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REVIEW

Xanomeline-Trospium and Muscarinic Involvement in Schizophrenia

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Abstract: Schizophrenia is a severe mental illness that has its onset in late adolescence or early adulthood and is associated with significant dysfunction across multiple domains. The pathogenesis of schizophrenia remains unknown, but physiologic understanding of the illness has been driven by the dopamine hypothesis. However, acetylcholine (ACh) clearly plays a role with mixed results regarding effect on psychosis. Selective muscarinic M_1 and M_4 agonists, such as xanomeline, originally developed to aid in cognitive loss with Alzheimer's, showed promise in proof-of-concept study in 20 patients with schizophrenia. Unfortunately, tolerability problems made muscarinic agonists impractical in either condition. However, coadministration of trospium, a lipophobic, non-selective muscarinic antagonist previously used for the treatment of overactive bladder, with xanomeline resulted in a significant reduction of cholinergic adverse effects. A recent randomized, placebo-controlled study of the antipsychotic effects of this combination in 182 patients with acute psychosis revealed improved tolerability with 80% of subjects staying to the end of the 5 weeks study. At the end of the trial, the treatment group saw a −17.4 change in the positive and negative symptom scale (PANSS) score from baseline compared to a −5.9 change in the placebo arm ($P < 0.001$). Furthermore, the negative symptom subscore, was also superior in the active arm ($P < 0.001$). These early studies are exciting because they suggest that the cholinergic system may be recruited to treat a severe and disabling disorder with suboptimal treatment options. Xanomeline-trospium combination is currently in phase III studies.

Keywords: muscarinic agents, M1 agonist, M4 agonist, non-selective anticholinergic

Introduction

Schizophrenia is a severe mental illness that has its onset in late adolescence or early adulthood and is associated with significant dysfunction across multiple domains. It manifests as both positive symptoms – predominantly psychosis, and negative symptoms – predominantly flatness and amotivation; as well as cognitive dysfunction. The pathogenesis of schizophrenia remains unknown, but physiologic understanding of the illness has been driven by the dopamine hypothesis which has evolved over time. The most commonly cited iteration of the hypothesis is prefrontal dopaminergic hypoactivity and limbic system dopaminergic hyperactivity.[1,2] The differential levels of activity have been explained by receptor distribution (D_1 versus D_2 receptors in the frontal and limbic areas),[2,3] and dopaminergic circuits (mesocortical versus mesolimbic pathway integrity).[1] In the latter model, negative symptoms are related to dysfunction of either the frontal cortex or mesocortical dopaminergic pathways. This essentially eliminates frontal cortical function and is the reason patients with schizophrenia are unable to abstract. In this model, psychosis results when a patient with schizophrenia comes across a life situation requiring significant frontal cortical decision-making effort (labeled frontal cortical demand),[4] the signal from the frontal cortex to the midbrain for additional dopamine to solve the problem[5] results in dopamine transport from the midbrain. Frontal lobe or mesocortical dysfunction results in an ongoing need for dopamine, and a continuous signal to increase cortical dopamine, resulting in excessive limbic dopamine and psychosis.[4]

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Alternatively, or additionally, glutamatergic dysfunction has also been reported in schizophrenia. Specifically, there is evidence of reduced glutamate dysfunction and schizophrenia can be worsened by glutamate antagonism.[6]

However, the "dual state theory" has several inconsistencies. For example, according to the theory of salience, D_2 is an autoreceptor which regulates dopaminergic inflow and filters out excess signaling,[7] and in the case of schizophrenia, should be downregulated. However, several positron emission tomography (PET) and single-photon emission computerized tomography (SPECT) studies using radiolabeled L-dopamine demonstrated *elevations* of presynaptic dopaminergic synthesis rate in schizophrenic patients, and revealed an upregulation of D_2 striatal receptors in neuroleptic-free schizophrenics.[8] Several lines of evidence suggest that the cholinergic system may be of particular interest in this condition.

Treatment-Resistant Psychosis

Theoretical mechanisms aside, there is a significant problem with suboptimal response to available treatments for schizophrenia (TRS). Twenty to 50% of patients with schizophrenia do not respond adequately to current antipsychotic medications.[9] As many as 70% of patients with TRS may have dopamine supersensitivity psychosis (previously known as tardive psychosis)[10] However, a meta-analysis of 11,958 patients with first episode psychosis found that one-third of patients responded poorly or relapsed quickly after initial treatment, suggesting that TRS is not always caused by prior to treatment.[11] Clozapine is frequently considered the only evidence-based treatment available for TRS due to its perceived superiority,[12] but it does not maintain that superiority in multiple analyses.[13,14] The current focus to address TRS is to develop medications that work with novel mechanisms such as inhibition of the vesicular monoamine transporter (VMAT2) (Hoare et al, 2023),[15] trace amine receptor (TAAR1) agonists,[16,17] or cholinergic stimulation.

