In Vitro Metabolism of CRV431, a Novel Cyclophilin Inhibitor for the Treatment of HBV

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BACKGROUND

CRV431 is a non-immunosuppressive cyclosporine derivative designed to bind cyclophilins but not calcineurin, and inhibit the action of cyclophilins in the life cycle of many viruses, including HBV. As it is known that cyclosporins are extensively metabolized via cyctochromes P450, the aim of this study was to characterize CRV_{431} metabolism in liver microsomes from several species *in vitro*.

METHODS

The *in vitro* metabolism of CRV_{431} was studied in microsomes from rat, monkey and human livers (Sekisui Xenotech). Microsomes were incubated at 37 °C for 0, 10, 20, 40, and 80 minutes with 0.1, 1 and 10 µg/mL CRV_{431} in the presence of an NADPH regenerating system, and the metabolite profiles were assessed utilizing electrospray ionization liquid chromatography mass spectrometry (LC-ESI-MS) in positive ion mode.

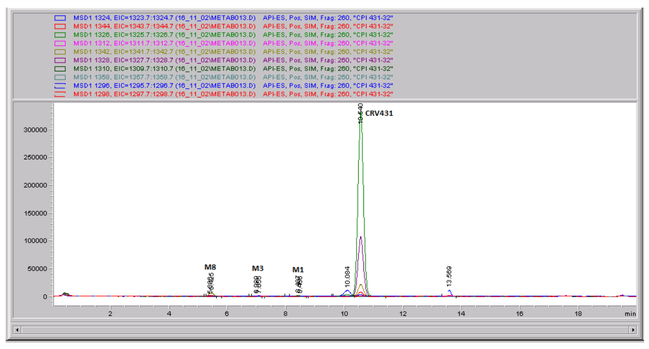
RESULTS

- CRV_{431} was extensively metabolized through oxidation to produce various hydroxylated and demethylated species.
- Metabolite species identified, as their sodium adducts, included monohydroxylated CRV_{431} (two distinct metabolites, 1342 m/z), di-hydroxylated CRV_{431} (1358 m/z), demethylated CRV_{431} (two distinct metabolites, 1312 m/z), demethylated and hydroxylated CRV431 (two distinct metabolites, 1328 m/z), didemethylated and hydroxylated CRV_{431} (1314 m/z), and didemethylated and dihydroxylated CRV_{431} (1316 m/z).
- The magnitude and extent of metabolism (20 min) was greatest in monkey (>95%) followed by human (>70%) followed by rat (<5%).
- Importantly, all metabolites identified in human microsomes were correspondingly identified in monkey and rat microsomes. Hence, qualitatively, metabolism was similar across species, whereas there were quantitative differences.
- An *in vitro* cytochrome phenotyping study indicated that cytochrome P450 3A4/5 is the major enzyme system involved. Enzymes 1A2, 2B6, 2C8, 2C9, and 2D6 are not involved in the *in vitro* metabolism of CRV_{431}.

CONCLUSION

CRV_{431} was metabolized *in vitro* in similarity to other analogs in the cyclosporine drug class, and was qualitatively similar among all species. Knowledge about the metabolite profiles will be useful for further preclinical and clinical development of CRV_{431} for chronic hepatitis B.

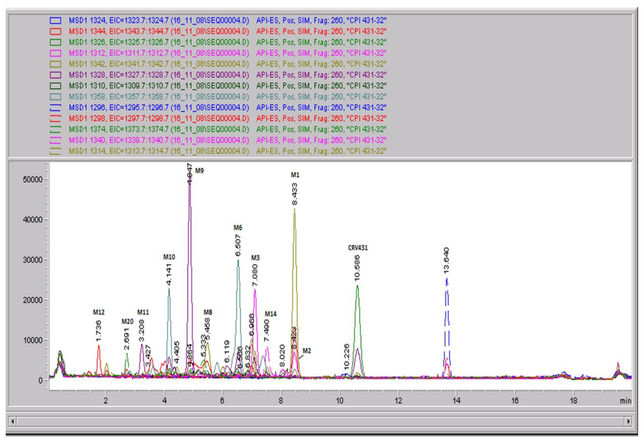
CRV_{431} Metabolism in Rat Liver Microsomes



