteristics). Therefore, some useful supplementary reading can be found from product information that is available from the Freedom of Information (US; http://www.fda.gov) and the European Public Assessment Reports (EU; http://www.emea.eu.int).

Overall, this book stands out in the row of extensive authorships of toxicology consultant Gad. Non-clinical safety pharmacology is an evolving non-clinical (regulatory and scientific) discipline and the book provides an up-to-date useful and practical introduction to the topic.

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