Acetylcholine in Schizophrenia

Acetylcholine (ACh) is an ester of choline and acetic acid that is used as a neurotransmitter in the body and brain. In the periphery, it is the major mediator of the parasympathetic system. In the central nervous system, its main actions are in learning, memory, attention, arousal and motivation.

Attention to ACh in schizophrenia was brought about by understanding some of the neurochemical effects of clozapine. Clozapine attracted attention because it appears to be particularly effective in patients with treatment-resistant schizophrenia[18] but has minimal blockade of the D_2 receptor[19] but is a potent anticholinergic.

Cholinergic afferent neurons and interneurons are present in the basal forebrain and striatum, respectively. Cholinergic receptors mediate many important sensory, motor, and higher functions, such as those affected by schizophrenia, like reward, arousal, attention, and motivation.[20] There are two types of cholinergic receptors, nicotinic and muscarinic receptors. Differing in structure as well as pharmacodynamics, nicotinic receptors possess ligand-gated ion channels, which are immediate in onset and fast in action, whereas muscarinic receptors are G protein-coupled receptors that have multiple components and produce a longer lasting action.[21] Muscarinic receptors, which are more abundant in the CNS when compared to nicotinic receptors, are of five types: M_1, M_2, M_3, M_4, and M_5. All the receptors appear to be region specific in the CNS, with the M_2 and M_4 receptors featuring predominantly on cholinergic interneurons within the neo-striatum (which includes the nucleus accumbens, an integral part of the mesolimbic pathway), while M_1 receptors are located on projection neurons in the neo-striatum and throughout the neo-cortex (which includes the prefrontal cortex).[22,23] It also should be noted that M_5 receptors were the only muscarinic receptors present on dopaminergic neurons in the midbrain. In the context of schizophrenia, muscarinic receptors were found to be decreased in chronically medicated patients with schizophrenia and cholinergic cell bodies reduced in the nucleus basalis of Meynert.[20,24] One neuroimaging study used SPECT with ^{123}I-quinuclidyl benzilate (IQNB), a radio-ligand with high affinity to all five receptor subtypes in patients suffering with schizophrenia for more than 12 years who had stopped antipsychotic and anticholinergic medications for 18 days prior to imaging. Receptor occupancy was reduced in these patients by 20–35% when compared to healthy controls.[20] Regarding specific receptors, radio-ligand binding studies using the procholinergic substance, pirenzepine, revealed that M_1 and M_4 receptors decreased in the frontal cortex in subjects with the disease compared to healthy controls.[25]

Clozapine appeared to improve cognitive function in people with either responsive or treatment-resistant schizophrenia[26] despite strong antagonism at the muscarinic M_1 and M_4 receptors,[27] a process which is known to impair cognition in patients with psychosis.[28] It appears that clozapine metabolites are important in its action.

Despite the fact that metabolites of clozapine are efficiently excreted by the kidney while clozapine itself is reabsorbed, the free (non-protein bound) concentrations of clozapine are only 5.5% of total while the major active metabolite, *N*-desmethylclozapine, is 9.7%.[29] Thus, in 15 patients treated with clozapine monotherapy whose whole plasma levels were 387 and 227 ng/mL, respectively, the free levels were slightly lower for clozapine (21.3 ng/mL) versus *N*-desmethylclozapine (22.0 ng/mL)[29] These numbers suggest a major role for the *N*-desmethylclozapine metabolite.

N-desmethylclozapine has been found to be a partial agonist (with intrinsic activity at 46 ± 9%) at human M_1 receptors.[30] The high level of intrinsic activity and affinity[30] suggest that it may compete sufficiently with clozapine itself to significantly reduce the blockade of the M1 receptor. Both clozapine and *N*-desmethylclozapine have over 500 times the affinity to muscarinic receptors as ACh itself.[31]

Regarding dopaminergic activity, muscarinic receptors may promote or depress dopamine transmission at the striatum. While M_1 stimulates the release of dopamine through dopaminergic afferents, the M_2 and M_4 receptors act as autoreceptors, putting a check on acetylcholine release through the cholinergic interneurons, and subsequent nicotinic acetylcholine receptor-dependent DA release at the striatum.[23] In a study done comparing M_4 receptor knockout (lacking the M_4 receptor) and wildtype mice, knockout animals were shown to have a considerable release of dopamine after amphetamine was administered.[32] This not only suggests that M_4 has a regulatory influence of cholinergic output within the striatum, but also how the cholinergic and dopaminergic systems work in tandem.