1 µg/ml CRV_{431} incubation for 40 minutes

Component	Proposed Biotransformation	Relative LCMS retention	m/z	Δ m/z	% of Total Drug-related Mass Versus Time (minutes)				
					0 min	10 min	20 min	40 min	80 min
CRV431	NA	1.0	1326	0	96.4	95.7	94.7	93.8	ND
CRV431 unsaturated impurity	NA	0.96	1324	NA	3.6	3.6	3.7	4.1	ND
M1	Hydroxylation	0.8	1342	+16	0	0.1	0.4	0.5	ND
M3	Demethylation	0.67	1312	-14	0	0	0.1	0.2	ND
M8	Hydroxylation	0.52	1342	+16	0	0.5	1	1.6	ND

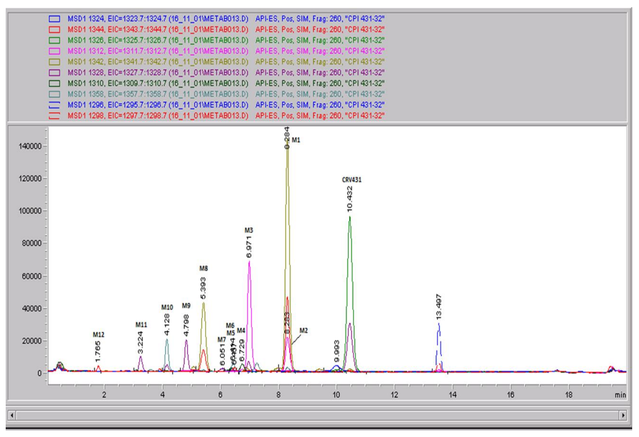
CRV_{431} Metabolism in Cynomolgus Monkey Liver Microsomes



1 µg/ml CRV_{431} incubation for 10 minutes

Component	Proposed Biotransformation	Relative LCMS retention	m/z	Δ m/z	% of Total Drug-related Mass Versus Time (minutes)						
					0 min	2.5 min	5 min	10 min	20 min	40 min	80 min
CRV431	NA	1.0	1326	0	96.3	59.4	21.5	12.3	4.3	6.6	6.7
CRV431 unsaturated impurity	NA	0.96	1324	-2	3.7	3.8	1.7	0.8	0	0	0
M1	Hydroxylation	0.8	1342	+16	0	18.1	24.2	16.1	3.2	1.7	0.9
M2	Demethylation	0.8	1312	-14	0	3.1	6.7	2.7	0.7	0	0
M3	Demethylation	0.67	1312	-14	0	3.8	7.6	8.3	5.4	3.3	0.7
M4		0.65	1310	-16	0	0.3	0.5	0.6	0.6	0	0
M5	Di-demethylation	0.62	1298	-28	0	0	0.2	0.4	0.7	0.6	0
M6	Di-hydroxylation	0.62	1358	+32	0	2.4	8.2	12.6	10.9	4.8	1.3
M7	Demethylation + Hydroxylation	0.58	1328	+2	0	0	0	1.1	0	0	0
M8	Hydroxylation	0.52	1342	+16	0	1.9	3.7	3.7	2.0	0	0.4
M9	Demethylation + Hydroxylation	0.47	1328	+2	0	1.9	8.6	15.6	24.8	25.2	17.6
M10	Di-hydroxylation	0.39	1358	+32	0	0.8	4.4	6.9	6.7	4.2	1.4
M11	Demethylation + Hydroxylation	0.30	1328	+32	0	0.2	1.3	2.8	6.5	8.7	11.7
M12	Di-hydroxylation + demethylation	0.17	1344	+ 18	0	0.1	0.7	1.8	6.2	11.7	21.6
Additional Metabolites Not Present in Human Liver Microsome Experiments											
M13		0.75	1340	+14	0	1.1	1.6	1.1	0	0	0
M14		0.70	1340	+14	0	0.9	2.4	3.2	1.7	0.6	0
M15	Hydroxylation	0.65	1342	+16	0	1.6	3.3	3.9	3.3	2.4	1.7
M16	Tri-hydroxylation	0.50	1374	+48	0	0.4	1.8	2.6	2.2	1.4	0
M17	Tri-hydroxylation	0.46	1374	+48	0		0.4	0.8	0.9	1.1	0
M18	Di-demethylation + hydroxylation	0.41	1314	-12	0	0	0.2	0.6	2.3	4.1	5.4
M19	Di-demethylation + hydroxylation	0.31	1314	-12	0	0	0.2	0.4	1.3	1.8	2.8
M20	Tri-hydroxylation	0.25	1374	+48	0	0.1	0.8	1.6	3.3	2.9	1.8
M21	Di-hydroxylation + demethylation	0.13	1344	+18	0	0	0	0	1.2	3.7	8.0
M22	Di-hydroxylation + demethylation	0.32	1344	+18	0	0	0	0	5.6	8.4	11.1
M23	Di-hydroxylation + demethylation	0.35	1344	+18	0	0	0	0	3.6	2.6	1.4
M24	Hydroxylation	0.18	1342	+16	0	0	0	0	2.3	4.0	5.7

