Improvement of liver fibrosis by a novel long acting Glucagon/GIP/GLP-1 triple agonist, efocipegtrutide (HM15211) in carbon tetrachloride-induced mouse model of liver injury and fibrosis

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ABSTRACT

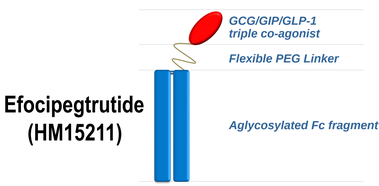
Background and Aims: Fibrosis is the pathologic result of chronic inflammation due to NASH, which can progress to liver cirrhosis, and causes liver-related complications. However, no drug has been approved to date. Thus, to provide a novel therapeutic option with more predictable therapeutic benefits, a novel long-acting Glucagon/GIP/GLP-1 triple agonist, efocipegtrutide, was developed and its efficacy has been extensively evaluated in various animal models of NASH and/or fibrosis such as MCD, AMLN, CDAHFD, and TAA mice. To generalize its potential therapeutic benefits, anti-fibrotic effect of efocipegtrutide was further evaluated in carbon tetrachloride (CCl_4)-induced fibrosis mouse, one of the well-established models of liver fibrosis.

Methods: To induce liver fibrosis, 20% CCl_4 was intraperitoneally injected 3 times/week to C57BL/6 male mouse for 10 weeks and efocipegtrutide was administrated during the last 6 weeks. To evaluate the therapeutic effect of efocipegtrutide on fibrosis, hepatic pro-collagen 1α1 contents, hydroxyproline contents (HP), and Sirius red staining area were measured. Additionally, blood surrogate markers and hepatic fibrosis related gene expression level were analyzed.

Results: CCl_4 mice showed various liver fibrosis profiles. However, treatment of efocipegtrutide effectively improved these fibrosis profiles such as hepatic pro-collagen 1α1 contents (0.89 μg/g vs. 1.22 μg/g for CCl_4, vehicle; $p < 0.05$), hydroxyproline contents (348.6 nmol/g vs. 430.5 nmol/g for CCl_4, vehicle; $p < 0.001$) and Sirius red positive area (2.46 % vs. 4.81 % for CCl4, vehicle; $p < 0.001$). Also, meaningful reduction of hepatic fibrosis marker genes expression (collagen 1α1, collagen 3α1 and α-smooth muscle actin) were observed in efocipegtrutide-treated group. Furthermore, blood surrogate biomarkers of fibrosis and inflammation (TIMP-1, PIIINP and TNF-α) were markedly improved by efocipegtrutide treatment.

Conclusion: Efocipegtrutide effectively improved liver fibrosis in CCl_4-induced fibrosis mice. These results are consistent with previous study results in various NASH/fibrosis animal models, in which further reinforcing the robust anti-fibrotic effect of efocipegtrutide via simultaneous action of GCG, GIP, and GLP-1. Human study is ongoing to assess the clinical relevance of these findings.

BACKGROUND



Efocipegtrutide (HM15211) is a long-acting Glucagon/GIP/GLP-1 triple co-agonist conjugated with a human IgG Fc fragment *via* flexible linker

- Rationally designed triple agonist optimized for liver targeting and effects
- Extended half-life allows once weekly dosing

- Rapid & potent liver fat reduction both in animal and human demonstrated
- Potent hepatic lipid lowering and MoAs elucidated *in vitro / in vivo*
- Inhibition of HSC activation and MoAs elucidated *in vitro / in vivo*

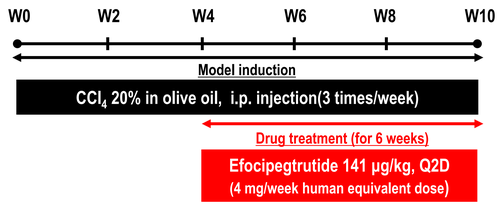
[The progress of preclinical studies on NASH for efocipegtrutide]

	NAFLD / NASH	Fibrosis	Reference (selected)
Diet induced	MCD-diet		*Diabetologia* **61** (Suppl 1); S61 (2018) Oral presentation at 2018 EASD (#119)
	CDAHF-diet		*Diabetologia* **63** (Suppl 1); S64 (2020) Oral presentation at 2020 EASD (#127)
	AMLN-diet		*Diabetologia* **64** (Suppl 1); S100 (2021) Oral presentation at 2021 EASD (#191)
Surgery induced		Bile duct ligation	*J Hepatol.* **73** (Suppl 1); S12 (2020) Oral presentation at 2020 EASL (AS015)
Chemical induced		TAA injection	Refer to WED-437
		CCl_4 injection	Newly presented