History of ACh in Treatment of Schizophrenia

Use of ACh to treat schizophrenia was described predominantly in Europe in the early part of the twentieth century.[33,34] Over the same time period, there was increasing recognition that procholinergic drugs with central nervous system access can cause psychosis.[35–38] The mixed results suggested that some muscarinic stimulation is helpful in schizophrenia, but excessive stimulation may not be desirable. However, the initial successful introduction of the phenothiazines and the introduction of the dopamine hypothesis muted the interest in utilizing the ACh system to treat schizophrenia. Nonetheless, the introduction of cholinesterase inhibitors for the treatment of dementia served as an impetus for studies examining their use in cognitive benefits in schizophrenia. Open-label studies were generally positive, while randomized, placebo-controlled studies with cholinesterase inhibitors generally failed.[38] In a post hoc analysis of some of the data (falsely labelled meta-analysis but which included only 13 [instead of 28] double-blind studies of which four [out of 6] with rivastigmine, six with donepezil [out of 14], and three with galantamine [out of 8]) there was a small effect in improvement of memory and Trail Making Part A.[39]

In addition to the work with cholinesterase inhibitors, there was an effort to develop selective muscarinic receptor agonists to aid with cognition in individuals with Alzheimer's disease. Eli Lilly and Novo Nordisk developed, xanomeline a selective M_1 and M_4 receptor agonist.[40] It was studied for Alzheimer's disease in a multicenter (17 sites; N = 343), double-blind, placebo-controlled study utilizing three doses of 25, 50, and 75 mg daily.[40] The treatment was administered for 6 months followed by a 1-month washout period. Xanomeline demonstrated a significant dose-dependent improvement in cognitive function as assessed by the Alzheimer's Disease Assessment Scale cognitive subscale. Maximal cognitive improvement occurred after 12 weeks of therapy. Additionally, dose-dependent improvements in coexisting psychotic symptoms of delusions, hallucinations, and suspiciousness were also observed.[40] Unfortunately, poor tolerability, particularly with gastrointestinal symptoms, precluded its introduction as a clinical treatment.[40,41]

At the same time, ongoing research into the role of the muscarinic system in schizophrenia utilizing other techniques (such as animal models)[42] and agents (such as the M_4 receptor agonist LY2033298)[43] continued to suggest an important role in modulation of the disease. Ultimately, a small proof of concept study done in 2008 showed a clear beneficial effect on positive symptoms.[44]

In this small pilot study, 20 patients with a diagnosis of schizophrenia or schizoaffective disorder, with a total positive and negative syndrome scale (PANSS) score of >60 and at least a 4 on one positive symptom item or a 3 on two positive items were

given placebo treatment for 7 days, after which they were randomized to xanomeline 25mg TID or placebo. Subjects were then titrated up to 75 mg TID according to tolerability. Patients were observed for 4 weeks. Total PANSS score was statistically significant across groups ($P = 0.039$) although the positive and negative symptoms scores separately did not separate ($P = 0.082$ and $P = 0.083$, respectively). Separation was observed in the brief psychiatric rating scale (BPRS) score by week 1 of treatment and was sustained. There was no difference in the CGI score ($P = 0.94$). Cognitive battery scores showed an improvement between groups across all categories, reaching clinical significance ($P < 0.05$) in Digit span memory scale, story recall memory scale, list learning total verbal learning scale, and delayed memory visual learning scale.[44] This study suggested an improvement in the schizophrenia symptoms, as well as in cognitive abilities, consistent with the previous Alzheimer's dementia study[40] but given the small size of the study, and lack of adjustments for multiplicity in the cognition, the value of the findings was limited. Regrettably, as with its use in Alzheimer's, the high incidence of gastrointestinal distress, including nausea vomiting, and indigestion across all doses, likely due to the peripheral muscarinic activity of xanomeline, ultimately resulted in discontinuing clinical development.[45]

Xanomeline-Trospium

As previously noted, xanomeline is a muscarinic cholinergic agonist that has no dopaminergic effects.[46] It has a high affinity to the M_1 and M_4 muscarinic receptors (K_i in the low teen range nM[46] compared to M_2, M_3, and M_5 receptors (K_i in the 30s or higher nM[47]) but still stimulates them at higher doses.[48] Xanomeline also exhibits an appreciable affinity to $5HT_1$ and $5HT_2$ receptors ($K_i > 120$ nM).[46] Despite not directly affecting dopamine receptors, M_1 and M_4 receptors, unlike the other muscarinic receptors, are primarily located in cortical and limbic regions of the brain that are commonly associated with a dopaminergic action involved in cognitive and affective functions.[49] Additionally, it seemed effective, just poorly tolerated.

