Late-breaking abstract
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THE REDUCTION OF BOTH CYCLOPHILIN B AND HCV-RNA BY THE CYCLOPHILIN INHIBITOR DEBIO-025 CONFIRMS THE IMPORTANCE OF CYCLOPHILIN B FOR HCV REPLICATION IN MAN

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Background: Cyclophilin B (CypB) has been suggested to play a role in HCV replication. The cyclophilin inhibitor, DEBIO-025, has shown anti-HCV activity in vitro. In a phase Ib study, DEBIO-025 monotherapy (1200 mg BID for 15 days) induced a strong anti-HCV effect (3.6 $\log_{10}$ reduction) in HIV-HCV co-infected patients. The aim of this ancillary study was to measure the levels of CypB in the PBMCs of these patients in order to investigate the relationship between inhibition of CypB and anti-HCV effect.
Methods: Using purified antibodies specific for CypB, an Enzyme-Linked Immunosorbent Assay (ELISA) technique was developed for the measurement of CypB in the PBMCs lysates of patients, collected at baseline (Day 45 628), at the end of treatment (Day 15) and at Day 42. Total protein concentration of cell lysates was measured with a Coomassie-based BioRad kit.
Results: CypB levels in the PBMCs of placebo patients (n = 3) remained unchanged during the treatment period. However, in DEBIO-025 treated patients (n = 16), CypB levels dropped from 67±6 (SE) ng/mg protein (baseline) to 5±1 ng/mg protein at Day 15 ($p < 0.01$) and did not return to baseline values at Day 42 (20±3 ng/mg protein). In all patients who showed a clinically relevant drop in viral load (>1 $\log_{10}$), CypB levels were consistently decreased (>90%). These data suggest that CypB reduction plays a key role in the mechanism of action of DEBIO-025 against HCV. The fact that one patient with a CypB diminution of 93% did not show a reduction of HCV-RNA indicates that other factors of viral origin and/or host related may modulate the influence of CypB inhibition in HCV replication.
Conclusion: DEBIO-025 induced a strong drop in CypB, coinciding with the decrease in HCV viral load. These are the first preliminary human data supporting the hypothesis that CypB is an important factor in HCV replication and that cyclophilin inhibition is a valid target for the development of anti-HCV drugs. Further research to elucidate the exact nature of the interaction between the HCV replicon and CypB is necessary for the complete understanding of the anti-HCV effect of cyclophilin inhibitors.

The Reduction of Both Cyclophilin B and HCV-RNA by the Cyclophilin Inhibitor Debio 025 Confirms the Importance of Cyclophilin B for HCV Replication in Man

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Background

Cyclophilin A (CypA) and cyclophilin B (CypB) have been suggested to play a role in HIV-1[1] and HCV[2] replication, respectively. The cyclophilin inhibitor Debio 025 has shown potent anti-HIV-1[3] and anti-HCV[4] activity *in vitro*. In a phase 1b study, Debio 025 monotherapy for 15 days induced a strong anti-HCV effect (3.6 $\log_{10}$ reduction) in HIV-1/HCV coinfected patients[5]. CypA and CypB levels were measured in patients' peripheral blood mononuclear cells (PBMCs) to investigate the relationship between CypA/CypB inhibition and antiviral effect.

Methods

Clinical study

Nineteen HIV-1/HCV coinfected patients received 1200 mg Debio 025 (n = 16) or placebo (n = 3) daily for 15 days (unbalanced randomization) in a double-blind, placebo-controlled, oral dosing, single arm, phase Ib study.

Cyclophilin assays

PRINCIPLE: Polyclonal IgG antibodies specific for CypA and CypB were obtained after rabbit immunization with human recombinant CypA and CypB, respectively. Enzyme-linked immunosorbent assays (ELISAs) were developed for measurement of CypA and CypB levels using a competitive format in 96-well microtiter plates. Cyclophilin (recombinant Cyp or cell lysate samples) was incubated and adsorbed onto plates previously coated with anti-Cyp IgG. Captured Cyp molecules were revealed by biotinylated anti-Cyp IgG.
Streptavidin-conjugated horseradish peroxidase was added to complex biotinylated IgG and finally a peroxydase substrate was added. Plates were read at 495 nm.