STUDY DESIGN and KEY RESULTS

Study design

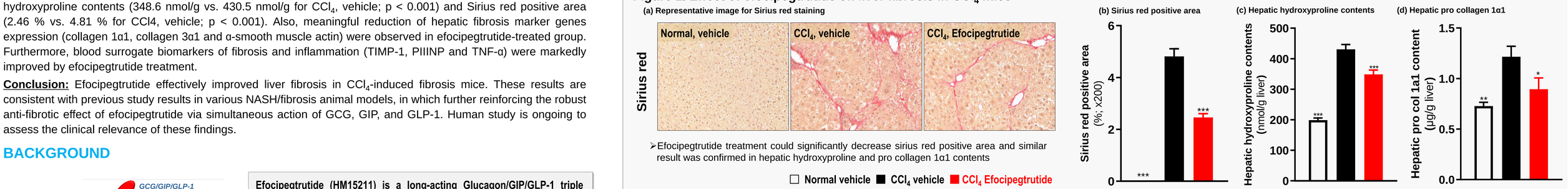
CCl_4 mice
(C57BL/6 strain)
(n=9-10/group)



- Therapeutic potential of efocipegtrutide in liver fibrosis was evaluated in CCl_4 mice. CCl_4 metabolized in the liver by the cytochrome P450 superfamily of monooxygenases (CYP family) to the trichloromethyl radical (CCl_3). It causes hepatocyte damage, necrosis, inflammation, and fibrosis. Based on that, many studies were performed for liver fibrosis study in CCl_4 model.
- In this study, CCl_4 (20% in olive oil, 4:1 ratio) was intraperitoneally injected to mouse for 10 weeks, and efocipegtrutide was subcutaneously administered during last 6 weeks. After treatment, liver tissue samples were prepared, followed by Sirius red staining to determine the liver fibrosis. Hepatic hydroxyproline contents and pro-collagen 1α1 were quantified and qRT-PCR was performed to measure the expression level of hepatic fibrosis-related genes. Also, blood collection was performed to evaluate surrogate markers.

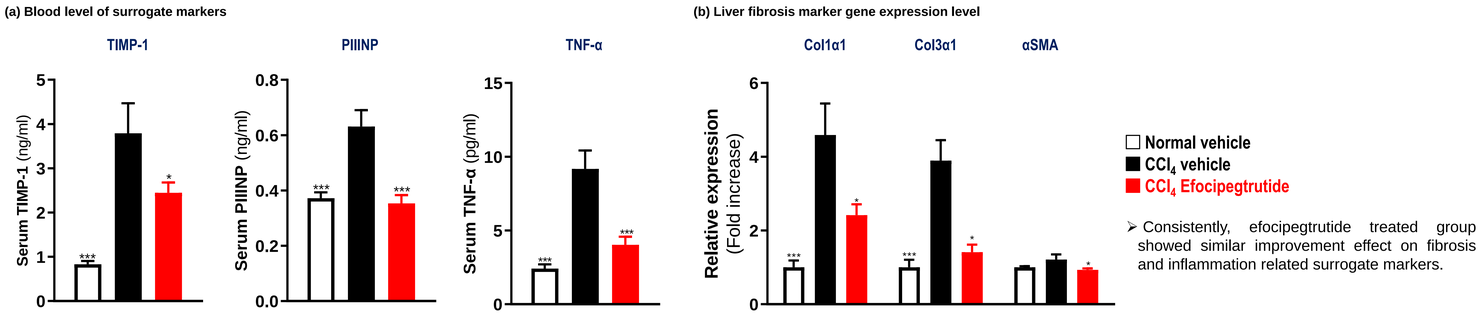
KEY RESULTS

Figure 1. Effect of efocipegtrutide on liver fibrosis in CCl_4 mice



➢Efocipegtrutide treatment could significantly decrease sirius red positive area and similar result was confirmed in hepatic hydroxyproline and pro collagen 1α1 contents

Figure 2. Effect of efocipegtrutide on blood (a) and liver (b) surrogate markers in CCl_4 mice



➢ Consistently, efocipegtrutide treated group showed similar improvement effect on fibrosis and inflammation related surrogate markers.

CONCLUSIONS

- **In CCl_4 mice, efocipegtrutide could improve liver fibrosis, which is in-line with previous results in various animal models of NASH and/or fibrosis**
- **Phase2b study in biopsy-proven NASH subjects is on-going in US to assess clinical relevance of non-clinical findings (fast-track granted, US)**

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