Official title of study: Nicotinic Cholinergic Modulation as a Novel Treatment Strategy for Aggression Associated With Autism

NCT number: 02552147

Document date: 8/29/2017

RESEARCH PLAN

1. **Statement of Purpose:**

The scientific aims of this pilot study are to determine the feasibility, tolerability, and efficacy of transdermal nicotine to treat irritability and aggression comorbid with Autism Spectrum Disorder in adults without intellectual disability.

2. **Background:**

Autism spectrum disorder (ASD) is a heterogeneous disorder characterized by deficits in communication, social interactions, and behavior, presenting in the early developmental period that causes significant impairment in functioning[1]. The constellation of aggression and related behaviors, which include irritability, agitation, and aggression, are highly comorbid with ASD, with a large recent study reporting over two-thirds of children with ASD have demonstrated aggressive behaviors toward caregivers[2]. Aggression in ASD, both toward others or as self-injurious behavior, significantly increases the risk for acute hospitalization or residential placement[3], increases parent and/or caregiver stress[4], and places the patient at risk for abuse[5].

Current treatment options for aggression in ASD include behavioral approaches and pharmacotherapy[6-8]. Atypical antipsychotics, especially risperidone and aripiprazole, have emerged as the accepted first-line treatment of aggression in ASD and pervasive developmental disorders (PDD), and are complimented by other antipsychotics and mood stabilizers[7, 9]. Risperidone demonstrated a large effect on aggression in children with ASD [10]. Despite this, treatment-resistant cases are common, with one large recent study of individuals of all ages with ASD finding almost 40% were resistant to pharmacotherapy over a 3-5 year period[11]. Complicating this problem are significant side effects of these medications, particularly metabolic derangements, which appear to be even more significant in an ASD population[12]. For instance, children prescribed risperidone gained on average 5.6 kg (16.7%) over 6 months[10, 13], increasing the risk for obesity and diabetes. *The reality of aggression and its consequent harms weighed against partially effective treatments with significant side effects is one faced every day for patients with ASD, their parents and caregivers, as well as healthcare providers.*

None of the pharmacotherapies used for aggression in ASD are specific for treating aggression in ASD, and only a limited understanding exists regarding the neurobiological mechanisms driving aggression and irritability in ASD. The study of aggression in rodent models has benefited substantially from novel technologies such as rodent fMRI[14] and optogenetic techniques[15, 16], yet further knowledge of neurobiological mechanisms of aggression in general, as well as in ASD specifically, is critically needed. We rationalized that a logical foundation on which to design novel treatments for aggression in ASD is to identify systems important for modulating aggressive behavior that are also consistently misregulated in ASD cohorts. Modulation of nicotinic cholinergic signaling through nAChRs represents a novel strategy for influencing aggression in ASD. Multiple lines of evidence have identified abnormal cholinergic signaling and changes in nAChRs in patients with ASD[17]. Neuropathological studies from adults with ASD demonstrate significant misregulation

of β2-containing and α7 nAChRs in diverse brain regions, including the cortex[18], cerebellum[19, 20], and thalamus[21], while studies of infantile autism identified abnormalities in basal forebrain cholinergic neuron development[22]. Genetic and epigenetic studies have also demonstrated that changes in nAChR subunits might contribute to ASD in humans, with the preponderance of evidence pinpointing changes in *CHRNA7*, the gene coding for the α7 subunit[23-25]. The 15q13.3 microdeletion syndrome results from a copy number variation encompassing roughly 6 genes, one of which is *CHRNA7*, and affected subjects can present with ASD, aggression, epilepsy, and schizophrenia[26, 27], which is replicated by a 15q13.3 mouse model[28]. Deletion of *CHRNA7* itself is likely responsible for at least some of the phenotypic findings of 15q13.3 microdeletion syndrome[29], directly linking abnormal α7 expression and signaling with ASD and aggression. Commonly used mouse models of ASD and related neurodevelopmental disorders, including the BTBR mouse[30] and the *Mecp2*-null mouse[31] demonstrate reduced prefrontal cortical acetylcholine levels and altered nAChR currents, respectively. The BTBR mouse also shows increased impulsivity[30], which could predispose to aggression in ASD. Taken together, human neuropathological studies, genetic studies, and animal models implicate alterations of nAChRs and cholinergic signaling in ASD throughout the lifespan.

