NKTR-181: Relationship Between Mu-Opioid Receptor Binding Kinetics and *In Vivo* Pharmacodynamics

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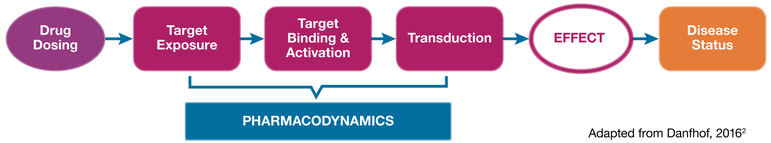
BACKGROUND

- NKTR-181 is a novel mu-opioid receptor agonist in clinical development for the treatment of moderate-to-severe chronic low back pain in opioid-naïve adult patients. We have previously reported that the unique physicochemical properties of NKTR-181 result in a slower rate of entry into the central nervous system (CNS) compared with standard opioids[1]
- Rapid entry into the CNS and the subsequent activation of mu-opioid receptors are important factors that make an opioid attractive for abuse; therefore, NKTR-181 is hypothesized to show less potential for abuse relative to conventional opioids while also achieving meaningful analgesia
- Here, we compare the temporal effects of NKTR-181 and oxycodone on mu-opioid receptor pharmacology using *in vitro* kinetic receptor binding and signaling assays as well as *in vivo* pharmacodynamic studies of neurotransmitter release and antinociception

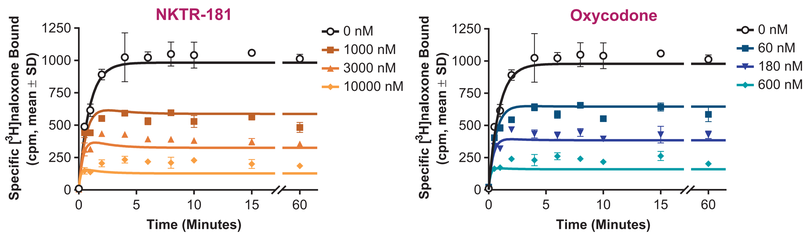


Adapted from Danfhof, 2016[2]

RESULTS

NKTR-181 and Oxycodone Binding Kinetics at Mu-Opioid Receptor

NKTR-181 displays slower association kinetics relative to oxycodone



Compound	k_{on} ($M^{-1}min^{-1}$)	k_{off} (min^{-1})	$t_{1/2}$ (min)	K_d (nM)
NKTR-181	5.45 x 10^5	0.443	1.56	813
Oxycodone	86.8 x 10^5	0.554	1.25	63.8

Figure 1. Binding kinetics at mu-opioid receptor. Studies were performed using membranes from Chinese hamster ovary (CHO) cells heterologously expressing recombinant human mu-opioid receptors. Receptor association and dissociation rates were determined in competition binding studies that measured kinetic binding of a fixed concentration of [^{3}H]Naloxone to mu-opioid receptor in the absence or presence of various concentrations of unlabeled test compound. The kinetic parameters k_{on} and k_{off} were calculated using the equations of Motulsky and Mahan[3]

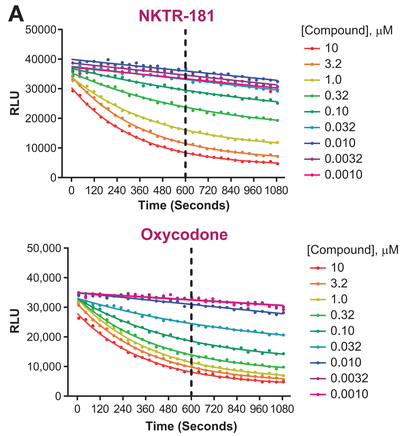
REFERENCES

1. Miyazaki T, et al. *J Pharmacol Exp Ther.* 2017;363(1): 104-113
2. Danhof M. *Eur J Pharm Sci.* 2016; 94: 4-14
3. Motulsky HJ, Mahan LC. *Mol Pharmacol.* 1984; 25(1): 1-9

RESULTS *(CONTINUED)*

Real-time Functional Effects of Mu-Opioid Receptor Activation by NKTR-181 and Oxycodone

Differential potency and rates of forskolin-induced cAMP inhibition by NKTR-181 and oxycodone



Compound	k (sec^{-1})
NKTR-181 (0.3 μM)	0.0013
Oyxcodone (0.3 μM)	0.0025
DAMGO (0.1 μM)	0.0028

