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CTX-343, A PERIPHERALLY RESTRICTED LPA RECEPTOR 1 ANTAGONIST FOR TREATING MASH AND OTHER FIBROTIC LIVER DISEASES

D. BAGNOL, J.S. SWANEY, D.S. LORRAIN, K.I. LORRAIN, A.R. BROADHEAD, C.BACCEI, L. EIDE, B. PAULSON, G. EDU, J. ATKINS, K.J. STEBBINS, M.M. POON, A. CHEN

Contineum Therapeutics, San Diego, CA, USA

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CONTINEUM therapeutics

INTRODUCTION

LPA is a well-known signaling phospholipid that mediates wound healing and tissue fibrosis via LPAR1[1-4]. Repeated liver injury results in progressive hepatocellular damage and hepatic stellate cell (HSC) differentiation into myofibroblasts, ultimately leading to tissue fibrosis and cirrhosis[5]. The underlying mechanism involves aberrant upregulation of LPA and increased LPAR1 expression on activated HSCs[5-7]. In preclinical in vivo models of liver fibrosis, LPAR1 antagonists are also efficacious in reducing fibrosis[7].

CTX-343 is a novel and potent small molecule LPAR1 antagonist. Given the link between LPAR1 and fibrotic disease, including clinical proof of concept in idiopathic pulmonary fibrosis, there is strong rationale for the exploration of CTX-343 as a therapeutic for fibrotic disease of the liver.

AIM

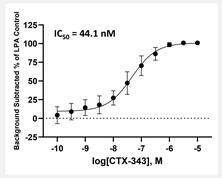
We characterized CTX-343, a peripherally restricted LPAR1 antagonist for *in vitro* potency and selectivity. The mouse PK/PD and rat PK profile of CTX-343 was evaluated, its efficacy investigated in several *in vivo* rodent models of liver fibrosis as well as in Precision Cut Liver Sections (PCLS) from a MASH patient.

METHODS

CTX-343 binding kinetics and functional activity were evaluated in B103 cells stably expressing human LPAR1. CTX-343 selectivity for human LPAR1-6 was evaluated using transiently transfected HEK293T cells. CTX-343 inhibition of primary human hepatic stellate cells (HSC) activation was evaluated by immunocytochemistry. Histamine release into plasma was measured in CD-1 mice that received a single oral gavage administration of Vehicle (1%HPMC/0.1%TWEEN80) or CTX-343 (0.03, 0.3, or 3 mg/kg; n=6/group), followed by intravenous administration of LPA (300 µg), 3 or 24 hr later. C57Bl/6N female mice (8-10 weeks old) were dosed intraperitoneally twice a week for 6 weeks with 1 mL/kg of CCl_4 dissolved in corn oil to induce liver injury. Cholestatic liver injury was induced in C57Bl6/N male mice (8-10 weeks old) by performing a double ligation of the common bile duct for 11 days. Wistar male rats (7-8 weeks old) were dosed intraperitoneally weekly with 50 mg/kg of diethyl-nitrosoamine (DEN) dissolved in PBS for 18 weeks to induce severe liver fibrosis and cirrhosis. CTX-343 with doses ranging from 0.003 to 3 mg/kg was formulated as a suspension in vehicle (1%HPMC/0.1%TWEEN80) and administered orally once a day in all models.

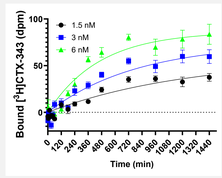
RESULTS

CTX-343 inhibits LPA-stimulated calcium mobilization



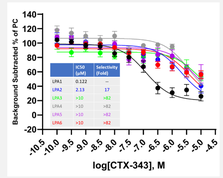
B103 cells were stably transfected with human LPAR1. Cells were preincubated with CTX-343 at different concentrations for 24 hours at 37°C and treated with LPA (EC_{80} or 3µM). Each data point represents the mean of the 7 individual experiments. Error bars are SD.

[^{3}H]-CTX-343 kinetic binding to hLPAR1



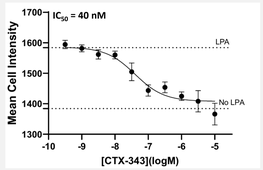
CTX-343 exhibited slow binding kinetics in a human LPAR1 membrane binding assay (3 h, K_i = 5.6 nM) with steady state reached at 12 h, a residence time ($1/K_{off}$) of 46 h, and a dissocia-tion constant (K_d) of 1.2 nM. Data are n=4-8 replicates per time point. Error bars are SEM.

CTX-343 selectivity against hLPAR1-6



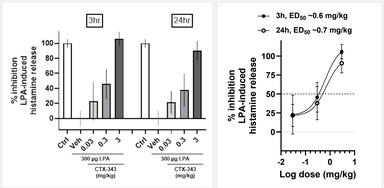
HEK293T cells were transfected with human LPAR1-6 and incubated with CTX-343 at different concentrations for 3 hours at 37°C and treated with LPA. µM = Micro-molar. Each data point represents the mean of 7-8 replicate wells. Error bars are SEM.

CTX-343 inhibits LPA-induced human HSC activation



LPA = 10 µM LPA in 0.1% DMSO in DMEM and no LPA = 0.1% DMSO in DMEM. n=6 to 12 replicate wells per data point. Error bars are SEM. Phospho-Myosin light chain staining was used as marker of HSC activation. HSC = Hepatic Stellate Cells.

