INTRODUCTION

Clinical pharmacology focuses on 'all aspects of the study and use of drugs in humans' with the objective to treat patients rationally. Rational pharmacotherapy revolves around two core questions: 'How does a drug reach its site of action in sufficient quantities?' (*i.e.* pharmacokinetics) and 'How does a drug exert an effect?' (*i.e.* pharmacodynamics), which relates both to intended and unintended effects. Typically, answering these question involves an integration of various areas of knowledge on a pharmaceutical, including molecular mechanisms (sub-cellular level), (patho)physiology of the intended indication(s), drug distribution, interactions, epidemiology, and individual characteristics.[1]

Although the execution of experiments with pharmacologically active substances in living beings dates back to ancient times, this practice became a more scientifical character starting in the renaissance and progressing throughout the 19th century.[2] Conversely, clinical pharmacology is a relatively young discipline with its roots in the 1950s.[3] At that time, the majority of pharmaceuticals was chemically derived, meaning that raw materials are converted to the active (drug) substance by a series of chemical reactions. It is therefore not surprising that pharmacological knowledge and theories are largely based on experience with these so-called 'small molecules'.

Since the 1980s biotechnologically derived drug substances have emerged.[4,5] These 'biopharmaceuticals' (or 'biologicals') are produced by manipulated organisms or living cell systems, usually via recombinant DNA techniques. Naturally occurring human proteins – including coagulation factors, hormones, enzymes, and plasma constituents – have been manufactured that way, as well as tailored or specifically developed proteins, mostly monoclonal antibodies (MABs), which can be directed against signal peptides, blocking receptors, or targeting cell types for destruction.[4–8]

Admittedly, drugs that could qualify as biopharmaceuticals, such as insulin, were already in use by the 1920s. However, these drugs were then extracted from animal tissues, which posed additional difficulties in terms of potency and safety.[9] Many other drugs also have an origin in nature; for example, those derived from (medicinal) plants (*e.g.* digitalis, morphine, salicylic acid, cytostatics) or from microbial secretions (*e.g.* many antibiotics and certain oncolytics), but such drugs are nowadays chemically synthesised.[10–14]

Biopharmaceuticals are among the most celebrated drugs; they have offered perspective to patients with previously incurable enzymatic or hormonal deficiencies, they can specifically target cancer cells as opposed to the whole-body cytotoxic attack by chemotherapeutical agents, and they can block the activity of signal peptides that play a role in the pathophysiology of – for example – rheumatological diseases. Because of these benefits and the blockbuster state usually associated therewith, biopharmaceuticals are increasingly being developed, with an expected share of 27% of the total pharmaceutical market in 2020 (73% of the top 20 product sales).[15]

In contrast to small molecules, biopharmaceuticals are much more complex in structure, with molecular weights of 3.5 (calcitonin) to 150 kDa (monoclonal antibodies), and sometimes consist of subunits (quaternary structure). As a result of these differences, biopharmaceuticals display other pharmacokinetic properties compared with small molecules. For example, they distribute more slowly over the body than small molecules, and are mainly eliminated via catabolism into amino-acids and target-mediated pathways.[16–19]

However, the pharmacokinetics of biopharmaceuticals are commonly described by models that were suitably applied to small molecules. In the ***first section*** of this thesis (*Sticky proteins*), some pharmacokinetic aspects of large therapeutical proteins will be explored, especially those that are not adequately covered by current pharmacokinetic models or theory. The focus will be on monoclonal antibodies, which represent the largest class of biopharmaceuticals, and because of their very slow plasma clearance, MABs are particularly useful in investigating the complex pharmacokinetics of biopharmaceuticals.

The ***first chapter*** studies the use of a population modelling approach in testing pharmacokinetic biosimilarity of a monoclonal antibody. This technique

is widely used during drug development to describe and predict drug concentration in the body over time, but in biosimilarity research a non-model approach is still favoured. The benefit of a pharmacokinetic model is that it can deal with the non-linear elimination pathways.

The following two chapters (***2 & 3***) discuss rises in plasma concentration after the cessation of intravenous administration, which have been frequently observed for various biopharmaceuticals. These observations are not in agreement with the current understanding of how infused molecules behave. Even the aforementioned, more sophisticated, mathematical models that incorporate the known complex elimination routes cannot account for these findings.

Apart from the higher level of complexity in pharmacokinetics, biopharmaceuticals are associated with other safety hazards than small molecules. First, as a result of the production in (non-human) host cell systems, other substances than the pharmaceutical (called impurities) are introduced during manufacturing, some of which are harmful when administered (*e.g.* endotoxin, peptidoglycans, or flagellin from bacterial hosts) or possess unintended ('pharmacological') activity. Second, safety pharmacology studies can be misleading, because the target that interacts with the biopharmaceutical or the impurity is exclusively expressed in human beings. Additionally, the toxicity often is the result of an intricate interplay of multiple cell types and effector pathways which can be difficult to simulate in the laboratory. Again, the preclinical testing strategy to detect safety concerns and prevent dangerous drugs from entering the clinical phases of development has not changed much since the introduction of biopharmaceuticals. The ***second section*** of this thesis (*Dirty proteins*) highlights shortcomings in assessments of adverse immunostimulation, which is a propensity of some monoclonal antibodies, but is also encountered as a result of impurities in the drug product.