ASSAY SPECIFICITY: Assay specificity was tested by depleting the PBMC lysates using anti-CypA and/or anti-CypB IgG directly coupled to beads. The presence of CypA and/or CypB was also analyzed in the lysate of wild-type (WT) Jurkat T cells (400'000 cells) containing both CypA and CypB, and in the lysate of CypA-knockout (KO) Jurkat T cells containing only CypB.

PBMC SAMPLES: Patients' PBMCs were collected and frozen at - 80°C at baseline (Day -28), at the end of treatment (Day 15) and 27 days after the end of treatment (Day 42). After thawing and washing, PBMCs were lysed in a Triton-X100 lysis buffer. Lysates were then cleared by centrifugation and frozen. Total protein concentration in cell lysates was measured with a Coomassie-based BioRad kit. Some PBMC lysates of selected patients we using anti-CypA and -CypB IgG.

in vitro experiment in Huh-7 cells

10 pg of *in vitro* transcribed genomic JFH-1 RNA was electroporated into Huh-7 cells. Seven days post-transfection, cells were treated with or without Debio 025 (1 µM). At the indicated time points, intracellular HCV RNA was analyzed by RT-QPCR and presented as genome equivalents (GE)/µg total RNA. Intracellular and extracellular CypB content was quantified by CypB ELISA.

Results

CypA ELISA did not measure CypA in lysates depleted of CypA by anti-CypA IgG or in CypA knockout cell lysate (Fig 1a). Furthermore, no signal was measured with recombinant CypB. Similar data was obtained with CypB ELISA, showing that the assay does not cross-react with CypA (Fig 1b).

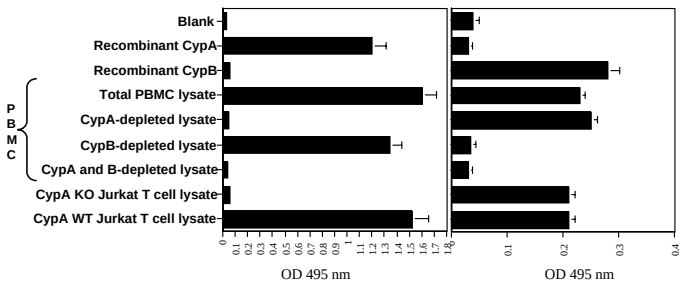

Fig. 1a ■ **CypA ELISA** **Fig. 1b** ■ **CypB ELISA**

Figure 1 CypA and CypB measurements in PBMC and Jurkat T cell lysates

Debio 025-treated HIV-1/HCV coinfected patients experienced a significantly greater maximum HCV viral load reduction compared to placebo (least square means of 3.6 versus 0.7 respectively, $P = 0.0045$).

Placebo patients did not change their viral load during treatment, while Debio 025 induced an important and continuous viral load decline without reaching a plateau. After treatment discontinuation, HCV viral load levels returned gradually to baseline values over the 4-week follow-up period (Fig. 2).

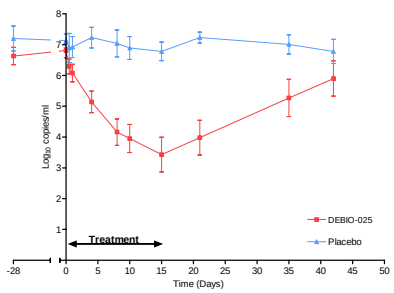

Figure 2 Mean $\log_{10}$ HCV RNA copies/mL (± SEM) in HIV-1/HCV coinfected patients before, during, and after treatment with Debio 025 or placebo.

PBMC CypA mean levels (± SEM) measured at baseline (Day -28) were similar in the placebo group (0.65 ± 0.25 µg/mg protein) and in the Debio 025 group (0.56 ± 0.05 µg/mg protein), and remained unchanged during the entire study in both groups (Fig. 3).