Inhibition of LPA-induced histamine release vs CTX-343 dose



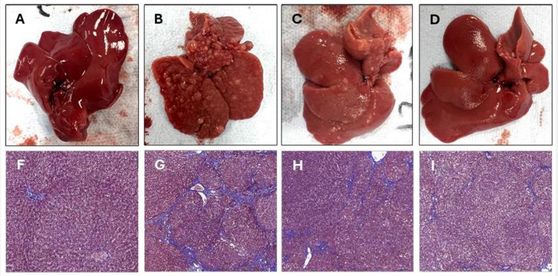
Intravenous administration of LPA increases histamine release in CD1 mice. CTX-343 administered 3 or 24 h prior to LPA dose-dependently inhibited histamine release with an ED_{50} of value 0.6 mg/kg and 0.7 mg/kg respectively. The corresponding EC_{50} values were 110 nM at 3h and 4.6 nM at 24h.

A summary table of the effect of CTX-343 on the reduction of liver soluble collagen, histological deposition of liver collagen fibers, hepatic lesions, and micronodular cirrhosis across three rodent models.

Species	Model	CTX-343 dose (mg/kg, po)	% Reduction of soluble collagen	% Reduction in fibrosis (Histology assessment)	% Reduction of hepatic lesions or micronodular cirrhosis incidence
Mouse	CCl4	0.03	29	11	*na*
		0.3	51*	40***	*na*
		3	74**	36**	*na*
Mouse	BDL	0.3	49.9*	0	46.2*,#
		3	54.5*	11.1	48.1*,#
Rat	DEN	0.3	42.9**	50.4*	54.8##
		3	36*	52**	59.2##

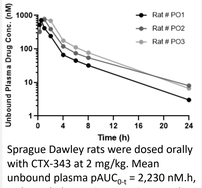
* p < 0.05; **, p < 0.01; *** p< 0.001. # hepatic lesions, ## micronodular cirrhosis. ANOVA with Dunnett's multiple comparisons test to vehicle group. *na* = not applicable.

CTX-343 improved DEN-induced liver micronodules (A-D) and cirrhosis in rats (F-I)



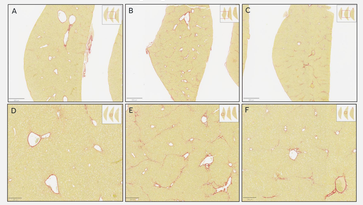
Liver sections from Saline (A, F) or DEN-treated rats dosed with vehicle (1% HPMC/0.1% Tween80) (B, G), 0.3 mg/kg CTX-343 (C, H) or 3 mg/kg CTX-343, po (D, I). Micronodules <3mm were evaluated. Liver fibrosis was evaluated by histology on liver

PK profile of CTX-343 in rats



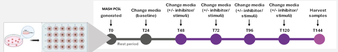
Sprague Dawley rats were dosed orally with CTX-343 at 2 mg/kg. Mean unbound plasma $pAUC_{0-t}$ = 2,230 nM.h, Unbound plasma C_{max} = 750 nM and T_{max} = 0.8 hours. Oral bioavailability (%F) = 60%. CTX-343 distributed to the liver with minimal brain penetration ($K_{p,uu}$ = 12.2 in liver & 0.066 in brain).

CTX-343 reduced PSR staining in CCl_4-induced liver injury in mice



Corn oil (A, D) and CCl4 treated mice dosed with vehicle (1% HPMC/0.1% Tween80) (B, E) or CTX-343, 3mg/kg, po (C, F).

CTX-343 reduces fibrosis and inflammation marker secretion from MASH tissue



Cells with ticks denote reduction in secretion of the marker at a specific time point and dose.		CTX-343			ALKSI (pos. ctrl)
All compared to vehicle control		0.03 µM	0.3 µM	3 µM	10µM
Collagen 1α1	72h	✓✓	✓✓✓	✓✓✓	✓✓✓
	96h		✓	✓✓	✓✓✓
	120h			✓	✓✓✓✓
	144h			✓	✓✓✓✓
Fibronectin	72h	✓	✓✓	✓✓	✓✓
	96h		✓	✓	✓✓
	120h				✓✓✓
	144h			✓	✓✓✓
TIMP-1	72h	✓	✓✓	✓✓	✓✓
	96h			✓	✓✓
	120h			✓✓	✓✓✓
	144h				✓✓✓
IL-6	72h		✓	✓✓	✓
	96h		✓✓	✓✓✓	✓✓
	120h			✓✓✓	✓✓✓
	144h	✓	✓✓	✓✓✓	✓
IL-8	72h	✓	✓	✓✓	
	96h			✓	
	120h			✓✓	✓✓
	144h		✓✓	✓✓	
MCP-1	72h		✓	✓✓✓	✓
	96h			✓✓	✓
	120h			✓✓	✓✓
	144h		✓	✓✓	✓✓

Attenuation	Symbol
0-10%	
10-25%	✓
25-50%	✓✓
50-75%	✓✓✓
75-100%	✓✓✓✓

Checks marks denote the amount of inhibition with CTX-343 or positive control ALKI

Precision Cut Liver Slices (PCLS) from 73 y/o male, F2 fibrosis donor (4mm diameter, 250µm thickness) were prepared and plated in 96 well plate. Tissue was allowed to rest for 24 hours, then cultured for additional 120 hours. Treatments include Control or lipid media +/- CTX-343. Pos Ctrl = Positive control.

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CONCLUSIONS

In adult male rats, CTX-343 exhibited good PK properties and distributed preferentially to peripheral tissues, especially the liver. Consistent with the PK, CTX-343 also inhibited liver fibrosis and fibrotic gene expression in mouse CCl_4 and BDL models, as well as rat DEN model of liver fibrosis. Moreover, CTX-343 reduced markers of inflammation and fibrosis in human MASH precision cut slices.

These data indicate that CTX-343 is a potent and selective LPAR1 antagonist that may serve as a novel treatment for MASH and other fibrotic liver diseases.

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CONTACT INFORMATION

Authors contact:
dbagnol@contineum-tx.com