Pharmacological strategies designed to normalize or augment the cholinergic misregulation in ASD might be beneficial for treating its symptoms. Donepezil, an acetylcholinesterase inhibitor, improves cognitive rigidity and social deficits in the BTBR mouse model of ASD[30, 32] and has demonstrated encouraging, though as yet inconclusive, results for certain core symptoms in humans with ASD[33-35]. Despite these promising results, research on the use of cholinergic agents in ASD will have greater impact if experiments focus on specific ASD-related behaviors that are most likely to be improved by cholinergic modulation. Substantial evidence demonstrates that nAChR agonists, especially nicotine, reduce aggression in animal models with heightened aggression, including cats, rats, and mice[36-41], thereby acting as a serenic agent[42]. The pharmacological or neural circuit mechanisms underlying such serenic effects are unknown. **Given the cholinergic changes in ASD and the ability of nAChR agonists to act as serenic agents, we hypothesize that nicotinic pharmacotherapies will be specifically therapeutic for aggression in ASD**. Other disorders with cholinergic dysfunction and persistent aggression have been successfully treated with transdermal nicotine, including dementia[43, 44] and schizophrenia[45]. Nicotinic AChR agonist therapy has not been systematically described to treat ASD, however use of varenicline, an α4β2 partial agonist with activity at α7, was reported in a 9-year old boy with ASD, with good tolerability and efficacy for social, communication, and repetitive behavior measures[46].

3. **Research Plan:**

The study will take the form of a small, randomized-controlled trial with a cross-over design. The basic study design is as follows:

1. **Participants:** Participants will be recruited through the Yale Child Study Center (YCSC) Autism Program, local support and advocacy groups for young adults

with Autism Spectrum Disorder (ASD), local therapeutic schools, and local mental health providers who work with individuals with ASD. We will invite these entities to inform their patients/students about our study, and invite them or their parents (if legal guardians) to contact the PI (Alan Lewis) if they are interested in learning more about the study, and potentially participating, or having their adult children participate. Pamphlet advertisements with contact information will be placed at the meeting sites of these groups, with permission. Clinicians or group leaders will also offer the participants the option of submitting their telephone numbers in order for us to contact them directly.

2. **Consent:** After telephonic contact is made, participants or parents who express interest in participation will be invited for an initial screening visit. At this point it will be determined whether the participants are able to give informed consent in accordance with the inclusion criteria of the study. Once this is established, informed written consent will be obtained. Participants will be invited to attend the screening visit with a primary caregiver. If participants are unable to provide consent, a parent, if still their legal guardian, will be allowed to provide informed consent on their behalf. In the case where the participant is unable to provide informed consent and it is provided by the participant's parent, assent will be obtained from the participant and the participant will still sign the consent form to confirm willingness to participate. Once participants are enrolled, this contact will be considered Visit 1, and relevant behavioral baseline data will be obtained, as discussed under study design.

3. **Study design:** This is a double blind study such that subjects and all investigators will be unaware of treatments during the data collection phase of the study. It will be placebo controlled using identical appearing transdermal patches that either contain or do not contain nicotine. After informed consent is obtained, participants will be scheduled for a total of three visits with a member of the study team at the YCSC or at Services for the Underserved (SUS), Bronx, NY, as noted above. The purpose of conducting the study at SUS is to enable a subject who would otherwise meet criteria for the study but clinical considerations make the logistics of transportation to YCSC unacceptably challenging.

    a. **Visit 1**: As described above, informed consent will be obtained from participants or their legal guardian parent on meeting inclusion criteria and without exclusion criteria. Assent will be obtained from the participant in cases where informed consent is provided by a parent. Baseline behavioral rating scales will be performed. Specifically, we will obtain the Aberrant Behavior Checklist (ABC), State/Trait Anxiety Inventory (STAI), and Social Responsiveness Scale-Adults (SRS-A). These will be obtained from patients and primary caregivers as appropriate. Vital signs will be obtained. All participants will be given three weeks of identical appearing transdermal patches. Participants are randomized into two groups, one of which receives nicotine patches first and the other receives placebo patches first. Randomization will be performed by block randomization

