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Summary, discussion and future perspectives

Summary
In Chapter 1, a short general introduction about Crohn’s disease (CD) and ulcerative colitis (UC) is presented, with a description of the medical therapy and especially treatment with mesalazine (5-ASA) (part I) and anti-TNF-alpha (part II). It starts in part I with 5-ASA which is the cornerstone for medical treatment in UC. We described the side effects and suggest options for a desensitization protocol and optimization of the treatment. In part II we focus on agents against TNF-alpha and their use in inflammatory bowel disease (IBD). We focus on trough levels and antibodies against these drugs to optimize the treatment, study genetic risk factors and explore the potential of oral treatment with Infliximab (IFX).

Part I: Mesalazine

In Chapter 2 we describe symptoms of patients who are intolerant for 5-ASA showing an inflammatory response after rechallenge with fever, increased CRP, vomiting and diarrhea. Our study showed that patients with UC and intolerance to 5-ASA can be safely rechallenged but only around 25% will tolerate the drug. We also described a blinded, randomized crossover rechallenge and a three days desensitization protocol for 5-ASA intolerance in UC patients. This is important as patients who report adverse reactions need careful evaluation as it may lead to discontinuation of an important drug for the treatment of UC. We show that patients with inflammatory side effects like fever, increased CRP, vomiting and diarrhea upon mesalazine administration cannot be desensitized with the presented three day desensitization protocol.

Rare side effects of 5-ASA are hepatotoxicity, nephrotoxicity and acute pancreatitis. In Chapter 3 we described a case of a UC patient with a 5-ASA induced auto-immune hepatitis/PSC overlap syndrome without signs of hypersensitivity. We performed a brief overview of current literature regarding 5-ASA induced hepatotoxicity which is a known but rare complication of 5-ASA. Nephrotoxicity is also a rare reaction to 5-ASA. In Chapter 4 an analysis was performed to assess the clinical features of patients with IBD who developed chronic renal damage after administration of 5-ASA compounds. This rare condition appears to be more common in male patients, can occur after many years of drug administration and, once recognised, is only reversible in approximately one third of patients. In the same study a genome wide association study was performed and identified a HLA class II allele to be associated with drug-induced renal injury. This genome-wide association study identified a suggestive association in the HLA region (P=1x10-7) with 5-ASA induced nephrotoxicity. A subgroup analysis of patients who had a renal biopsy demonstrating interstitial nephritis significantly strengthened this association.
Part II: Anti-TNF-alpha

Measurement of trough levels and antibodies against anti-TNF-alpha agents is useful in the treatment of IBD with anti-TNF-alpha agents. This is of importance as therapeutic drug monitoring (TDM) can lead to optimization of anti-TNF-alpha therapy. The presence of anti-drug antibodies is associated with infusion reactions and loss of response and it is therefore important to have reliable assays available. In Chapter 5 we determined that there is a good correlation of IFX and antibody to IFX level measurements between the assays developed by Sanquin Research (Amsterdam), the Laboratory for Pharmaceutical Biology (Leuven) and the commercially available kit from BMD. Nevertheless, the BMD kit detected false positive IFX levels in 18% of the samples, including samples only containing antibodies to IFX and antibodies to ADA. IFX-biosimilars are available. These biosimilars are expected to have the same specificity but are marketed at a much lower price. In Chapter 6 we showed that the second generation anti-TNF-alpha drugs (like Golimumab, Etanercept and Certolizumab) show increased TNF-alpha neutralizing potential compared to first generation anti-TNF-alpha drugs. We also showed that the originator IFX (Remicade) and the IFX biosimilar CT-P13 (Inflectra) have the same neutralizing capacity. Furthermore, we show that antibodies to IFX (ATI) show cross-reactivity toward the IFX biosimilar CT-P13 (Inflectra) proving that the CT-P13 (Inflectra) IFX biosimilar also has the same antigenic properties. As described, some patients treated with IFX or Adalimumab (ADA) develop antibodies against the drug. Although the co-administration of an immunosuppressant prevents the development of antibodies to a certain level we are currently unable to predict which patients are at risk of developing these antibodies. Recently genetic studies identified risk variants that play a role in the development of these antibodies. In Chapter 7 we were able to replicate the HLA-DQA1*05 allele associated with formation of antibodies to IFX and ADA in IBD patients. We also identified eight suggestive association signals in non-HLA regions that need to be replicated in larger cohorts. The population pharmacokinetics of IFX have been described earlier. In chapter 8 we developed a pharmacokinetic model that could be used to optimize TDM of IFX in IBD patients. Simulations from the model show that dosing every 12 weeks can be considered in the treatment of patients with IBD with IFX, but only in those that do not show ATI. This strategy reduces IFX-therapy related visits to the hospital with one third. Studies have shown that tissue IFX concentration correlates with a better and sustained response in CD and that IFX exerts its effect at least partly by local anti-inflammatory and immunomodulatory effects in the bowel. Local IFX treatment has also been proven effective in fistulising perianal disease. Furthermore, the development of a subcutaneous formulation of CT-P13 IFX is described. Also, oral administration of anti-TNF-alpha has been shown to be safe in healthy volunteers. In this thesis we describe the colopulse technology which is a capsule specifically developed to target the ileo-colonic region (chapter 9). This local administration could therefore be ideal for CD patients with active disease of the terminal ileum. In Chapter 10 we present a protocol Towards Mucosal Application of IFX. The objective of this protocol is to describe a proof of concept.
study to treat patients with active ileo-colonic CD with orally administered ColoPulse IFX tablets instead of intravenously administrated IFX. Enrollment of participants to this study will start as soon as possible.

