4906

Effect of Gender on the Rodent Pharmacokinetics of ON 123300, A Dual Inhibitor of ARK5 and CDK4/6, for the Treatment of Cancer

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PURPOSE

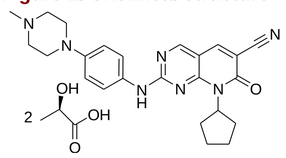
- ON 123300 is a novel third generation cyclin-dependent kinases 4/6 (CDK4/6) inhibitor with dual inhibition of c-MYC activated kinases ARK5 controlling cellular metabolism and survival with low nanomolar potency[1].
- ON 123300 has the potential to be effective in patients developing resistance to second generation CDK4/6 inhibitor compounds.
- CYP450 reaction phenotyping studies suggested that ON 123300 is susceptible to metabolism by CYP3A4 and CYP2C8[2].
- CYP3A4 constitutes about 30% of the total CYP 450 in liver[3]. They are found to be more actively secreted in males compared to females in certain species[4].
- This study was undertaken to investigate the gender differences in the metabolism of ON 123300 in rats, a preclinical toxicological species.

METHODS

- *In vitro* metabolism experiments were performed in rat liver microsomes from male and female donors.
- ON 123300 (final 10 µm) was incubated with microsomes, and samples (100 µl) were withdrawn at specified incubation times over 60 minutes and immediately quenched and centrifuged.
- The supernatant was analyzed for ON 123300 and its metabolites by HPLC.
- An *in vivo* pharmacokinetic study was performed in male and female SD rats using intravenous (bolus over 30 sec; n =3/gender) or oral route of administration (n=5/gender). Intravenous doses were 5 mg/kg and 10 mg/kg; whereas oral dose was 100 mg/kg. Blood samples were collected over 4 hours and 24 hours for IV and oral route of administration, respectively.
- ON 123300 plasma concentrations were measured by LC-MS/MS method[5] and PK parameters were estimated by non-compartmental analysis.

RESULTS

Figure 1. Chemical structure



ON 123300 Bislactate
Mol. Formula: $C_{30}H_{39}N_7O_7$
Mol. Wt.: 609.67

Figure 2. First pass metabolism

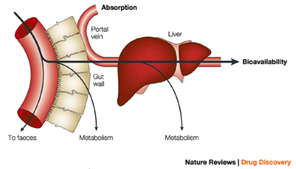


Image source:
https://canna-pet.com/first-pass-effect/

Table 1. ON 123300 pharmacokinetic parameters from *in vitro* liver microsomal metabolism study from male and female rat donors

Parameter[a]	Males	Females
$t_{1/2}$ (min)	10.8	38.2
$Cl_{int,vitro}$ (µL/min/mg)[b]	130	36.7
$Cl_{int,vivo}$ (mL/min/Kg)[c]	239	67.5
Predicted Cl in vivo (mL/min/Kg)[d]	2.33	0.670

[a]data presented as the average of duplicate experiments
[b] $Cl_{int} = k \times V$ where k is the degradation rate constant and v is the volume of sample per mg of microsomal protein (2.0 mL/mg)
[c]Estimated using equation $Cl_{int,vivo} = Cl_{int} \times MPPGL \times Wt_{liver}$ where MPPGL (microsomal protein per gram of liver) is 46 mg/g and average rat liver weight is10 g (normalized by average body weight of 0.25 kg)[6].
[d]Estimated using equation $Cl = \frac{Q \times f_u \times Cl_{int,vivo}}{Q + f_u \times Cl_{int,vivo}}$ where fu is the fraction of drug unbound in blood (f_u= 0.01) and Q is the rat hepatic blood flow (85 mL/min/kg)[7].