Recently, a new co-formulation of xanomeline and trospium has been introduced as a clinical strategy to access clinical benefits with a tolerable level of adverse consequences.[50,51] Karuna Therapeutics, a small pharmaceutical company established in Boston, Massachusetts, in 2009, added trospium chloride, a non-selective muscarinic antagonist that has been previously used for the treatment of overactive bladder for years,[52] to xanomeline. Trospium has a highly polarized tertiary amine structure that prevents it from entering into the central nervous system.[53] Coadministration of trospium and xanomeline is believed to block the unwanted peripheral cholinergic side effects of xanomeline. Indeed, the combination appears to be associated with a 50% reduction of cholinergic side effects in healthy volunteers.[54,55]

This was examined in a recently published 5-week multisite (12 sites), phase II trial; 182 patients with a diagnosis of schizophrenia confirmed with a Mini-International Neuropsychiatric Interview, and with a PANSS score exceeding 80 at baseline were randomized to receive either the combination medication (xanomeline-trospium) twice daily or placebo.[50] Dosing schedule was flexible, starting with 50mg/20mg combination (xanomeline/trospium) increased to a maximum of 125/30 and returning to 100/20 if higher doses were not tolerated. Most of the patients, 91% (72/90), in the active medication group reached the highest dose of the drug. Over 80% of the patients completed the study and the rates were not different (72/90 of active medication and 73/92 in the placebo group). At the end of the trial, the treatment group saw a −17.4 change in the PANSS score from baseline compared to a −5.9 change in the placebo arm ($P < 0.001$).[50] Secondary outcomes of PANSS positive symptom subscore, CGI-S scale, and negative symptom subscore, were also superior in the active arm ($P < 0.001$). Discontinuation rates due to adverse effects (AEs) were comparable in both groups (20% versus 21% in treatment and placebo groups, respectively). AE reports were also similar with AEs being reported in 54% vs 43% in treatment vs placebo groups ($P = 0.14$, $z = 1.5$). Most commonly reported AEs included constipation (17%), nausea (17%), with dry mouth, vomiting and dyspepsia being reported in 9% of the patients.[50] These findings suggest the promising efficacy of the medication combination, and the comparable discontinuation rates suggest tolerability of the cholinergic and anticholinergic side effects. Some of the limitations with the study include the short duration and the inpatient setting of the study.

Phase III studies have been completed but have not been published. One phase III study has been presented in poster form.[56] The study recruited 252 hospitalized patients with schizophrenia in 22 sites across the USA. The average age for

those randomized to xanomeline-trospium was 45.6 +/- SD 10.4 (75.4% male) compared to 46.2 +/- sD 10.8 (75.5% male) of those randomized to placebo. Basline PANSS scores were similar at 98.3 +/- SD 8.9 and 97.9 +/- 9.7, respectively. Patients on xanomeline-troospium were statistically improved by week 2 ($P < 0.05$), and had a greater than 20 point reduction in total PANSS by study end at week 5 ($P < 0.0001$). Response rates, defined as a greater that 30% improvement, occurred in 35.7% of xanomeline-trospium-treated patients versus 17.9% of placebo-treated patients by week 3 ($P = 0.0027$) and 54.8% verus 28.3% ($P < 0.0001$) by week 5. Improvement in the negative symptom subscale was small (−4.2 points versus −2 points) but still statistically superior ($P = 0.002$) in the xanomeline-trospium group. There were 2 (1.6%) severe adverse events in each group. Any adverse events were more common in the active medication group (75.4% vs. 58.4), but few of those led to early study termination (7.1% vs. 5.6%). Simpson-Angus Scale Parkinsonian measures, Barnes Akathisia Scale, and prolactin measures were not different in the two groups. An additional phase III study was also completed with similar data and the results are available from Karuna Therapeutics.

Conclusion

Schizophrenia is a severe psychotic disorder that is associated with poor outcome and a high rate or treatment resistance and partial response. Furthermore, currently available treatments are associated with significant adverse consequences. The dopamine hypothesis has dominated the thinking about pathophysiology and treatment development for nearly half a century. While a potential role of cholinergic agents is not a product of new insight into disease process, clever merging of available agents may lead to new approaches to treatment. Specifically, combining an M1 and M4 agonist (xanomeline) with a peripheral nonselective anticholinergic (trospium) to reduce peripheral cholinergic AEs has produced a drug combination that appears to reduce antipsychotic symptoms, improve cognitive deficits, and is adequately tolerated. The xanomeline-trospium amalgam is currently in phase III study, and may eventually become a clinical tool. Proper utilization will require the development of predictive biomarkers that inform which approach might be preferred in a specific patient (dopamine blockade or muscarinic stimulation). Furthermore, there will be a need for head-to-head studies of different agents early in the course of illness, and augmentation studies that may inform the use of multiple mechanisms later in the illness or in treatment-resistant patients.

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