Discussion and future perspectives

Mesalazine and intolerance

In recent years there are more and more strategies in the treatment of IBD. 5-ASA is a very effective and safe drug in the treatment of UC, but unfortunately some patients develop adverse reactions.\textsuperscript{17,18} These patients might benefit from 5-ASA rechallenge or with another formulation of 5-ASA. Ideally a rechallenge should be performed in a blinded fashion. However, as shown in this thesis this showed to be hard to realize in clinical practice. A rechallenge with another formulation of 5-ASA is probably a more feasible strategy in clinical practice. Our study identified patients with an inflammatory response upon 5-ASA that has not previously been reported. The mechanism behind this inflammatory response is unclear. Successful desensitization in patients with 5-ASA intolerance has been described. These patients had a variety of side-effects, e.g. urticaria, fever, exanthema and diarrhea.\textsuperscript{19-24} In our study all patients with this rapid inflammatory response upon 5-ASA administration could not be desensitized with a three days desensitization protocol. There are several explanations why our desensitization protocol could have failed. First, we found no evidence for mast cell activation or an immune mediated reaction. Second, a three day desensitization protocol may be too short. However, with the development of an inflammatory response within two hours after on average 5-ASA dose of 200 mg, it is unlikely that a longer desensitization protocol would be effective. Additionally, it is possible that the dosages of 5-ASA were increased to fast. However, we recommend not to rechallenge UC patients with an inflammatory response upon mesalazine administration and these patients cannot be desensitized with a three day desensitization protocol (Chapter 2). Beside these side effects every physician should be familiar with other adverse effects associated with mesalazine therapy. Rare side effects like a mesalazine induced auto-immune hepatitis/PSC overlap syndrome could be recognized by assessing liver enzymes and renal function after initiating mesalazine treatment (Chapter 3 and 4).

Additional (genetic) studies have to be conducted to predict these side effects. A so called genetic passport in the future could potentially predict the development of these rare adverse effects so these drugs can be avoided, or monitoring intensified, in high-risk patients. These pharmacogenetic testing before the start of treatment may be useful to maximize the benefit of the treatment in any particular patient. Pharmacogenetic testing also has the potential to optimize drug selection and dosing as well as to minimize potential harm of drug toxicity. Furthermore, it may be possible to avoid drugs that are ineffective or harmful for a particular patient, optimize the use of (cheaper) conventional drugs and achieving optimal dosages faster which ultimately will lead to a reduction of healthcare costs.
Optimizing anti-TNF-alpha therapy