Table 2. ON 123300 pharmacokinetic parameters following IV administration (5 mg/kg and 10 mg/kg) to male and female rats

Parameter[a]	Dose			
	5 mg/kg		10 mg/kg	
	Males	Females	Males	Females
$AUC_{0-\infty}$ (ng-hr/mL)	1020 ± 128	1795 ± 217	2200 ± 136	4399 ± 542
Cl (mL/min/Kg)	82.67 ± 11.09	47.15 ± 6.20	75.90 ± 4.66	38.44 ± 4.60
V (L/kg)	5.99 ± 0.81	3.07 ± 0.16	4.72 ± 0.99	2.85 ± 0.17
$t_{1/2}$ (hr)	0.84 ± 0.03	0.76 ± 0.06	0.71 ± 0.10	0.87 ± 0.12

[a]data presented as mean ± SD of 3 animals per group

Table 3. ON 123300 pharmacokinetic parameters following oral administration (100 mg/kg) to male and female rats

Parameter[a]	Males	Females
C_{max} (ng/mL)	321 ± 58.9	1253 ± 590
T_{max} (hr)	2.3 ± 1.4	0.6 ± 0.2
$AUC_{0-\infty}$ (ng-hr/mL)	1965 ± 749	5617 ± 1914
Cl/F (mL/min/Kg)	971 ± 329	372 ± 228
$t_{1/2}$ (hr)	1.9 ± 0.5	3.0 ± 0.5

[a]data presented as mean ± SD of 5 animals per group

Figure 3. ON 123300 plasma concentration vs. time profile following IV administration to male and female rats (n=3/group).

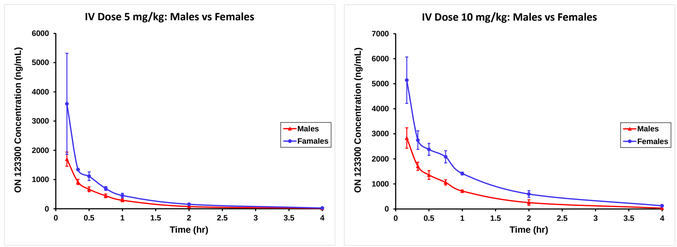
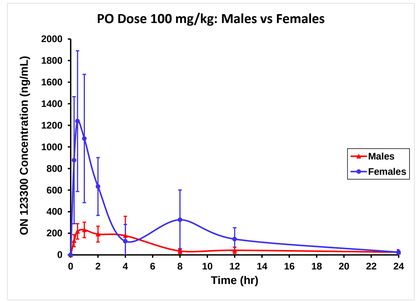


Figure 4. ON 123300 plasma concentration vs. time profile following oral administration (100 mg/kg) to male and female rats (n=5/group).



CONCLUSIONS

- **The *in vitro* intrinsic clearance was ~3.5 fold higher in male liver microsomes compared to female liver microsomes suggesting a differential expression of CYP's responsible for the metabolism of drug.**
- **The observed clearance (Table 2) in male and female rats was significantly higher than the predicted *in vivo* clearance based on intrinsic clearance data from the *in vitro* studies in liver microsomes (Table 1). This may be due to extrahepatic drug metabolism, active drug uptake into the liver and renal elimination of unchanged drug[8].**
- **Drug exposure was dose proportional. The clearance in male rats (~80 mL/min/kg) approximated rat hepatic blood flow (85 mL/min/kg) suggesting that the compound has a high hepatic extraction ratio.**
- **Consistent with *in vitro* liver microsome study, ON 123300 displayed significantly higher exposure (~3 fold increase of AUC) in female rats compared to male rats after oral administration.**
- **Gender differences in the pharmacokinetics of the drug should be taken into account while selecting the relevant species for toxicological evaluation of the compound; and designing the dosing strategy for further development.**

REFERENCES

[1]Reddy MV, et al. J Med Chem. 2014;13; 57(3):578-99.
[2]Hoffman BS, et al. Mol Cancer Ther. 2015; 14:LB-A21.
[3]Bertz RJ, et al. Clin Pharmacokinet. 1997; 32(3):210-258.
[4]Tetsuya A, et al. Biol Pharm Bull. 2005; 28(2):311-315.
[5]Mudunuru J, et al. AAPS. 2017; W4101.
[6]Obach RS, Drug Metab Dispos. 1999; 27 (11):1350-1359.
[7]Kajbaf M, et al. Eur J Drug Metab Pharmacokinet. 2013; 38:33-41.
[8]Iwatsubo T, et al. Pharmacol Ther. 1997; 73(2):147-171.