21 Voriconazole Inhibition of Tacrolimus Metabolism in Human Liver Microsomes and After Liver Transplantation

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BACKGROUND

Voriconazole is known to inhibit cytochrome P450 3A4 enzyme, however, the extent of the interaction between tacrolimus and voriconazole has not been defined. We assessed the effect of voriconazole at steady state on the pharmacokinetics of tacrolimus after liver transplantation and examined the interaction between voriconazole and tacrolimus using human liver microsomes to determine its effect on CYP3A inhibition in comparison to other azoles.

METHODS

Clinical Study Design: This was planned as an open, randomized, two period, two treatment, placebo controlled pharmacokinetic study. Prior to enrollment in this study, patients were to have stabilized on individualized doses of tacrolimus. During the first treatment period, the first enrolled subject who received voriconazole and tacrolimus showed elevated tacrolimus blood concentrations (>15ng/mL). In accordance with the protocol, these elevated tacrolimus blood concentrations qualified the subject for withdrawal from the study.

Drug Administration

Dosing: Voriconazole 200 mg bid or 0 mg bid

Laboratory Methods: Human liver microsomes were prepared by differential centrifugation from adult organ donors.

Assessment of inhibition of tacrolimus metabolism: Tacrolimus was incubated at various concentrations with human microsomal protein (0.4mg/ml), MgCL2 (10mM) for ten minutes at 37oC in 0.1mM phosphate buffer (mM, PH 7.4). The reaction was initiated with the addition of NADPH (1mM). After 30 minutes the reaction was terminated by placing the tubes into ice, quickly followed by addition of 5 ml of cold ethyl ether.

Tacrolimus Analysis: Cyclosporine (0.1mg/ml) was used as the internal standard. Tacrolimus and cyclosporine were extracted in to ether; ether layer was separated and evaporated under nitrogen. The residue obtained was reconstituted in actonitrile/H2O (6:4) for HPLC analysis. The change in the concentration of Tacrolimus was quantified by reverse phase HPLC. A C-18 column (3.9x150mm, 10 mm, Bondapack, Part Number 86684) was equipped with a C-18 guard column and was maintained at 70oC. A mobile phase consisting of acetonitrile/water (6:4) at a flow rate of 1.5 ml/min was used. The column eluent was monitored at 214nm. Low concentration of tacrolimus was measured by MEIA method in IMx analyzer (Tacrolimus II assay).

Assessment of CYP3A activity:

Microsomal Incubations: Formation of 6b hydroxytestosterone from testosterone was used as a measure for CYP 3A4 activity in these microsomes. Testosterone was incubated with human microsomal protein (0.4mg/ml), MgCL2 (10mM) and NADPH (1mM) for 15 minutes at 37oC in 0.1mM phosphate buffer (mM, PH 7.4). The reactions were terminated by placing the tubes in ice, quickly followed by addition of 500ml of cold methanol Samples were vortexed and centrifuged at 30,000 rpm for 4 minutes. And supernatant analyzed by HPLC for 6b-hydroxytestosterone.

HPLC Analysis: Concentration of 6b-hydroxytestosterone was measured by reverse phase HPLC using 100 ml of supernatant injected into a LiChrospher 100 RP-18 column (4.6x250 mm, 5 mm) with a mobile phase of methanol:water (60:40) and a flow rate of 1.2ml/min. The product was detected by its absorbance at 242 nm and quantitated by comparing the absorbance to a standard curve of 6b-hydroxytestosterone.

Km and Vmax determinations for testosterone and tacrolimus: A range of substrate concentration was incubated as outlined above to determine the Km and Vmax. The substrate concentrations were 10-250mM for testosterone and 3-15mg/ml for tacrolimus. For testosterone, the inhibitor concentrations were as follows: voriconazole (5, 200mg/ml), itrconazole (0.1, 2.5mg/ml) and ketoconazole (2, 25 ng/ml), the formation rate of the metabolite, 6b-hydroxytestosterone was calculated (nmol/mg protein/min). For tacrolimus, voriconazole concentration was 0, 200mg/ml, the loss of parent tacrolimus was measured and rate of metabolism was calculated. Michaelis-Menten equation for a one-enzyme model was fitted to the data using the iterative non-linear regression program, Prism.

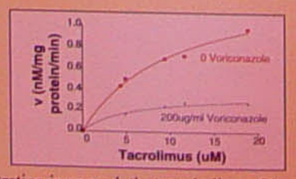
IC50 values determinations for testosterone and tacrolimus: A single concentration of each substrate was used: 200mM for testosterone and 50 ng/ml for tacrolimus. Inhibitors were prepared as methanolic stock solutions and inhibitor concentration range in the reaction mixture were as follow: voriconazole (2-500mg/ml for testosterone, 1-100mg/ml for tacrolimus), itraconazole (0.001-10mg/ml for testosterone, 0.0003-2.5mg/ml for tacrolimus) and ketoconazole (0.1-100ng/ml for testosterone, 0.06-25ng/ml for tacrolimus). Concentrations of the inhibitor to cause 50% inhibition of original enzyme activity were determined by Prism.