Anti-TNF-alpha have proven to be effective in the treatment of CD and UC.25,26 These drugs are administered at fixed dose and intervals derived from dose finding studies for IFX.27 Observational studies showed that approximately 10% of patients per year lose their response to these anti-TNF-alpha antibodies.28 One factor associated with loss of response is immunogenicity, whereby the production of antidrug antibodies is associated with infusion reactions and an accelerated antibody clearance resulting in lower anti-TNF-alpha antibody titers.29 In this thesis we determined the correlation between academically developed assays (Leuven and Amsterdam) that were used in several studies to detect IFX drug levels and anti-IFX-antibodies.30-32 The sensitivity of the three assays to detect antibodies to IFX was comparable (Chapter 5). We did not correlate the results of the tests with the clinical situation of the patient. Observational studies have demonstrated a relationship between anti-TNF-alpha drug concentrations, the presence of ATI and clinical outcome.31-36 IFX exposure below 3 μg/mL increases risk of developing ATI’s. Identification of influential pharmacokinetics and ATI factors improves prediction of IFX levels, potentially allowing individualized dosing and cost reduction.37 The place of TDM with early serial trough and ATI level measurements is doubtful. It will probably optimize anti-TNF-alpha treatment.18,39 Other studies contradict the clinical importance of TDM. The TAILORIX study of biologic naïve luminal CD patients receiving IFX compared two groups where dose escalation was based on trough levels to one clinically-based dose escalation group. At one year no differences between clinical and endoscopic outcomes were observed between groups. However, the results may have been affected by the large proportion of the clinical care group receiving dose escalation.40,41 Easier assessment of IFX through levels might be performed by using a capillary fingerstick blood test. An early report showed that IFX concentration in capillary fingerstick blood as determined with a rapid test is equal to the concentration found in venous blood. This may allow rapid adaptation of the drug regime or infusion period.42 TDM does have an important place in identifying ATI. ATI may be neutralizing or non-neutralizing, depending on the binding site. Neutralizing ATI produced in response to anti-TNF-alpha bind to the epitope binding (Fab’)2 region of the anti-TNF-alpha, thereby reducing the agents’ therapeutic activity.43 By contrast, non-neutralizing antibodies do not prevent binding of the agents to target molecules, and hence do not reduce the efficacy of biologic agents; they do, however, impact the pharmacokinetics, by accelerating clearance of the agent.44 The clinical implications of immunogenicity are a concern for effective treatment; further research, particularly into the more recently approved biologics, is required. IFX-biosimilars, such as CT-P13 (Inflectra/ Remsima), Celltrion) and Flixabi (Biogen) are available now.45-48 These biosimilars are expected to have the same specificity and the same sequence as the original molecule IFX (Remicade), but are marketed at a much lower price than the first-generation anti-TNF-alpha blocking agents, which will make treatment of these diseases more cost-effective. We show that IFX and the IFX biosimilar CT-P13 (Inflectra) have the same neutralizing capacity as the original IFX. Furthermore, we show that ATI show cross-reactivity toward the IFX biosimilar CT-P13 (Inflectra) proving that the CT-P13 (Inflectra) IFX biosimilar
also has the same antigenic properties and therefore the clinical implication is that patients with ATI should not be switched to IFX biosimilar (Chapter 6). The difference between IFX biosimilar and IFX originator is the glycosylation profile. In samples with originator IFX and biosimilar IFX there is a significantly different N-glycosylation profile that showed no significant variations in biological activity, suggesting that the differences are probably not therapeutically significant. The NOR-SWITCH clinical trial showed that switching from IFX originator to IFX biosimilar CT-P13 was not inferior to continued treatment with IFX originator. Other clinical trials showed also no clinical impact on clinical outcomes after switching to IFX biosimilar. As the field of IBD is moving towards personalised medicine, it is important to conduct studies that focus on immunogenicity because future immunogenicity assays might serve as biomarkers to predict treatment response to anti-TNF-alpha therapy (Chapter 7). As discussed before a genetic passport may be useful in the future to predict which patients will not tolerate certain medications or who has increased immunogenicity and should be treated with combination (thiopurine or methotrexate with biological). However, small studies investigating primary non-response by genetic determinant have reported conflicting data or were underpowered. HLA-DQA1*05 haplotype was identified as a genetic determinant of immunogenicity to anti TNF-alpha. Pre-treatment genetic testing has not yet been considered for clinical use. The mechanism of anti-TNF alpha is still a matter of debate. In a study of the in vitro and in vivo mechanisms of anti-TNF alpha, it was found that FcγR engagement by anti-TNF-alpha was obligatory for improvement of colitis in mice and development of regulatory CD206+ macrophages. A hypo-fucosylated form of anti-TNF binds FcγRIIIa with more affinity and induced development of CD206+ macrophages in human PBMCs, particularly PBMCs that express low-affinity FcγRIIIa. Therefore, hypo-fucosylated anti-TNF might be more effective in patients with IBD. The University Medical Center Groningen (UMCG) developed a fluorescent tracer for labelling biologicals. With these studies we will gain insight into the biological distribution and concentrations in the inflamed gut. Furthermore we can identify the biological target cells in the inflamed gut inflammation using near-infrared fluorescence molecular endoscopy (NIR-FME). By gaining insight in local biological concentrations, distribution and target cells, we will better understand the mechanism of action and can optimise the dosing of biologicals. Currently there are no good clinical or biochemical predictors of response to a biological. In the previous years the majority of studies that have tried to investigate these factors have provided controversial results. Primarily, this could be due to different experimental settings and to the different definitions of disease remission used. Moreover, many studies included a small number of patients and most of them were retrospective. This has produced so far a low use of predictive factors in daily clinical practice. Examples of clinical parameters which predict response to anti-TNF-alpha are younger age, higher CRP and Hb baseline levels which are associated with better response to anti TNF-alpha. A recent study showed that the major modifiable factors associated with treatment ineffectiveness were low drug concentrations and immunogenicity.
Discussion