using blocks of 4 subjects, and allocation concealment performed via the use of sequentially numbered, sealed opaque envelopes, on which the participant's identifying information is written prior to opening the envelope. In collaboration with the Investigational drug service at YNHH, we will link the allocation to a set of patches that are numbered in a simple fashion to help participants use the correct patch on the correct day. The first group will receive 7 days of nicotine patches, dose = 7 mg patches followed by 14 days of identically appearing placebo patches. The second group will receive 14 days of placebo patches, followed by 7 days of 7 mg nicotine patches. This design allows each participant to serve as his/her own control, with a seven-day washout period, which will eliminate carryover effects for those subjects receiving nicotine during the first 7 days of the study. Subjects will be asked to place the nicotine patch on their skin in the morning and remove the patch prior to sleep at night prior to sleep. This counter-balanced design is aimed to control for expectation bias and maximize validity. *Duration of visit: 90 minutes.*

b. **Visit 2**: Participants will return for a subsequent visit at day 8, at which point the above rating scale data will again be collected to measure both primary and secondary outcomes. Tolerability will be assessed as determined by vital signs, open-ended inquiry, and screening for common adverse effects of transdermal nicotine as observed in previous studies with non-smoking subjects. Open-ended inquiry for tolerability will be performed by asking subjects "What types of side effects or discomfort did you experience while wearing the patch during the preceding week?" and subject responses will be recorded by detailed notes. This approach may allow the investigators to gain a better understanding of the experience of the patch in individuals with ASD who might experience side effects differently than neurotypical individuals. Brief qualitative reports of the patient's subjective experience will be recorded by asking "In what ways did you find the patch helpful or harmful during the past week, if at all?". Detailed notes of responses will be written. *Duration of visit: 60 minutes*

c. **Visit 3**: Participants will return for a third and final visit at day 22, at which point data will again be collected to measure primary and secondary outcomes, tolerability, and subjective experience (as detailed in Visit 2, above). *Duration of visit: 60 minutes*

4. **Data analysis:** Study results will be tabulated and subjected to statistical analysis to determine the presence of significant differences in primary and secondary outcomes between control and intervention. Statistical methods are outlined under 'statistical considerations' and performed by a trained statistician the YCCI and Yale Center for Analytical Sciences. Data regarding tolerability will be compared between control and interventions arms.

5. **Deviations from standard of care**: Current pharmacological standard of care for treatment of agitation and irritability in the population from which we are recruiting include mood stabilizers and antipsychotics[7, 9]. Patients currently taking these agents will not be required to discontinue this treatment, and the intervention is not expected to interfere with this treatment. Therefore, during the placebo period patients will not experience treatment below the standard of care, and during the active period they will continue to receive the standard of care, plus the active agent with its potential positive and negative effects.

4. **Subject Population:** Provide a detailed description of the types of human subjects who will be recruited into this study.

   Our study population will consist of adult subjects with ASD who have primary caregivers. Specifically, our population is individuals over the age of 18 participating, or with parents participating in local support and advocacy groups for ASD. Participants in this study will have prior, confirmed diagnoses of ASD in order to be considered for inclusion. In this pilot study we will include both higher functioning individuals with ASD (who will provide their own consent), as well as less cognitively able individuals (who will have consent provided by a guardian parent). All participants will be living with or closely engaged with a primary caregiver, and are expected to have at least moderate impairment related to social and communication deficits. Please see the study inclusion and exclusion criteria for a quantitative description of the study subjects.

5. **Inclusion/Exclusion Criteria:** What are the criteria used to determine subject inclusion or exclusion?

Inclusion criteria:

- Age: 18-60
- Gender: All
- Language: Communicative in English
- Participants with a prior diagnosis of DSM-5 ASD at some point in their lifetime OR a DSM-4 diagnosis of Autism OR a DSM-4 diagnosis of Asperger's syndrome OR a DSM-4 diagnosis of Pervasive Developmental Disorder Not Otherwise Specified.
- Symptoms of irritability, agitation or aggression as reported by parent and/or participant
- Aberrant behavior checklist – Irritability Subscale (ABC-I) score of 16 or higher
- No changes in psychotropic medications within the past 14 days.
- Either lives with a primary caregiver or closely engaged with a primary caregiver who interacts with the patient daily
- BMI $\geq$ 17.5 and $\leq$ 45

Exclusion criteria:

- Age < 18 or > 60

- BMI < 17.5 or > 45
- Currently using tobacco or any nicotine products (transdermal, gum, e-cigarettes)
- Changes in psychotropic medication management within the past 14 days
- Previous allergy to transdermal patches
- Patients with heart rate > 100 or < 50 or known history of cardiac rhythm abnormalities
- Systolic blood pressure > 150 or < 95; diastolic blood pressure > 90 or < 50
- No symptoms of irritability, agitation, or aggression as reported by parent and/or participant
- ABC-I score of less than 16
- No primary caregiver, or primary caregiver unable to assist with rating scales

6. How will **eligibility** be determined, and by whom?

Eligibility will be determined by the investigators. Once potential participants or a parent makes contact telephonically, we will explain briefly some information about the study, and offer them the opportunity to present for a screening visit. We will explain that a diagnosis of ASD is required for participation in the study, and encourage participants or their parent to bring information about their prior diagnosis to the screening visit, but will not request any PHI over the phone. At the screening visit, we will again explain the study procedures, and determine whether participants meet inclusion and exclusion criteria. In order to confirm diagnosis, we will require potential participants to provide evidence of a prior diagnosis done by a psychiatrist or clinical psychologist.

7. **Risks:** Describe the reasonably foreseeable risks, including risks to subject privacy, discomforts, or inconveniences associated with subjects participating in the research.

The study has a number of risks:

- **Medication related:** Side effects of transdermal nicotine exposure have been well studied, and participants may experience nausea, insomnia, headache, skin irritation, palpitations, and dizziness. These side effects were found to be dose related in healthy, non-smoking subjects, with 15 mg nicotine patch significantly less likely to cause side effects than higher dose such as 30 mg[47]. Specifically, in this study 8 non-smoking patients were given a 15 mg nicotine patch. Five of 8 patients experienced mild nausea and lightheadedness after one hour, while 3 of 8 did not have any side effects. Seven of 8 patients continued with the study. Studies of chronic transdermal nicotine in non-smoking populations are very encouraging regarding tolerability. In a large study of transdermal nicotine for treating mild cognitive impairment in older adults, tolerability was excellent in the over 30 subjects randomized to nicotine treatment [48]. It is not known whether subjects with ASD will be more or less susceptible to the common side effects of transdermal nicotine, however, transdermal nicotine use has been reported in case series or trials of patients with advanced dementia and age, a population that is likely to be increasingly sensitive to nicotine than younger, healthier subjects[43, 44, 48-50].

The question of development of physiological dependence or even nicotine use disorder secondary to patch exposure in non-smoking participants is an important one. We can base the likelihood on previous studies in non-smoking participants that were conducted for significantly longer periods of time than in the proposed study. Nicotine patch has been used for 4-weeks in patients with mild-moderate Alzheimer's Disease for 16 hours per day, initially using 5 mg patches for 7 days, 10 mg patches for 14 days, and finally 5 mg patches for 7 days [49]. In general, patches were well tolerated, with only one of 8 subjects discontinuing. There were no observed nicotine withdrawal symptoms in these participants. Sleep interruption was not problematic as the patches were removed at bedtime. An almost identical study was performed with 15 subjects with age-associated memory impairment using the same nicotine patch dose[50]. Only one of 15 subjects withdrew from this study due to nicotine-related side effects, in this case, nausea. In this study, the most common side effects were local skin irritation, mild nausea and abdominal discomfort, and lightheadedness. Two subjects reported palpitations, but there was no change in body weight, blood pressure, or heart rate in subjects receiving nicotine compared to receiving placebo. No subjects appeared physiologically or psychologically dependent on the nicotine patches. In a 6-month trial of transdermal nicotine or placebo to treat mild cognitive impairment, no subjects (0 of 34 subjects) developed withdrawal or continued to use nicotine products following the study[48]. Data from studies of smokers, who might be predisposed to difficulty discontinuing nicotine-containing products, demonstrated that difficulty with discontinuation of transdermal nicotine after use for smoking cessation was rare (2%), with the percentage of smokers continuing to use nicotine replacement products proportional to the rate of nicotine delivery[51]. Taken together, these previous studies are reassuring regarding the safety of nicotine products from an addiction potential standpoint in non-smoking subjects. Whether addiction potential differs in ASD populations remains to be seen, but epidemiological data demonstrating reduced smoking in ASD subjects argues that this group will not be predisposed to development of nicotine use disorder[52].

