C105SR, a novel non-peptidic small-molecule cyclophilin inhibitor with potent mitoprotective and hepatoprotective properties in the context of hepatic ischemia/reperfusion injury

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Introduction

Hepatic ischemia/reperfusion injury (IRI) is a severe complication of various clinical conditions, including hemorrhagic shock, liver resection and liver transplantation. Hepatic IRI is a leading cause of early allograft dysfunction and a major risk factor for acute and chronic rejection. IRI occurs as the result of a biphasic phenomenon in which cellular damage caused by hypoxia is paradoxically exacerbated by the restoration of oxygen delivery. Mitochondrial dysfunction plays an important role in the pathogenesis of IRI. In particular, mitochondrial permeability transition (mPT) is thought to be a critical mediator of the damage that accompanies reperfusion of organs following prolonged ischemia. mPT is defined as a sudden increase in permeability of the inner mitochondrial membrane to solutes with a molecular mass less than 1.5 kDa. mPT is mediated by the opening of the mPT pore (mPTP), a high-conductance channel involved in Ca2+ homeostasis.

Cyclophilin D (CypD) is a member of a family of highly homologous peptidyl-prolyl cis-trans isomerases (PPIases) that catalyze the interconversion of the two energetically preferred conformers (*cis* and *trans*) of the planar peptide bond preceding an internal proline residue. The PPIase domain catalyzes cyclophilin isomerase activity. It contains two binding pockets: the PPIase catalytic site (S1 pocket) and the so-called « gatekeeper pocket » (S2 pocket), the functional role of which remains unknown. CypD is localized in the mitochondrial matrix and acts as a key regulator of mPTP opening. Persistent CypD-dependent mPTP opening induces necrotic cell death, which plays a major role in IRI. Thus, mPTP opening inhibition by compounds that target CypD represents an attractive strategy for cellular protection in the context of hepatic IRI.

Aim

We have recently developed a new family of non-peptidic, small-molecule cyclophilin inhibitors (SMCypIs), chemically different from all known cyclophilin inhibitors (1). We have established that one of the SMCypIs, compound C31, exerts mitoprotective effects *in vitro* and protects cells in an *in vivo* murine model of liver IRI (2). Here, we aimed at chemically improved C31 to identify a new candidate for hepatic IRI therapeutic development.

Methods

In order to improve the potency of our previously reported SMCypI C31, chemical modifications of its three functional regions, including R1 that binds the S1 pocket of CypD, R2 that binds the S2 pocket of CypD and R3 that interacts with residues between the two pockets were performed. The newly generated compounds were evaluated for their ability to inhibit CypD PPIase activity and for their mitoprotective properties, assessed by measuring calcium retention capacity in mouse liver mitochondria. The ability of the selected compounds to inhibit mPTP opening was evaluated in cells subjected to hypoxia/reoxygenation using a calcein/cobalt assay. Their ability to inhibit cell death was evaluated in cells subjected to hypoxia/reoxygenation by measuring LDH release, propidium iodide (PI) staining and cell viability with a MTT assay. The best performing compound *in vitro* was selected for *in vivo* efficacy evaluation in a mouse model of hepatic IRI.

Conclusion

We identified a novel cyclophilin inhibitor with strong mitoprotective and hepatoprotective properties both *in vitro* and *in vivo* that represents a promising candidate for cellular protection in hepatic IRI.

References

1. Ahmed-Belkacem A, Colliandre L, Ahnou N, Nevers Q, Gelin M, Bessin Y, Brillet R, Cala O, Douguet D, Bourguet W, Krimm I, Pawlotsky JM, Guichou JF. Fragment-based discovery of a new family of non-peptidic small-molecule cyclophilin inhibitors with potent antiviral activities. Nat Commun. 2016;7:12777.
2. Panel M, Ruiz I, Brillet R, Lafdil F, Teixeira-Clerc F, Nguyen CT, Calderaro J, Gelin M, Allemand F, Guichou JF, Ghaleb B, Ahmed-Belkacem A, Morin D, Pawlotsky JM.Small-Molecule Inhibitors of Cyclophilins Block Opening of the Mitochondrial Permeability Transition Pore and Protect Mice From Hepatic Ischemia/Reperfusion Injury. Gastroenterology. 2019;157(5):1368-82.

