Small molecules, big consequences

Impact paragraph

Social-economical and clinical relevance

Chronic low-grade inflammation is a critical factor in progression of metabolic diseases including type 2 diabetes, NASH and cardiovascular diseases. Chronic inflammatory disorders have been identified as the leading cause of death worldwide today and their prevalence is going to steadily increase in the coming years. In European countries, the forecasted prevalence of NASH is 29.5% by 2030 [1]. Moreover, NASH and IBD patients incur substantial financial costs besides poor quality of life. In Europe, the annual healthcare costs incurred by IBD patients were estimated to be around €5.6 billion [2], while those related to NAFLD were estimated to be €35 billion [3] placing high economic burden on the society.

The shortage of early markers that can accurately differentiate NASH from steatosis and the lack of detailed mechanistic knowledge on NASH progression are the main obstacles in diagnosing and treating NASH, making liver transplantation the only therapeutic resort [4]. Although recent years have shown advancements in non-invasive imaging, to date, no (cost)effective methods are available to establish novel non-invasive diagnostic tools and therapeutic targets in the context of NAFLD. New pipelines of drugs should not only focus on reversing steatohepatitis but should also prove to be non-toxic and economical.

The current thesis demonstrated the specific inhibition of extracellular CTSD activity to be a novel strategy for the prevention and treatment of NASH. Even though cathepsin inhibitors are being extensively investigated for their therapeutic application in cancer and bone disorders, most clinical trials involving these inhibitors are abandoned due to their off-target effects [5]. In contrast to this, the targeted cathepsin inhibition followed in this thesis is promising since it specifically blocks the extracellular cathepsin fraction while preserving the essential physiological functions of intracellular cathepsin fraction, thus limiting toxic side effects. This approach thus can decrease the costs spent in clinical development and increase the chances of success in clinical trials. Furthermore, these small molecules are valuable for both liver disease and obesity-related pathologies since extracellular CTSD inhibition improved metabolic parameters including insulin resistance and dyslipidemia. Since extracellular CTSD activity is markedly impaired in atherosclerosis, cancer and lysosomal storage diseases such as NPC1, the findings from the current thesis can be extended to such co-morbidities thereby further increasing the clinical relevance. In addition to its therapeutic role, plasma CTSD has enormous potential as an early non-invasive biomarker for NASH, thus helping in early diagnosis and treatment of NASH.

Another exciting approach to alleviate lipid storage problems is to use cholesterol extracting compounds such as cyclodextrins, which are already being extensively evaluated as treatment options in Alzheimer's, atherosclerosis, Krabbe and Niemann-Pick type C1 diseases [6]. Concomitant with the beneficial cholesterol-depleting effects, the current thesis uncovered the time-dependent pro-inflammatory effects of 2-hydroxypropyl-β-cyclodextrin (CD) in several models of metabolic inflammation. While CD can help to reduce the disease burden in patients, our results warrant careful monitoring of CD in clinic to prevent unwanted effects. These results thus have high clinical impact and pave a way to optimize time and dose regimes for future use of cyclodextrins. Greater affordability and improved safety of CDs can further augment their medical potential.

Besides the above-mentioned contemporary therapeutic approaches, this thesis also discussed the importance of non-pharmacological stress-reduction strategies which can help in societal cost saving. Effective translation of these findings in larger patient populations can help draw more definite and detailed conclusions. Altogether, this endeavor can lead to reduction in socioeconomic burden and consequently improve quality of life in patients suffering from chronic inflammatory disorders.

Novelty of the concept

In this thesis, we investigated novel intervention strategies to treat metabolic inflammation. Along with our previous findings, the current thesis mechanistically demonstrated the pivotal role of cathepsin D in lipid-induced hepatic inflammation. Using the state of the art *in sillico* approach, we designed and validated specific small-molecule inhibitors against cathepsin D in a preclinical setting. Here, we show for the first time that specific inhibition of extracellular CTSD has beneficial effects in diminishing hepatic steatosis, inflammation and insulin resistance associated with NAFLD progression. Besides exploiting the function role of CTSD, we uncovered the potential mechanisms regulated by intra and extracellular CTSD fractions in the content of metabolic inflammation.

