Xanomeline's Activity in Rodent Models of Psychosis: Role of Central Muscarinic Receptors and Augmentation by Risperidone and Aripiprazole

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Background

All marketed antipsychotic drugs for schizophrenia directly antagonize dopamine D2 receptors[1] and have substantial efficacy limitations and safety limitations[2]. Xanomeline, a M_1/M_4-preferring muscarinic receptor agonist, has shown antipsychotic activity in schizophrenia[3] and Alzheimer's disease trials[4] but does not bind D2 receptors[5]. The investigational antipsychotic KarXT combines the peripheral antimuscarinic trospium with xanomeline to mitigate its associated pro-cholinergic adverse effects. KarXT exhibited robust antipsychotic activity in a Phase 2 clinical trial[6] and is currently in Phase 3 development for schizophrenia. Here we demonstrate the potential for antipsychotic response augmentation by combining agents with different mechanisms of action, using the Conditioned Avoidance Response (CAR) and MK-801-induced Locomotor Assay (LMA) in mice to compare dose-escalation of xanomeline with the D_2 antagonist antipsychotics risperidone or aripiprazole, alone and in sub-threshold dose combinations. Finally, we confirm that the avoidance response to xanomeline is centrally mediated.

Methods

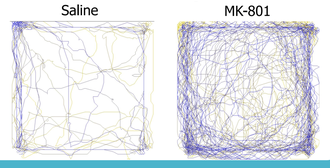
Animals: Adult male, C57BL/6J mice

Conditioned Avoidance Response (CAR): CAR is a validated preclinical model to study antipsychotic drugs[7]. Mice were first trained to avoid a foot-shock (i.e., crossing from one side of a shuttle box to another within 10 secs) to a performance criterion of ≥85% avoidance responses. A multiple-effects repeated-measures, counter balanced design was used. *Post hoc* analysis was conducted with Dunnett's (to vehicle group comparisons) or Sidak family-wise *post hoc* analysis (more than 1 comparison). Experiments evaluated the effects of blocking central (scopolamine) vs. peripheral (N-methylscopolamine) muscarinic receptors. Additional experiments evaluated augmentation by combining subthreshold subcutaneous doses of xanomeline plus risperidone or aripiprazole.

Locomotor Activity (LMA): LMA is a preclinical model for evaluation of antipsychotic-like activity[8]. In this study, varying doses of xanomeline and risperidone were co-formulated and dosed i.p. 30 minutes before a challenge dose of the NMDA receptor antagonist MK-801, followed by evaluation of open-field activity for 60 minutes. Results were analyzed *post hoc* using a one-way ANOVA with Dunnett's post-hoc tests. Plasma and brain tissue were collected to determine drug exposure following the experiment. Below limit of detection (BLD) values: 0.2 ng/mL risperidone and MK-801 and 0.4 ng/mL xanomeline.

Conditioned Avoidance Response

Locomotor Activity



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Funding of this work was provided by Karuna Therapeutics. The CAR study was conducted by the Indiana University School of Medicine (IUSM) Behavioral Phenotyping Core and the MK-801 LMA study by Charles River labs.

Xanomeline, Risperidone, and Aripiprazole Dose-Dependently Reduce Conditioned Avoidance Responding

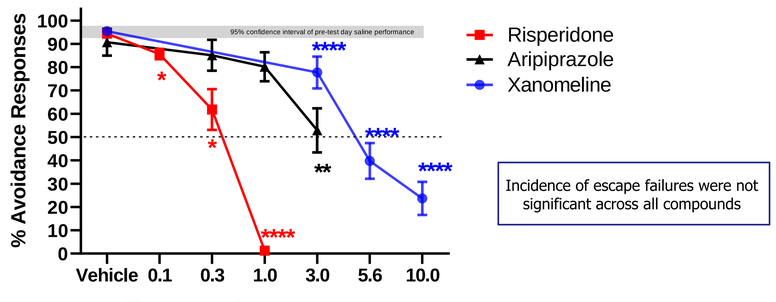

Figure 1: Compiled data over independent studies, with each compound analyzed separately via one-way repeated-measures ANOVA. Xanomeline [F(2.8, 55.4)=30.62, p<0.0001], N = 21; Risperidone [F(1.17, 9.34)=83.96, p<0.0001]. N = 9; Aripiprazole [F(2.16, 21.65)=7.12, p=0.0036], N = 11. Values are mean ± SEM percent avoidance responses on test day. *p<0.05, ** p<0.01, **** p<0.0001 vs. Veh.

Xanomeline's Antipsychotic Effect in CAR is Dependent Upon Central Muscarinic Receptor Activity

Figure 2: A. Xanomeline's (Xan) effect following blockade of central and peripheral muscarinic receptors by scopolamine (SCOP) and by peripheral-only muscarinic receptors by N-methyl-scopolamine (NMS). Scoplamine dose-dependently blocked xanomeline's effects, but NMS had no effect. [F(2.93, 38.15) = 58.09, p<0.0001], N=14. **B.** Scopolamine (0.3 mg/kg) failed to attenuate risperidone's (RIS) antipsychotic-like effect in CAR [F(1.55,23.22)=78.82, p<0.0001], N = 16. No incidence of escape failures were significant across any treatment condition. Values are mean ± SEM. *Post hoc* comparisons ** p<0.01, **** p<0.0001 vs. Veh/Veh; # p<0.05, ### p<0.001, #### p<0.0001 vs. Veh/Xan.