CRV_{431} Metabolism in Human Liver Microsomes



1 µg/ml CRV_{431} incubation for 20 minutes

Component	Proposed Biotransformation	Relative LCMS retention	m/z	Δ m/z	% of Total Drug-related Mass Versus Time (minutes)				
					0 min	10 min	20 min	40 min	80 min
CRV431	NA	1.0	1326	0	96.4	57.0	27.5	10.4	4.0
CRV431 unsaturated impurity	NA	0.96	1324	NA	3.6	2.9	1.8	0.7	0.2
M1	Hydroxylation	0.8	1342	+16	0	21.2	28.3	27.4	18.3
M2	Demethylation	0.8	1312	-14	0	3.6	4.9	4.6	3.1
M3	Demethylation	0.67	1312	-14	0	5.3	13.8	21.9	27.4
M4		0.65	1310	-16	0	0.3	1.1	1.7	2.0
M5	Di-demethylation	0.62	1298	-28	0	0	0.4	1.3	2.5
M6	Di-hydroxylation	0.62	1358	+32	0	0	1.3	1.4	1.1
M7	Demethylation + Hydroxylation	0.58	1328	+2	0	0.1	0.6	1.2	1.7
M8	Hydroxylation	0.52	1342	+16	0	5.9	10.3	13.0	13.5
M9	Demethylation + Hydroxylation	0.47	1328	+2	0	1.1	3.2	5.6	8.6
M10	Di-hydroxylation	0.39	1358	+32	0	1.2	3.3	5.3	6.6
M11	Demethylation + Hydroxylation	0.30	1328	+32	0	0.4	1.6	4.4	8.9
M12	Di-hydroxylation + demethylation	0.17	1344	+ 18	0	0.1	0.5	1.0	1.9

Cytochrome P450 Phenotyping

Targeted CYP-P450 enzyme	Inhibitor/solvent	Inhibitor/ solvent concentration	Incubation time (min)	CRV431 detected (pmol)	Percent loss of substrate	Percent CRV431 remaining	Rate of substrate disappearance (pmol/mg/min)	Percent of control	Percent inhibition
NA	Water (solvent control)	NA	0	131	NA	100	NA	NC	NA
			15	78.5	40.2	59.8	17.6	100	
NA	Acetonitrile (solvent control)	0.5% v/v	0	129	NA	100	NA	NC	NA
			15	76.9	40.4	59.6	17.4	100	
NA	Acetonitrile with 0.1% formic acid (solvent control)	1.0% v/v	0	129	NA	100	NA	NC	NA
			15	91.1	29.4	70.6	12.6	100	
NA	40:60 Methanol:0.1 M Tris, pH 9.0 (solvent control)	0.5% v/v	0	141	NA	100	NA	NC	NA
			15	80.0	43.4	56.6	20.5	100	
CYP1A2	Furafylline in acetonitrile	10 µM	0	139	NA	100	NA	NC	No inhibition
			15	84.7	39.1	60.9	18.2	104	
CYP2B6	Phencyclidine in water	30 µM	0	137	NA	100	NA	NC	No inhibition
			15	83.0	39.5	60.5	18.1	103	
CYP2C8	Gemfibrozil glucuronide in acetonitrile with 0.1% formic acid	100 µM	0	137	NA	100	NA	NC	No inhibition
			15	92.5	32.3	67.7	14.7	117	
CYP2C9	Tienilic acid in acetonitrile	20 µM	0	136	NA	100	NA	NC	6.9
			15	87.3	35.7	64.3	16.2	93.1	
CYP2C19	Esomeprazole in 40:60 methanol:0.1 M Tris, pH 9.0	10 µM	0	138	NA	100	NA	NC	37.3
			15	99.0	28.0	72.0	12.8	62.7	
CYP2D6	Paroxetine in water	5 µM	0	138	NA	100	NA	NC	No Inhibition
			15	80.8	41.3	58.7	19.0	108	
CYP3A4/5	Troleandomycin in acetonitrile	50 µM	0	131	NA	100	NA	NC	100
			15	155	No loss	118	NA	NA	