- **Inconvenience:** Participants with significant symptoms of irritability may be negatively affected by not being able to make other medication changes for the duration of the study unless they withdraw from the study. In this instance, subjects will be withdrawn from the study and instructed to seek further treatment from their medical providers.

8. **Minimizing Risks:**

- **Dependance:** Please see above (#8) for a detailed discussion regarding the risk of development of dependence. To further reduce the risk in our study we will administer the active agent for no longer than one week, which is substantially shorter than the trials cited above that demonstrated no risk of dependence with transdermal nicotine. We chose transdermal delivery of nicotine because its slow rate of delivery significantly reduces the risk of dependence development[51]. Finally, our protocol involves removal of nicotine patch prior to sleep, which significantly reduces the total exposure to nicotine within the study.

- **Discomfort:** The potential for discomfort will be clearly explained to subjects and where appropriate and permissible, their caregivers. In order to minimize the risk of discomfort, participants will be provided a low dose of 7 mg patches to be used for 16 hours a day while the subjects are awake. Similar to a previous study[53], study subjects who experience unpleasant side effects above and beyond those on which subjects were counseled during the first visit will be asked to contact study staff and report their symptoms. Subjects experiencing expected, non-dangerous side effects such as moderate nausea will be instructed to remove the patch for that day and resume using the patch the next morning. Individuals experiencing side effects considered medically concerning, including severe headache, chest pain, or severe palpitations, will be instructed to discontinue patch use altogether and will be withdrawn from the study. Placebo patches will be handled in exactly the same manner, with the same ability to remove as described above. These data will be important for not only understanding tolerability of nicotine in subjects with ASD, but also tolerability of skin patches in populations with ASD, who are known to have altered somatic sensation.

  It should be noted that nicotine patches have been used successfully in populations with cardiovascular disease [54]. Although subjects with known cardiovascular disease, including hypertension, history of myocardial infarction, heart failure, or arrhythmia will be excluded, these data are reassuring given the possibility of enrolling a subject with a previously undiagnosed cardiovascular condition.

- **Inconvenience:** In order to be included in our study, participants should not be undergoing active changes in their psychotropic medications, reducing the likelihood that this may become a problem in the course of the study. We have limited the number of visits to three, which we believe to be the minimum number of visits yielding scientifically useful data.

9. **Statistical Considerations:** Describe the statistical analyses that support the study design.

a) We plan to enroll approximately 16 subjects in this *pilot* study.

b) The major hypothesis of the study is that transdermal nicotine will *reduce* symptoms of aggression as quantified by the Aberrant Behavior Checklist (ABC) – irritability subscale. For comparison, risperidone in a large clinical trial (n=63) had a large effect size of d>1.0 as determined by an ABC-irritability subscale reduction from a mean baseline score of 26.6 to a score of 9.5 after 8 weeks of medication exposure[10]. To determine power estimates for our study, we used 26.6 as our mean estimate for the placebo group. Based on a literature review, we conservatively estimated a standard deviation of 10. We then calculated power for a range of effect sizes, estimating a correlation of 0.70 for repeated measures. Alpha was set at 0.05.The power estimates are displayed in Table 1.

Table 1: Power estimates

Placebo Mean (SD)	Treatment Mean (SD)	Effect size (d)	Power
26.6(10)	22 (10)	0.59 (small-medium)	0.57
26.6(10)	20 (10)	0.85 (medium)	0.86
26.6(10)	15 (10)	1.27 (large)	0.99

These figures demonstrate our study is highly powered to detect medium and large-sized differences between placebo and treatment groups.

c) For the major hypothesis we will collect ABC rating scale data as well as at both baseline as well as at two other timepoints: after 7 days of transdermal nicotine treatment as well as after 7 days of placebo patch. Repeated measures analysis of variance (ANOVA) will be used with *treatment* (nicotine or placebo) as within-subjects factor and *order of treatment* (nicotine-placebo-placebo or placebo-placebo-nicotine) as a between-subjects factor. We will first test models with between and within subjects factors only. We will then test the interaction between *treatment* and *order*, in order to test whether treatment effects varied by administration order. McNemar's test or similar will be used for categorical variables such as whether or not a common, pre-defined side effect is reported. We will collect and report subjective experiences from patients and their caregivers but these will not be subjected to statistical analysis.

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