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CILIP - A NEW RECOMBINANT MICROBIAL LIPASE FROM *TETRAHYMENA THERMOPHILA* FOR TREATMENT OF EXOCRINE PANCREATIC INSUFFICIENCY

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INTRODUCTION

Exocrine pancreatic insufficiency (EPI) caused by inflammation or pancreatic tumors results in nutrient malfunction by a lack of digestive enzymes and neutralization compounds. A replacement therapy with enzymes derived from pancreatin requires high dosages, bares risks of impurities, viral contamination as well as intolerances and ethical or religious rejection. Despite satisfactory clinical results with current enzyme therapies, a normalization of fat absorption in patients is rare. In addition, high-dose application forms have not been available in several countries for years, which further exacerbates the problem of high pill burden for patients. An improved therapy is required which allows high dosage of enzymatic units with a high activity and stability in the gastrointestinal tract under the conditions of an EPI without any enteric coating which may lead to delayed release in some patients.

Cilian develops a microbial lipase from *Tetrahymena thermophila* (CiLip) which can be purified very easily and inexpensively via crystallization. The high specific activity lowers the pill burden significantly compared to pancreatin based products. In contrast to 3.3 g of enteric coated pancreatin products per day only 0.37 g of **uncoated crystallized CiLip** is sufficient for the same coefficient of fat absorption (CFA). In addition, the crystallization process enables a cost-effective production and an easy scale-up to meet the constantly growing demand.

AIM

- **High safety** due to the contained production process in a non-mammalian organism.
- **Lower pill burden** with significantly reduced pill size.
- **Better supply** security through scalable process without dependence on other production chains.

REFERENCES

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[2]: **Alexander Brock, Ingo Aldag, Stella Edskes, Marcus Hartmann, Torsten Herzog, Waldemar Uhl, Juergen Schnekenburger**. Novel ciliate lipases for enzyme replacement during exocrine pancreatic insufficiency. *Eur J Gastroenterol Hepatol*. 2016 Nov;28(11):1305-12.

[3]: **Andreas Minh Luu, Alexander Brock, Sabrina Ritz, Sandra Junghänel, Ingo Aldag, Stella Edskes, Marcus Hartmann, Michael Hessler, Michael Praktiknjo, Philip Arnemann, Christian Ertmer, Waldemar Uhl, Juergen Schnekenburger, Torsten Herzog.** Long term follow-up of a simplified and less burdened pancreatic duct ligation model of exocrine pancreatic insufficiency in Goettingen Minipigs. *BMC Gastroenterol*. 2020 Nov 30;20(1):403

RESULTS

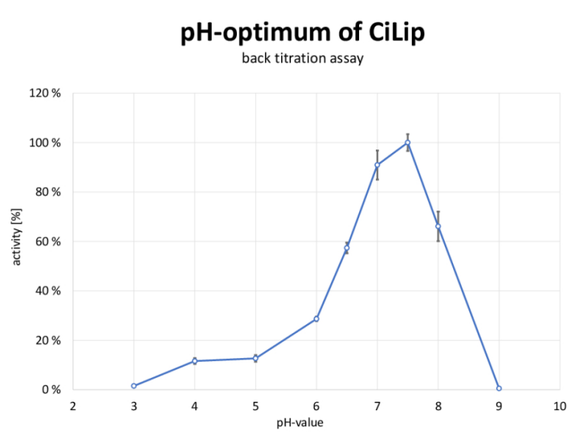

Figure 1: pH-optimum of CiLip-Lipase measured with back titration (n=3). Lipase activities are given as percent values relative to the pH-optimum at 7.5.

In vitro data of CiLip showed a **broad pH-optimum** between pH 6.5 and 8.0 with more than 60 % of the maximum activity (fig.1). Since CiLip is already active at pH 4.0, fat digestion can begin postprandially directly in the stomach.

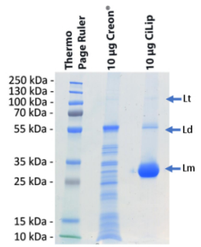

Figure 2: SDS-Gel with 10 µg of CiLip purified by crystallization compared to 10 µg of pancreatin from Creon® after staining with Coomassie Brilliant Blue.

Crystallization process

The enzyme can be purified to a **purity above 95 %** by a crystallization process that is cost-effective and readily scalable. In figure 2, after crystallization, only the bands for the lipase monomer (Lm), a dimer (Ld), and a trimer (Lt) band remain. This finding could be confirmed by immunoblot (data not shown).
Crystalline lipase is highly soluble and can be stored without much loss of activity even at room temperature (data not shown).