this thesis we developed a pharmacokinetic model that could be used to optimize TDM of IFX in IBD patients, but it needs to be confirmed in a prospective clinical trial. Considering the high percentage of patients in remission with trough levels ≤ 3.0 mg/L, dose intensification or modification in dose intervals should always be combined with clinical and/or endoscopic disease activity parameters (Chapter 8). Recently great progress has been made single cell analysis of the infiltrate in IBD. These new techniques can be used to reveal tissue specific characteristics and drug target but can potentially also predict the response to treatment in individual patients.\textsuperscript{58}

**Anti-TNF-alpha therapy in the future**

Previous studies showed that local treatment with IFX is efficacious like topic IFX injection for rectal stenosis in CD patients.\textsuperscript{59} Local IFX treatment has also been proven effective in fistulising perianal disease.\textsuperscript{1-5} Topical IFX treatment presumably results in a continuous IFX exposure at the site of inflammation with fewer side effects compared to systemically administered IFX. The development of a subcutaneous formulation of CT-P13 IFX for rheumatoid arthritis is described.\textsuperscript{7} These patients had more stable steady state therapeutic blood levels of IFX and have lower rate of ATI compared with patients receiving IFX IV treatment. In IBD patients also efforts has been made to use subcutaneous IFX. Preliminary results suggest that one year treatment with subcutaneous CT-P13 IFX is similar in efficacy and safety compared to intravenous administration in CD patients.\textsuperscript{6} There are gut specific oral agents in treatment of IBD like slow release mesalazine and budesonide. Efficacious oral IFX treatment has major advantages for patients with CD. First, patients no longer need to visit the hospital for medical treatment. Second, patients will not be punctured for infusion therapy and also the personnel do not have to receive a training for the procedure. Third, oral treatment eliminates infusion quality of life influencing related complications, such as extravasations and infusion reactions. Finally, local treatment of anti-TNF-alpha reduces the risk of developing of antibodies to the anti-TNF-alpha containing agent. An oral administration of the non-absorbable recombinant anti-TNF-alpha fusion protein, PRX-106 has been shown to be safe in healthy volunteers.\textsuperscript{8} A novel polyclonal anti-TNF-alpha antibody (AVX470) was effective in treating mouse models of colitis, delivering the anti-TNF-alpha to the site of inflammation with minimal systemic exposure.\textsuperscript{60} Another novel anti-TNF-alpha domain antibody (V565) could be detected by ELISA in post-dose serum of colitis mice, but not in naïve mice, demonstrating penetration of disrupted epithelium.\textsuperscript{9} The colopulse capsule is specifically developed to target the ileo-colonic region (chapter 9) and may therefore be perfect for CD patients with active disease of the terminal ileum (chapter 10) with possible less immunogenicity and enhanced patient satisfaction.

In conclusion, this thesis studies side effects and complications of 5-ASA and anti-TNF-alpha therapy and suggests strategies to optimise treatment with these drugs in IBD. A genetic passport to predict adverse effects, detection of immunogenicity, dose optimisation and the use of gut selective delivery systems harbours great promise for the near future in the treatment of IBD.
Chapter II

References


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