The benefits of suggested pharmacotherapies can be maximized by adhering to healthy lifestyle habits. While the information on lifestyle interventions for metabolic diseases is mostly limited to diet and exercise, this thesis consolidates the preclinical and clinical evidence on the role of stress reduction therapies in modulating inflammation that have enormous benefits in improving the quality-of-life in patients and reducing the healthcare burden. Also, this thesis provided critical data on the harmful pro-inflammatory effects of CD in the context of cholesterol-induced metabolic inflammation, which provides a novel view on CD as therapeutic compound. As CD shuttles cholesterol directly from the trapped sites and has effective renal clearance, it is currently considered promising for patients who are intolerant to statin therapy or cannot maintain a low-calorie diet. As such, our novel observations challenge the clinical use of CD and call for a careful reassessment of the drug's safety profile.

Future perspectives and potential application

Although the results in this thesis provide new diagnostic and therapeutic approaches for chronic inflammatory disorders, further work needs to be done to ensure that our findings are clinically translated to patients and the general public. In addition to other pre-clinical models, the results of the current project will be validated in blood-derived monocytes from healthy volunteers and samples from NASH. Since NASH patients have high risk of developing hepatocellular carcinoma (HCC), we planned to examine the cathepsin inhibitors in experimental models of HCC. As NASH shares the common pathogenic link with atherosclerosis [7], it is worthwhile to confirm and broaden the findings of the current thesis in atherosclerosis models. The next important step in human cathepsin research would be designing a personalized therapy with cathepsin inhibitors. For instance, optimizing the dosage of inhibitors by evaluating them in different cohorts would help a great deal in formulating efficient treatment regimens with even less side-effects [8].

Besides the role of CTSD, investigating the role of other lysosomal enzymes in the context of inflammation seems attractive. Several extracellular cathepsins are shown to mediate inflammatory

responses by means of their proteolytic activity on the extracellular matrix components, chemokines, cytokines and different membrane receptors. In line, overexpression and secretion of cathepsins is one of the common denominators in several inflammation related pathologies including cancer, atherosclerosis, auto-immune diseases, and arthritis [9]. For instance, cathepsin B is a well-known prognostic marker for several cancers [10]. Moreover, CTSD secreted by breast cancer cells is known to degrade endogenous cysteine protease inhibitor, cystatin C which subsequently leads to increased proteolytic activity of cathepsin B, thus exacerbating tumor progression and metastasis. Besides, blocking CTSD as well as CTSB/CTSL reduced inflammation in experimental colitis [11]. Therefore, it is reasonable to infer that extracellular cathepsins might synergistically contribute to the inflammatory responses. Future experiments with extracellular inhibitors of CTSB/L along with CTSD can clarify such speculations.

Lifestyle-induced weight loss and physical activity are highly recommended first line treatments for NASH patients. However, persistent lifestyle habits and weight loss are hard to reach and, unfortunately, lifestyle modifications alone are not effective in every individual. Nevertheless, it would be interesting to use combination treatment of the proposed pharmacological targets with the lifestyle interventions.

Lastly, instead of interfering with the inflammatory pathways, it is worthy to directly target the origin sites of inflammation, for example the gastrointestinal tract. High fat diet and obesity are known to disrupt the intestinal microflora composition and induce intestinal barrier dysfunction. Ultimately, these modifications lead to leaky mucosal barrier and release of bacterial metabolites that trigger a pro-inflammatory response leading to metabolic inflammation as seen in type 2 diabetes, NASH and atherosclerosis [12]. Therefore, it is worthwhile to study the gut microbiota–metabolism axis in the context of NASH. Collectively, the intricate relationship between lysosomal lipid metabolism and inflammation is gaining momentum and the therapeutic route for this field looks very promising.

References

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