Use of Midazolam Microdosing in an Entry Into Human (EiH) Study Allows Early Determination of Clinical Drug Interaction Liability Through CYP3A4

Yuyan Jin[1], Annelieke Kruithof[2], Zhigang Yu[3], Hisham Abdallah[1], Jacobus Burggraaf [2,4], Ingrid Kamerling[2], Jun Shi[1], Ilia Folitar[1], Suchat Wongcharatrawee[3], Richard Peck[1]

1. Hoffmann-La Roche, Pharma Research and Early Development; 2. Centre for Human Drug Research; 3. Former Hoffmann-La Roche Employee; 4. Leiden Academic Center for Drug Research, Netherlands.

1 – Background and Objectives

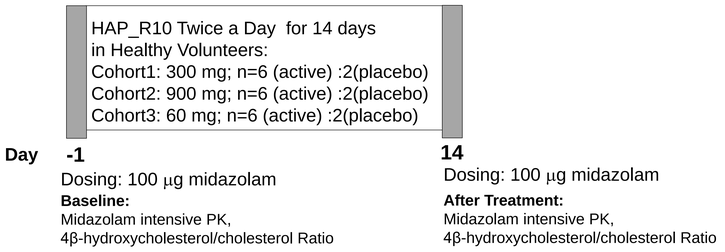
- HAP_R10 is an inhibitor of Hepatitis B virus (HBV) capsid assembly that significantly induced CYP3A4 *in vitro*.
- Microgram doses of midazolam can be used in early clinical trials as a safe, sensitive probe of CYP3A4 activity [1,2].
  - Micro dose of midazolam is sub-therapeutic and has no particular safety concerns.
  - Microdosing of midazolam can reliably predict Pharmacokinetics (PK) at therapeutic doses to evaluate *in vivo* CYP3A activity.
- 4β-hydroxycholesterol is a metabolite formed by CYP3A-catalyzed metabolism of cholesterol. 4β-hydroxycholesterol/cholesterol ratio in blood can be measured as an endogenous marker for the activity of hepatic CYP3A4 [3].
- ***The objective of the research was to evaluate the effect of multiple oral dosing of HAP_R10 on CYP3A4 activity in an EiH study assessed by the PK of a single oral microdose of midazolam and by blood 4β-hydroxycholesterol/cholesterol ratio.***

[1] Clin Pharmacol Ther. 2013 Jun;93(6):564-71
[2] Br J Clin Pharmacol. 2014; 79(2): 278-85
[3] Drug Metab Dispos 41:1488–1493

2 – Clinical Study Design

- Single-ascending dose (SAD, 30 – 2000 mg, n=39) and multiple-ascending dose (MAD, 60 – 900 mg BID for 14 days, n=18) of HAP_R10 study in healthy volunteers (HVs).
- A single oral dose of 100 μg midazolam was administered prior to and after 13 days of HAP_R10 dosing in all MAD cohorts to evaluate induction potential on CYP3A4 as measured by midazolam systemic exposure.
- 4β-hydroxycholesterol and cholesterol levels were measured in MAD cohorts prior to and after HAP_R10 dosing as an endogenous marker for hepatic CYP3A4 activity.

Figure 1. Multiple-Ascending Dose Part of HAP_R10 EiH Study Design



3 – Study Results

3.1 Midazolam Results

Figure 2. Mean (SD) Midazolam Plasma Concentration vs. Time Profiles Before and After Multiple Dose of HAP_R10

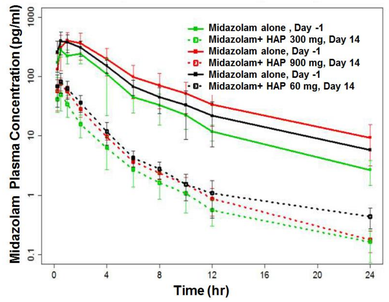
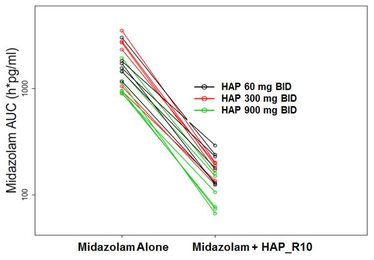