Contact information

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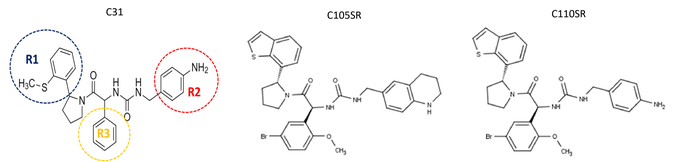
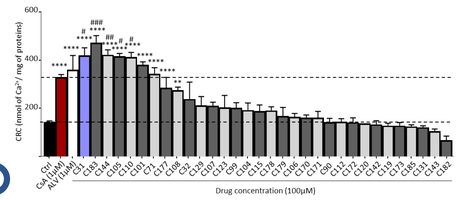
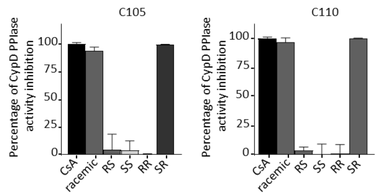
Results



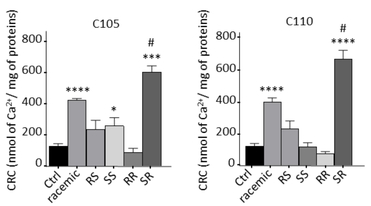
A. Chemical structures of compounds C31, C105SR and C110SR.



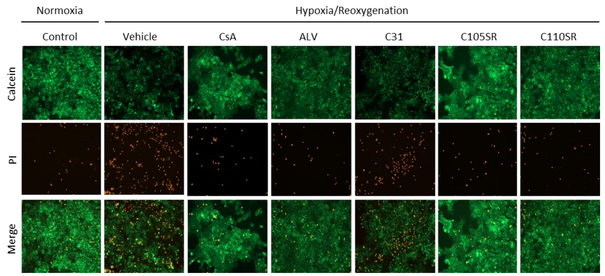
B. Calcium retention capacity (CRC) of mouse liver mitochondria in the absence (Ctrl) or in the presence of CsA, ALV, C31 or C31 derivatives, ranked by decreasing level of CRC.



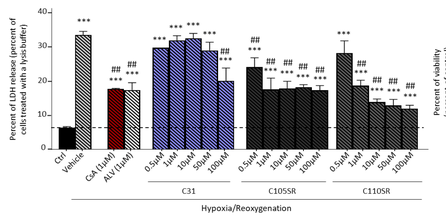
C. Inhibition of CypD PPIase activity by C105 (left) and C110 (right) racemic mixtures and their diastereoisomers at 10 µM.



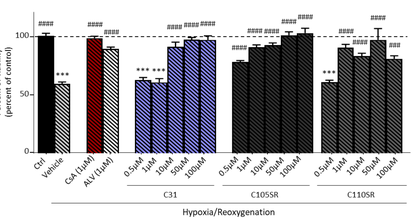
D. Mitochondrial calcium retention capacity (CRC) of mouse liver mitochondria in the absence (Ctrl) or in the presence of C105 (left) and C110 (right) racemic mixture and their diastereoisomers at 100 µM. $^{*}p < 0.05$ *vs* Ctrl ; $^{***}p < 0.001$ *vs* Ctrl ; $^{****}p < 0.0001$ *vs* Ctrl ; $^{\#}p < 0.05$ *vs* racemic mixture.



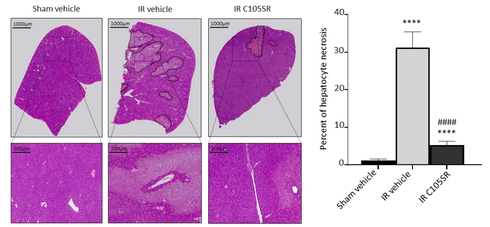
E. Representative images of calcein (green) and PI (red) labeling in cells exposed to normoxia (control) or hypoxia/reoxygenation in the absence (vehicle) or in the presence of CsA, ALV, C31, C105SR or C110SR at 1 µM (original magnification × 400).



F. LDH release from cells exposed to normoxia (Ctrl) or hypoxia/reoxygenation in the absence (vehicle) or in the presence of CsA, ALV or increasing concentrations of C31, C105SR or C110SR expressed as percentage of LDH release in cells treated with a lysis buffer. $^{***}p < 0.001$ *vs* Ctrl; $^{\#\#}p < 0.01$ *vs* hypoxia/reoxygenation vehicle.



G. Cell viability in cells exposed to normoxia (Ctrl) or hypoxia/reoxygenation in the absence (vehicle) or in the presence of CsA, ALV or increasing concentrations of C31, C105SR or C110SR expressed as percentage of control. $^{***}p < 0.001$ *vs* Ctrl ; $^{\#\#\#}p < 0.001$ *vs* hypoxia/reoxygenation vehicle ; $^{\#\#\#\#}p < 0.0001$ *vs* hypoxia/reoxygenation vehicle.



H. Representative hematoxylin and eosin staining images (magnification x200) of liver lobes (left) and percent of hepatocyte necrosis (right) in mice subjected to laparotomy without (sham vehicle) or with ischemia/reperfusion (IR) in the absence (vehicle) or in the presence of C105SR.

I. Serum ALT levels in mice subjected to laparotomy without (sham vehicle) or with ischemia/reperfusion (IR) in the absence (vehicle) or in the presence of C105SR.

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