Emricasan, a potent pan-caspase inhibitor, rapidly reduces caspase activity and biomarkers of apoptosis in patients with hepatic impairment but not in healthy volunteers: implications for safety, selectivity and mechanism of action.

Alfred P. Spada*, Patricia C. Contreras, MiRa C. Huyghe, Mark Morris and Gary C. Burgess
Conatus Pharmaceuticals, San Diego, California

Introduction:

Emricasan (IDN-6556, PF-03491390) is a potent irreversible pan-caspase inhibitor with the ability to rapidly reduce elevated levels of serum ALT, AST and caspase mediated cleavage of cytokeratin-18 in HCV infected patients.[1,2,3] To date, emricasan has been studied in more than 550 individuals and has exhibited a safety profile similar to placebo.

Emricasan is currently in three Phase 2 clinical trials, including two trials in patients with liver cirrhosis. Here we report the effect of emricasan in healthy volunteers on measures of serum caspase activity and apoptosis. These results are compared with the effect of emricasan in subjects with varying degrees of hepatic impairment, as well as the effect of emricasan in subjects with severe renal impairment.

Background:

Caspases play a central role in the processes of apoptosis and inflammation. As such, caspases are attractive targets for the treatment of variety liver diseases.[4] In liver disease, chronically elevated apoptosis results in the accumulation of apoptotic cells as well as the release of apoptotic bodies and other subcellular fragments such as microvesicles.[4,5] These cellular end-products of apoptosis, which contain a wide variety of biologically active substances, are engulfed by neighboring tissue and promote disease pathology.[6]

Caspase mediated apoptosis is driven by the enzymatic action of caspase 3 and 7 on a wide variety of cellular substrates. One target substrate of caspases is the intermediate filament protein, cytokeratin-18 (CK18). Caspase cleavage of CK18 yields a protein fragment, cCK18, which is generally recognized as a specific mechanistic biomarker of apoptosis. Elevated serum levels of cCK18 have been associated with severity in a variety of liver diseases including NAFLD / NASH and HCV[7]. In patients with cirrhosis, cCK18 has been reported to increase progressively with disease severity[8]. Inhibition of caspases may therefore reduce the disease-driven loss of hepatocytes and production of apoptotic bodies and microparticles that promote disease progression, Figure 1.

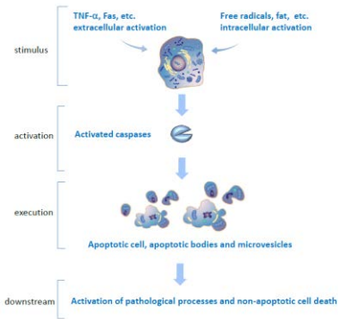

Figure 1: Hepatocyte cell death leads to amplification of disease pathology

Elevated serum levels of full-length CK18, (flCK18), are also associated with liver disease. Full-length CK18 is released following cell wall rupture, as occurs in necrosis, and as such is considered a mechanism independent, generic biomarker of overall cell death. Serum levels of cCK18 and flCK18 are measured by the specific ELISA based monoclonal antibody assays, M30-Apoptosense® ELISA, and M65® ELISA (VLVbio), respectively. Caspase 3/7 activity was measured using Caspase-Glo® 3/7 (Promega).

Inhibition of caspase activity has long been recognized as a potentially attractive approach for the treatment of a of variety liver diseases. However, a longstanding concern associated with the theoretical potential of the mechanism to disrupt normal homeostatic mechanisms and promote tumor formation, has understandably hampered development in this field. We show here that emricasan does not affect caspase activity and apoptosis in healthy subjects and is unlikely to affect normal homeostatic processes that may be modulated by caspase enzymes. Additionally, emricasan was found to have no tumorigenic potential in a recently reported carcinogenicity study.

Methods:

Emricasan was administered as a single 50 mg oral dose to subjects with mild, Child Pugh A (n=12), moderate Child Pugh B (n=8) or severe Child Pugh C (n=8), hepatic impairment and 8 healthy controls matched demographically with subjects in the severe cohort. In a second study, emricasan was administered as a single 50 mg dose to 8 subjects with severe renal impairment and 8 matched healthy controls. In both studies, serial blood samples were collected over a 48 hour period and analyzed for markers of apoptosis, cell death and caspase enzymatic activity.

On Study Day 1, a pre-dose blood sample was collected followed by administration of a single 50 mg dose of emricasan to all subjects. Eight blood samples were then collected over a 12 hour period post-dose with further samples collected at 24 and 48 hours post-dose.

Results:

Median baseline levels of caspase 3/7 activity in subjects with hepatic impairment were elevated in all subject groups, (mild, moderate and severe), relative to matched healthy control subjects. In addition, baseline levels of caspase 3/7 enzymatic activity were elevated in subjects with severe renal impairment relative to matched healthy control subjects. The data for baseline caspase 3/7, cCK18 and flCK18 for respective controls and severe cohorts are presented in Table 1.