RESULTS

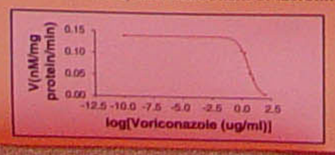
	Drug (dosing)	Tacrolimus dose, trough level	Whole blood tacrolimus level
Patient 1	Voriconazole 200 mg po bid (day 0 to 5)	2mg/d, 2.3ng/ml	12.5ng/ml (day 3), 23.4ng/ml (day 5)
Patient 2	Placebo (day 0 to 6)	4mg/d, 4.3ng/ml	3.4 to 6.2ng/ml (day 0-6)

Human liver microsome data results:

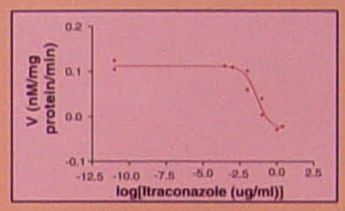
Metabolism of tacrolimus was assessed in the absence and presence of voriconazole. In the presence of voriconazole the metabolism of tacrolimus was inhibited.



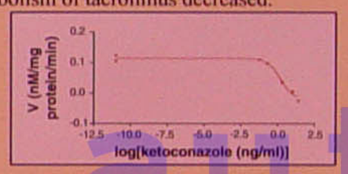
As voriconazole concentration increased, the metabolism of tacrolimus decreased



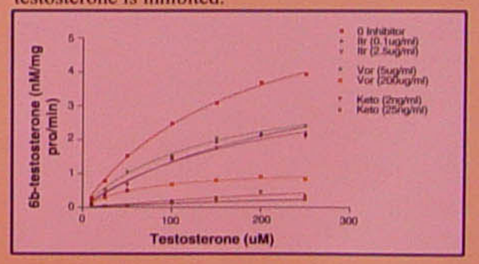
As itraconazole concentrations increased, the metabolism of tacrolimus decreased



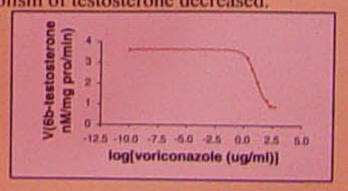
As ketoconazole concentration increased, the metabolism of tacrolimus decreased.



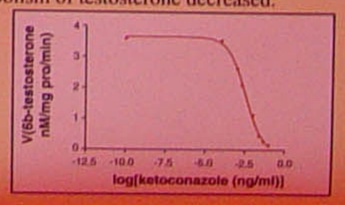
In the presence of inhibitor, the metabolism of testosterone is inhibited.



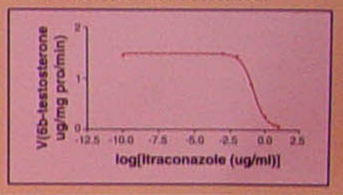
As voriconazole concentration increased, the metabolism of testosterone decreased.



As ketoconazole concentration increased, the metabolism of testosterone decreased.



As voriconazole concentration increased, the metabolism of testosterone decreased.



Kinetic Parameters for inhibition of metabolism

Inhibitor	Substrate			
	Tacrolimus (3.7-18.8uM)		Testosterone (10-250uM)	
	Vm	Km	Vm	Km
Control	1.473	10.64	6.867	180.2
Voriconazole (200 ug/ml)	0.37	5.43	1.161	70.1
Voriconazole (5 ug/ml)	-	-	3.816	140.5
Itraconazole (2.5 ug/ml)	-	-	0.68	473
Itraconazole (0.1 ug/ml)	-	-	4.918	266
Ketoconazole (25 ng/ml)	-	-	1.62	700
Ketoconazole (2 ng/ml)	-	-	4.05	204

IC50 determinations for CYP 3A4

	Tacrolimus (50 ng/ml)	Testosterone (200uM)
Voriconazole (ug/ml)		
IC50	4.02	11.46
Concentration range	1-100	2-500
Itraconazole (ug/ml)		
IC50	0.05	0.15
Concentration range	0.0003-2.5	0.001-10
Ketoconazole (ng/ml)		
IC50	1.52	3.4
Concentration range	0.06-25	0.1-100

CONCLUSIONS

Voriconazole inhibited the metabolism of tacrolimus. The rank order of potency for inhibition of tacrolimus and CYP3A metabolism was ketoconazole > itraconazole > voriconazole. Thus, while voriconazole was less potent than itraconazole and ketoconaozle in vitro in inhibiting CYP3A, coadministration of voriconazole with tacrolimus resulted in a significant increase in tacrolimus blood concentrations after liver transplantation.