Xanomeline Augments Risperidone and Aripiprazole Avoidance Responses

Figure 3: **A.** Effects of subthreshold doses of risperidone (RIS) and xanomeline (Xan) on avoidance response [F(3.80,60.74) = 56.64, p<0.001], N=17. Data are planned comparisons vs. xanomeline alone (*) and risperidone dose alone (#). **B.** Effects of subthreshold doses of aripiprazole (Arip) and xanomeline on avoidance responding [F(3.32,53.07)=39.54, p<0.0001], N=17. Administration of either risperidone or aripiprazole alone did not significantly differ from vehicle-vehicle control conditions (data not shown). No incidence of escape failures were significant across any treatment condition. *Post hoc* analysis vs. xanomeline alone (*) and vs. comparator dose alone (#). Values are mean± SEM. ** p<0.01, **** p<0.0001; ## p<0.01, ### p<0.001, #### p<0.0001.

Xanomeline Augments Risperidone Locomotor Assay Response Following MK-801 Challenge

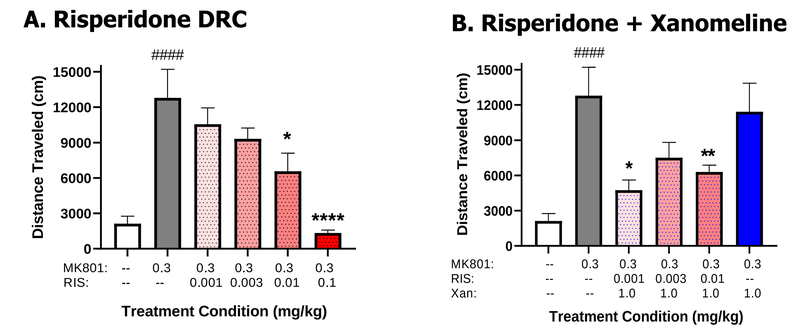

Figure 4: Effects of subthreshold doses of (A) risperidone (RIS) and (B) xanomeline (Xan)± RIS on distance traveled following MK-801 challenge [F(9,90)=7.34, p<0.0001; Sidak post-hoc comparisons], N=10 per condition. Values are mean ± SEM. #### vs. Veh/Veh, p<0.0001; * p<0.05; ** p<0.01; **** p<0.0001 vs. MK801/Veh; DRC=dose response curve.

Pharmacokinetic Interaction Between Risperidone and Xanomeline Does Not Explain Augmented Reduction in MK-801-Induced LMA

Treatment (mg/kg)			Brain (ng/g)		
MK-801	RIS	Xan	MK-801	RIS	Xan
0.3			386.9 ± 20.7		
0.3		1	373.5 ± 12.6		126.0 ± 6.6
0.3	0.001		381.9 ± 21.3	BLD	
0.3	0.001	1	409.3 ± 23.5	BLD	143.4 ± 4.8
0.3	0.003		394.7 ± 19.0	BLD	
0.3	0.003	1	405.7 ± 18.2	BLD	154.6 ± 9.3
0.3	0.01		416.6 ± 20.5	BLD	
0.3	0.01	1	424.1 ± 26.8	BLD	184.9 ± 15.5*
0.3	0.1		512.7 ± 28.7*	22.9 ± 3.0	

Table 1: Brain levels of xanomeline (Xan), risperidone (RIS), and MK-801 collected immediately after the LMA study in Figure 4 (mean ± SEM; N=10 per condition). Comparing 1.0 mg/kg xanomeline alone with xanomeline + risperidone dose groups revealed a significant effect [F(3,36)=6.25, p=0.0016], with 0.1 mg/kg risperidone + xanomeline differing from 1.0 mg/kg xanomeline alone (Dunnett test;*p<0.05). However, the 0.001 mg/kg risperidone + xanomeline group – which exhibited an augmented blockade of activity – was comparable to brain levels of xanomeline alone. MK-801 brain levels in the 0.1 mg/kg risperidone group were elevated compared to MK-801 alone, but MK-801 was unaffected in the risperidone + xanomeline groups. Exposures of risperidone or xanomeline alone groups were not measured. BLD= below level of detection; 0.2 ng/mL for risperidone and MK-801; 0.4 ng/mL for xanomeline. Technical limitations prevented measuring risperidone at the active doses.

Conclusions and Future Directions

- Xanomeline's antipsychotic activity in CAR, an animal model of psychosis, is mediated by central muscarinic receptors.
- Combined low doses of xanomeline + risperidone or aripiprazole significantly augmented CAR and LMA effects over those observed for each agent alone and did not appear to drive motor adverse effects commonly observed with risperidone and aripiprazole.
- Compound exposures were similar in single agent and combination groups, suggesting the LMA treatment effect with xanomeline + risperidone could not be attributable to any change in exposures.
- Additional studies are needed to assess chronic vs. acute dosing of xanomeline augmentation in animal models of psychosis and extend these findings to female animals.
- These data support further preclinical and clinical research evaluating xanomeline's ability to augment the effects of atypical antipsychotics.