Group (n)	Median Caspase 3/7 Activity (RLU)	Median cleaved cytokeratin 18 (M 30) (U/L)	Median full length cytokeratin 18 (M 65) (U/L)
Renal Control (8)	919.5	240.0	334
Hepatic Control (8)	963.5	161.5	284.0
Severe Renal (8)	1290.0	223.5	427.5
Severe Hepatic (8)	1662.5	517	851.0

Table 1: Baseline caspase activity and biomarkers of cell death and apoptosis in subjects with severe renal and severe hepatic impairment and matched healthy control subjects

Healthy control subjects in both studies exhibited similar baseline levels of serum caspase 3/7 enzymatic activity, serum levels of cCK18 and flCK18. Treatment with a 50 mg dose of emricasan had no effect on serum levels of caspase activity in either cohort of healthy controls, Figure 1. In addition, there were no statistically significant reductions in either cCK18 or flCK18 in these healthy subjects.

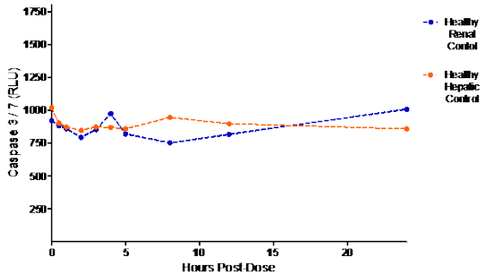

Figure 1: Median of serum levels of caspase 3/7 enzymatic activity following a single 50 mg oral dose of emricasan to healthy control subjects.

This observation is consistent with our previous experience in which treatment of healthy volunteers with emricasan had no effect on markers of apoptosis.[9] Taken together these results suggest that the pan-caspase inhibitor, emricasan, has little, if any, effect on physiologically normal serum levels of caspase activity.

In hepatic impaired subjects, baseline levels of caspase 3/7 were elevated relative to healthy controls. Treatment with a single 50 mg dose of emricasan rapidly reduced caspase activity levels in all subjects with liver disease, Figure 2. A maximal response was observed approximately 4 hours post-dose.[10]

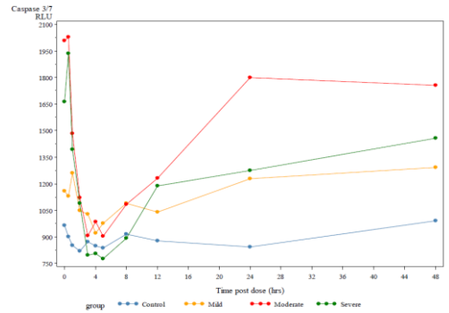

Figure 2: Median of serum levels of caspase 3/7 enzymatic activity following a single 50 mg oral dose of emricasan to subjects with hepatic impairment and healthy controls

Importantly, caspase activity approached levels observed in the healthy control cohort and returned to pre-dose levels approximately 24 to 48 hours.

Unlike subjects with hepatic impairment, elevated serum levels of caspase enzymatic activity were not reduced in subjects with severe renal impairment following a 50 mg dose of emricasan, Figure 3. Comparing

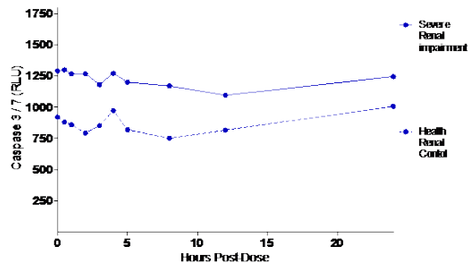

Figure 3: Median of serum levels of caspase 3/7 enzymatic activity following a single 50 mg oral dose of emricasan to subjects with severe renal impairment or healthy control subjects.

these results with the rapid and pronounced response in subjects with hepatic impairment, strongly suggests that emricasan is not inhibiting caspase activity in the serum, but rather is reducing the efflux of elevated levels of active caspase from the diseased organ. In the case of hepatic impairment, it is well documented that emricasan is rapidly taken up into liver and data suggests that this may largely be due to active transport mechanisms involving the hepatic uptake transporters OATP1B1, OATP1B3 and possibly NTCP. The expression of these transporters is generally restricted to the liver with limited expression in other tissues such as the kidney.[11] Passive diffusion of emricasan across cell membranes is low and does not play a major role in the uptake of emricasan. The fact that emricasan did not reduce elevated caspase activity in subjects with severe renal impairment is consistent with this model. There were no statistically significant reductions of either cCK18 or flCK18 in these subjects with severe renal impairment.

Importantly, a recently reported six month carcinogenicity study conducted with emricasan in transgenic, (Tg.rasH2), tumor prone mice, concluded that emricasan had no carcinogenic potential in man.[12]

Collectively these data provide important new insight and increase our understanding of the effect of a small molecule pan-caspase inhibitor in man. Furthermore these observations are also consistent with the large pre-clinical database of safety information along with the clinical experience with emricasan, both of which are remarkably devoid of theoretically anticipated mechanism-driven side effects.

Conclusions:

- Emricasan does not affect serum caspase enzymatic activity in healthy subjects.
- This observation suggests that in healthy individuals, on-going caspase activity and apoptosis is largely unaffected by emricasan.
- The lack of effect in subjects with severe renal impairment suggests emricasan possesses a high degree of tissue specificity.
- Emricasan may provide clinical benefit to patients with liver disease including those with advanced stages of liver disease.

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* aspada@conatuspharma.com

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