**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 cyclophilins are extensively metabolized via cytochromes P450, the aim of this study was to characterize CRV431 metabolism in liver microsomes from several species in vitro.

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**METHODS**

The in vitro metabolism of CRV431 was studied in microsomes from rat, monkey and human livers (Sekisui Xenotech). Microsomes were incubated at 37 °C for 0, 10, 20, 30, and 80 minutes with 0.1, 1 and 10 µg/ml CRV431 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.

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**RESULTS**

- CRV431 was extensively metabolized through oxidation to produce various hydroxylated and demethylated species.
- Metabolite species identified, as their sodium adducts, included monohydroxylated CRV431 (two distinct metabolites, 1342 m/z), di-hydroxylated CRV431 (1538 m/z), demethylated CRV431 (two distinct metabolites, 1312 m/z), methyalted and hydroxylated CRV431 (two distinct metabolites, 1328 m/z), demethylated and hydroxylated CRV431 (1314 m/z), and demethylated and dihydroxylated CRV431 (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 CRV431.

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**CONCLUSION**

CRV431 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 CRV431 for chronic hepatitis B.