PBMC CypB mean level (± SEM) in the placebo group at baseline was 73 ± 23 ng/mg protein and remained unchanged during the entire study (76 ± 25 ng/mg protein on Day 15 and 77 ± 25 ng/mg protein on Day 42). In the Debio 025 group, the mean level at baseline was 67 ± 6 ng/mg protein. It decreased to 5 ± 1 ng/mg protein on Day 15, with a statistically significant difference between baseline and the Day 15 assessment ($P<0.001$). On Day 42, the mean level (20 ± 3 ng/mg protein) was higher than the Day 15 mean level, but remained inferior to the mean baseline level (Fig. 3).

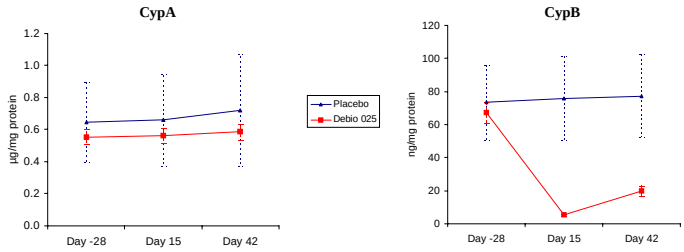

Figure 3 Mean (± SEM) PBMC CypA and CypB levels

Western blot analysis of the PBMC lysates of two Debio 025 treated patients confirmed that the drug drastically reduced CypB levels at Day 15 (end of treatment), but not CypA levels. No change in CypA and CypB levels were observed in the PBMC lysates of placebo treated patients (Fig. 4).

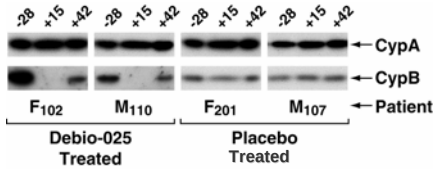

Figure 4 Western blot analysis of selected patients on study Days -28, 15 and 42

In Huh-7 cells transfected with JFH-1 RNA, treatment with Debio 025 induced a strong reduction of HCV RNA (Fig. 5a) which was concomitant with an intracellular CypB depletion and a corresponding CypB release within the extracellular medium (Fig. 5b).

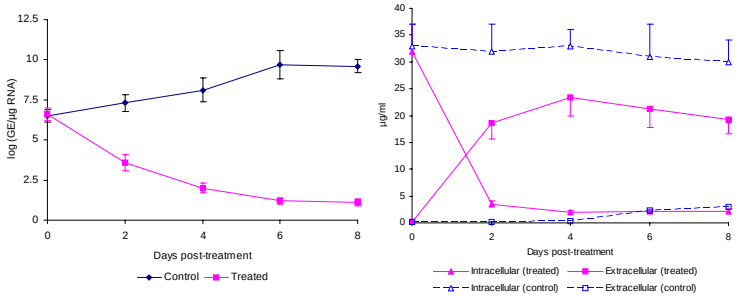

Fig. 5a Intracellular HCV RNA **Fig. 5b CypB**

Figure 5 Relationship between *in vitro* Debio 025-mediated HCV RNA block and intracellular CypB depletion (mean ± SD)

Conclusion

Debio 025 treatment of HIV-1/HCV coinfected patients induced a significant decrease in intracellular CypB levels in PBMCs, coinciding with a significant decrease in HCV viral load, while intracellular CypA levels remained unchanged.

These data confirm results from previous studies demonstrating that Cyp inhibition leads to the release of CypB from HeLa cells[6], while CypA levels remain unchanged. Our *in vitro* data in Huh-7 hepatoma cells indicate that the same process occurs at the hepatocyte level and support the hypothesis that depletion of intracellular CypB is important for the anti-HCV effect of Debio 025.

Further research to elucidate the exact nature of the interaction between the HCV replication complex and CypB is necessary for the complete understanding of the anti-HCV effect of Cyp inhibitors.

These are the first preliminary human data supporting the hypothesis that intracellular CypB depletion is associated with significant anti-HCV activity and that CypB inhibition is a valid target for the development of anti-HCV drugs.

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