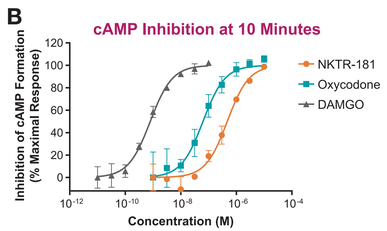


Figure 2. Onset of mu-opioid receptor activation. CHO cells stably expressing the human mu-opioid receptor were transiently transfected with pGloSensor-22F cAMP plasmid (Promega, Madison, WI). Adenylyl cyclase inhibition studies were performed by incubating cells with 10 μM forskolin for 10 min prior to addition of agonists, then real-time changes in cAMP levels were monitored as luminescent signal at indicated time points. (A) Kinetic traces of the effect of NKTR-181, oxycodone, and DAMGO on the inhibition of forskolin-induced cAMP. The rates of forskolin-induced cAMP inhibition (k) were calculated from one-phase decay non-linear regression analysis of time-response curves. (B) Concentration-response data obtained at 10 min were fitted using nonlinear regression analysis

NKTR-181 Induces Durable Antinociceptive Effect

NKTR-181 peak latency effect delayed relative to oxycodone

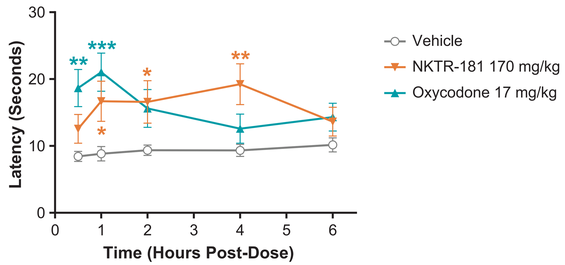
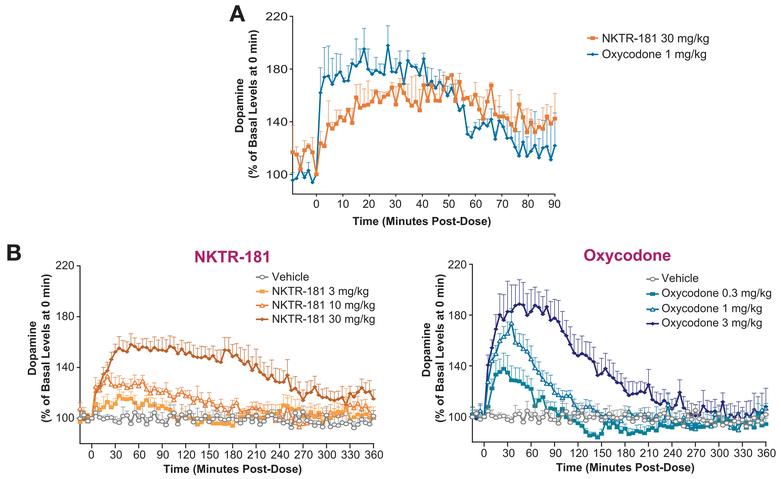


Figure 3. Hot Plate latency time course effect. The time course of hot plate latency was observed following oral administration of a single dose (50% effective dose) of NKTR-181 or oxycodone in Sprague-Dawley rats. Latency measurements were taken at 0.5, 1, 2, 4 and 6 hours post-dose. Data represent mean ± SEM (n=10). *P<0.05, **P<0.01, ***P<0.001; Two-way ANOVA, Dunnett's post-test with respect to saline

Dopamine Release in the Nucleus Accumbens in Response to NKTR-181 and Oxycodone

NKTR-181 induces slower onset of dopamine release in rat nucleus accumbens



Dopamine Release	Oxycodone (n=6)			NKTR-181 (n=6–7)			Vehicle (n=6)
	0.3 mg/kg	1 mg/kg	3 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	
Emax (%)	137.7	173.7	188.6	117.6	133.1	158.1	100
Relative $AUE_{0\text{-}5min}$	103.3	122.1*	134.7***	106.6	119.6	115.4	100
Relative $AUE_{0\text{-}10min}$	112.2	132.3***	143.8***	106.5	120.4*	119.9*	100
Relative $AUE_{0\text{-}20min}$	123.2	143.5***	159.2***	108.7	125.5*	128.1**	100

Relative AUE, area under the effect curve, % relative to vehicle
*p<0.05, **p<0.01, ***p<0.001; One-way ANOVA, Dunnett's multiple comparison test with respect to vehicle

Figure 4. Extracellular dopamine levels in the nucleus accumbens of awake rats. Test compound was administered as single intravenous doses to male Sprague-Dawley rats. Microdialysates were collected from a probe implanted in the nucleus accumbens shell of awake animals in (A) 90-second or (B) 5-minute intervals, and extracellular dopamine was quantified by HPLC using electrochemical detection

CONCLUSIONS

- NKTR-181 exhibits a slower pharmacodynamic profile compared to oxycodone across multiple *in vitro* and *in vivo* models
- These data support a differentiated mechanism of action for NKTR-181 relative to traditional opioids such as oxycodone

ACKNOWLEDGMENTS

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