In ***chapter 4***, an attempt is made to reproduce the observed clinical response following administration of the monoclonal antibody trastuzumab in an *ex vivo* stimulation test, which included material from 'responders' and 'non-responders'. Trastuzumab is an interesting example, because only 40% of patients shows signs of an inflammatory reaction[20] and even lower percentages have been reported in healthy volunteers.[21–23]

Chapter 5 describes the history of a biopharmaceutical where so-called host cell impurities, including flagellin, induced unanticipated adverse immunostimulation, which resulted in a severe delay in clinical development. In this case, an *ex vivo* simulation test could detect cytokine release caused by the impurities. Thus, the question is raised why preclinical testing failed in generating a safety signal, especially since all the applicable guidelines were meticulously followed. The next chapter (***6***) answers this question and illustrates several shortcomings of the current testing strategy based on two examples of adverse immunostimulation caused by impurities.

REFERENCES

1 Aronson JK. A manifesto for clinical pharmacology from principles to practice. *Br J Clin Pharmacol* 2010; **70**: 3–13.
2 Ivy AC. The history and ethics of the use of human subjects in medical experiments. *Science* 1948; **108**: 1–5.
3 Miller RR. History of clinical pharmacy and clinical pharmacology. *J Clin Pharmacol* 1981; **21**: 195–7.
4 Buckel P. Recombinant proteins for therapy. *Trends Pharmacol Sci* 1996; **17**: 450–6.
5 Nagle T, Berg C, Nassr R, Pang K. The further evolution of biotech. *Nat Rev Drug Discov* 2003; **2**: 75–9.
6 Walsh G. Biopharmaceutical benchmarks 2010. *Nat Biotechnol* 2010; **28**: 917–24.
7 Walsh G. Biopharmaceutical benchmarks 2014. *Nat Biotechnol* 2014; **32**: 992–1000.
8 Sanchez-Garcia L, Martín L, Mangues R, Ferrer-Miralles N, Vázquez E, Villaverde A. Recombinant pharmaceuticals from microbial cells: a 2015 update. *Microb Cell Fact* 2016; **15**: 33.
9 Sönksen PH. The evolution of insulin treatment. *Clin Endocrinol Metab* 1977; **6**: 481–97.
10 Wachtel-Galor S, Benzie IFF. Herbal Medicine: An Introduction to Its History, Usage, Regulation, Current Trends, and Research Needs. In: Benzie IFF, Wachtel-Galor S, eds. Herbal Medicine: Biomolecular and Clinical Aspects, 2nd edn. Boca Raton, Florida: CRC Press/Taylor & Francis, 2011.
11 Mann J. Natural products in cancer chemotherapy: past, present and future. *Nat Rev Cancer* 2002; **2**: 143–8.
12 Peláez F. The historical delivery of antibiotics from microbial natural products—can history repeat? *Biochem Pharmacol* 2006; **71**: 981–90.
13 García Jordá E. Sacred herbs. *Clin Transl Oncol* 2008; **10**: 685–7.
14 Zaffiri L, Gardner J, Toledo-Pereyra LH. History of antibiotics. From salvarsan to cephalosporins. *J Invest Surg* 2012; **25**: 67–77.

15 Gene Techno Science. Overview of global pharmaceutical market. 2015. http://www.g-gts.com/en/business/market/ (accessed Aug 1, 2017).
16 Keizer RJ, Huitema ADR, Schellens JHM, Beijnen JH. Clinical pharmacokinetics of therapeutic monoclonal antibodies. *Clin Pharmacokinet* 2010; **49**: 493–507.
17 Dostalek M, Gardner I, Gurbaxani BM, Rose RH, Chetty M. Pharmacokinetics, pharmacodynamics and physiologically-based pharmacokinetic modelling of monoclonal antibodies. *Clin Pharmacokinet* 2013; **52**: 83–124.
18 Shi S. Biologics: an update and challenge of their pharmacokinetics. *Curr Drug Metab* 2014; **15**: 271–90.
19 Tibbitts J, Canter D, Graff R, Smith A, Khawli LA. Key factors influencing ADME properties of therapeutic proteins: a need for ADME characterization in drug discovery and development. *MAbs* 2016; **8**: 229–45.
20 Chung CH. Managing premedications and the risk for reactions to infusional monoclonal antibody therapy. *Oncologist* 2008; **13**: 725–32.
21 Wisman LAB, De Cock EPM, Reijers JAA, *et al.* A phase I dose-escalation and bioequivalence study of a trastuzumab biosimilar in healthy male volunteers. *Clin Drug Investig* 2014; **34**: 887–94.
22 Yin D, Barker KB, Li R, *et al.* A randomized phase 1 pharmacokinetic trial comparing the potential biosimilar PF-05280014 with trastuzumab in healthy volunteers (REFLECTIONS B327-01). *Br J Clin Pharmacol* 2014; **78**: 1281–90.
23 Morita J, Tanaka M, Nomoto M, *et al.* Pharmacokinetic bioequivalence, safety, and immunogenicity of DMB-3111, a trastuzumab biosimilar, and trastuzumab in healthy Japanese adult males: results of a randomized trial. *BioDrugs* 2016; **30**: 17–25.