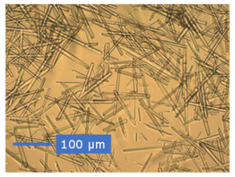

Figure 3: Crystallized CiLip-Lipase from *Tetrahymena thermophila*. Under the right conditions, crystallization starts quickly and spontaneously. In the process, the lipase crystallizes almost quantitatively and can be easily harvested and washed with a filtration centrifuge after completion of the crystallization process.

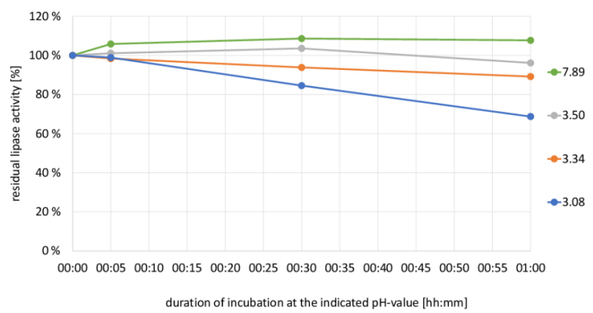

Figure 4: CiLip-Lipase measured with a Rhodamine fluorescence assay. Samples were incubated at the indicated pH-value for 5 to 60 minutes. Residual lipase activity was measured at pH 7.0.

CiLip also proved to be significantly more **stable to low pH-values** than Creon®. In pH-stability tests, CiLip was very stable over a period of 60 min. While for pH-values of 3.5 and above no decrease of activity was observable, at pH 3.34 the activity decreased to 90 % after 60 min while the activity at pH 3.08 decreased to approx. 70 % within 60 min (fig. 4).
Due to its stability to low pH-values, an enteric coat can be omitted for CiLip, which significantly extends the time available for the digestion of fats.
Substrate specificity studies showed a much more consistent activity towards long, middle and short chain fatty acids compared to Creon® (data not shown).

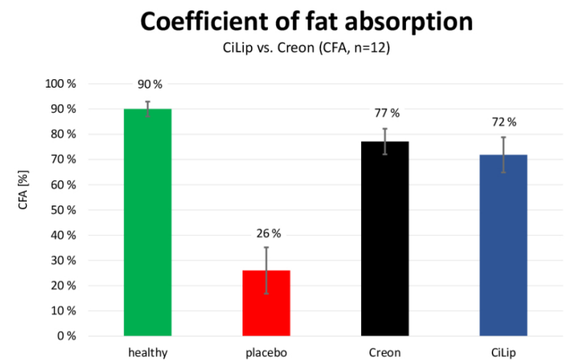

Figure 5: *In vivo* test of the coefficient of fat absorption (CFA) in 12 Goettingen Minipigs. Two meals with 300 g of pig diet with a fat content of 63 g each were given for seven days. Creon® with a pancreatin content of 1.4 g was given per meal (130,000 units per meal, 2,000 units per gram of fat). One dose of CiLip consisted of 826 mg of unpurified lipase. During the last two days of the seven-day medication period, the animals' complete stools were collected and subsequently analyzed.

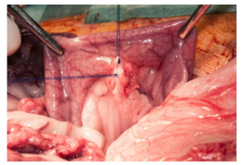

Figure 6: Exocrine pancreatic insufficiency was induced by ligation of the pancreatic duct in 14 Goettingen Minipigs. 12 pigs developed exocrine pancreatic insufficiency while the symptoms in two animals were not clear. Since about 10 % of all pigs have a second pancreatic duct, it is likely that this was the case in these two animals.

For relevant *in vivo* tests, we established an EPI-animal model with pancreatic duct ligated Goettingen Minipigs [3]. The **Latin Square crossover design** of the studies ensures that individual variations in digestibility do not have a negative impact on the study results. The excellent transferability of the results to humans could be proven in many studies by comparing the digestibility after Creon® ingestion.
After pancreatic duct ligation the CFA dropped from 90.0 % to 26.0 %. Both, Creon® and CiLip led to a **significant, comparable increase in CFA** (fig. 5). Because of the very high specific activity of CiLip the pill burden could be reduced significantly. CiLip was able to exert its effect even without enteric protection.

CONCLUSIONS

CiLip offers a new therapy option for EPI-patients. The highly efficient drug formulation overcomes the limitations of pancreatin products by a constant **high quality** and **activity**, **pH-stability** and **purity**. Compared to a Creon® capsule, with 40,000 units which weighs about 780 mg, a CiLip tablet of comparable activity weighs approx. 150 mg. Due to the high pH-stability, an **enteric coat is not required**, which means that the enzyme can already become active in the stomach.

Figure 7: Size comparison between a capsule of Creon® with 40,000 units (left) and a CiLip tablet (right) with comparable efficacy. The CiLip tablet shown is a symbolic image.

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