Table 1. Midazolam PK Parameters Before and After Multiple Dose of HAP_R10

Parameters (Unit)	HAP_R10 +Midazolam 100 μg					
	60 mg		300 mg		900 mg	
	Day −1 N = 6	Day 14 N = 6	Day −1 N = 6	Day 14 N = 6	Day −1 N = 6	Day 14 N = 6
C_{max} (pg/mL)	409 (31.7)	78.7 (44.6)	424 (51.5)	74.6 (23.2)	301 (18.1)	49.1 (41.5)
t_{max} (hours)	0.50 (0.5 – 2.0)	0.50 (0.3 – 0.5)	1.50 (0.5 – 2.0)	0.50 (0.3 – 1.0)	0.51 (0.5 – 2.0)	0.50 (0.3 – 0.5)
AUC_{0-tlhr} (pg • hr/mL)	1260 (26.4)	163 (33.8)	1340 (51.6)	143 (19.5)	884 (31.9)	85.0 (41.0)
AUC_{0-last} (pg • hr/mL)	1610 (31.2)	179 (35.6)	1850 (58.2)	158 (20.4)	1110 (31.6)	94.7 (40.0)
AUC_{0-inf} (pg • hr/mL)	1650 (33.7)	182 (35.4)	1910 (59.7)	160 (20.5)	1130 (31.2)	96.0 (39.6)
$t_{1/2}$ (hours)	4.57 (33.0)	3.02 (52.0)	5.06 (27.6)	3.54 (43.9)	4.20 (22.4)	2.57 (51.4)
CL/F (L/hr)	60.6 (33.7)	550 (35.4)	52.3 (59.7)	624 (20.5)	88.8 (31.2)	1040 (39.6)

* PK parameters are presented as geometric mean (coefficient of variation) except for t_{max} which is shown as median (range).

Figure 3. Individual Midazolam AUC_{0-inf} Changes Before and After Multiple Dose of HAP_R10 (n=18)



- Midazolam AUC_{0-inf} were substantially reduced by 89%, 91%, and 91%, respectively, when given with 60 mg BID, 300 mg BID, and 900 mg BID HAP_R10.
- The decreases in midazolam exposure confirmed clinical CYP3A4 induction by HAP_R10.
- All HAP_R10 doses resulted in maximal induction and no dose response was characterized.

3.2 *4β-hydroxycholesterol/cholesterol Ratio Results*

- Compared with values prior treatment, the ratios of 4-*β* hydroxycholesterol/cholesterol were increased by 1.9, 2.6 and 4.9 fold after 14 days of 60 mg, 300 mg, and 900 mg BID dosing of HAP_R10, respectively.
- The increases in 4-β hydroxycholesterol/cholesterol ratio across the dose range tested indicate corresponding dose-response in activity of hepatic CYP3A4 following multiple doses of HAP_R10.

Figure 4. Individual 4β-hydroxycholesterol/cholesterol Ratio Before and After Multiple Dose of HAP_R10 (n=18)

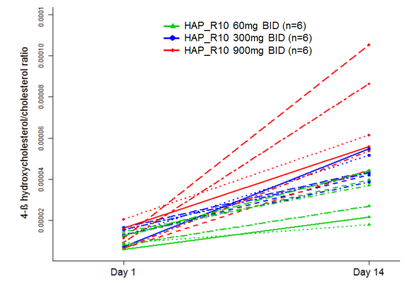
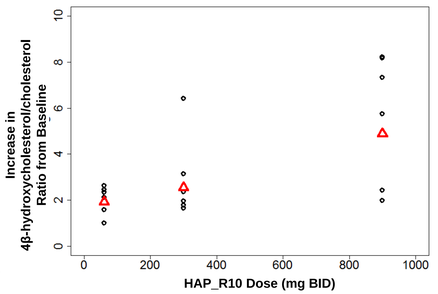


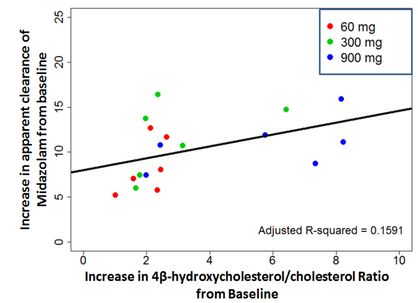
Figure 5. Dose Response in 4β-hydroxycholesterol/cholesterol Ratios Before and After Multiple Dose of HAP_R10 (n=18)



* Red triangles represent geometric mean at each dose level.

3.3 Midazolam vs. 4β-hydroxycholesterol/cholesterol Ratio

Figure 6. Correlation Between Increases in Apparent Clearance of Midazolam and Increases in 4β-hydroxycholesterol/cholesterol ratio (n=18)



- The increases in 4β-hydroxycholesterol/cholesterol ratio, which reflected induction on hepatic CYP3A4, were lower than the increases in apparent clearance of midazolam, where a combined induction effect on both gut and liver CYP3A4 was estimated.
- However, linear regression shows a significant correlation between increases in midazolam clearance and increases in 4β-hydroxycholesterol/cholesterol ratio.

4 – Conclusion

- The decreases in midazolam exposure and increases in 4β-hydroxycholesterol/cholesterol ratio indicated clinically significant CYP3A4 induction by HAP_R10.
- Use of Midazolam microdosing in an EiH study allows early determination of clinical drug interaction liability through CYP3A4.
- The corresponding dose-response increases in 4-β hydroxycholesterol/cholesterol ratio suggested its potential use as endogenous marker of hepatic CYP3A4 activity.