Chapter 2

CHOLINERGIC THERAPY FOR AUTISTIC SPECTRUM DISORDERS: REVIEW AND CASE REPORT

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ABSTRACT

The causes of autism are heterogeneous and still largely unknown. Currently available treatments especially for the behavioural problems frequently reported in children and adults with autistic spectrum disorder (ASD) are largely symptomatic. The cholinergic abnormalities are rather consistently reported in various molecular pathological studies, both in children and adults with ASD, and they may underlie the numerous cognitive and behavioural changes seen in ASD, e.g. cognitive changes, memory problems, attentional dysfunction etc. This raises the prospect of the use of cholinesterase inhibitors and other cholinomimetics (chemicals that can act by either directly stimulating the nicotinic or muscarinic receptors, or promote acetylcholine release) in ASD for treatment of both cognitive and behavioural changes, as well as activities of daily living and improving the overall global functioning in these subjects, similar to the effect these treatments have in various neurodegenerative and neurodevelopmental conditions (e.g. dementia and schizophrenia). We provide an overview of the current use of cholinesterase inhibitors (donepezil, galantamine and rivastigmine) and cholinomimetics (e.g. nicotine) in the treatment of some of the cognitive and behavioral symptoms in ASD. We also discuss the potential use of cholinomimetics in these subjects and review the experience of Mr. A, a now twenty-eight year-old non-smoking male with a history of severe behavioral dysfunction who has been wearing a nicotine patch since July, 2005.

Keywords. ASD, Autism, cholinergic receptors, cholinergic therapy, nicotine.

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ABBREVIATIONS

ASD, autism spectrum disorders, ATEC, Autism Treatment Evaluation Checklist, CDCP, Center for Disease Control and Prevention, ChAT, Choline acetyltransferase, ChEI, acetylcholinesterase inhibitor, DS, Downs syndrome, HA, hyperammonemia, mAChR, muscarinic acetylcholine receptor, nAChR, nicotinic acetylcholine receptor, PET, Positron emission tomography, SJS, Stevens Johnson Syndrome, SPECT, Single-photon emission computed tomographs, SSRI, Selective serotonin reuptake inhibitor, VPA, valproic acid.

INTRODUCTION

Autism represents a clinically heterogeneous group of disorders, commonly referred to as “autism spectrum disorders” (ASD). The characteristic clinical phenotype of ASD is associated with developmental delays in multiple areas of cognitive and behavioural functioning, including abnormalities in social interactions, disturbed sensorimotor and perceptual performance, impairment in attentional processes and motivation, aberrant communication skills, and restricted and repetitive stereotyped behaviours. Autism incidence rates are currently predicted as 1:150 births (CDCP, 2007), with a 3-fold higher prevalence in males.

The extent of the characteristic ASD clinical symptoms varies significantly, from profoundly mentally retarded to high-functioning Asperger’s syndrome individuals. The diversity of the variety and severity of symptoms associated with ASD contributes to difficulties in studying this disorder. This is even more pronounced for the profoundly mentally retarded individuals who do not develop spoken language. In addition to the abundance of characteristic behavioural and cognitive changes, a variety of additional medical and psychiatric comorbidities are frequently reported in autism. These include movement abnormalities, obsessive compulsive disorder, epilepsy, Tourette syndrome, attention deficit hyperactivity disorder (Gillberg and Billstedt, 2000, reviewed in Mukaetova-Ladinska et al, 2011)

Since the causes for autism still remain largely unknown, various forms of treatments have been used over the years to lessen the deficit of autistic individuals, increase their quality of life and functional independence, as well as decrease the family and carers’ distress. These treatments fall largely in two categories:

1. psychosocial/educational interventions and
2. medical management

Although psychosocial interventions seem to be the preferable management option (especially for young children with autism), their clinical effectiveness has not been largely supported in systematic reviews (Seida et al, 2009, Kasari and Lawton, 2010). In contrast, the educational interventions appear to have a better response, especially in relation to enhancing global functioning in children with autism (reviewed in Swiezy et al, 2008, Kanne et al, 2008). However, the comprehensive treatment of children and adults with ASD should consist of the combination of educational, psychosocial and pharmacological treatments and interventions (Tuchman et al, 2010), since the treatment needs to address also the underlying
comorbidities, including their impact on the already existing behavioural and cognitive changes associated with ASD.

At present, the pharmacological treatments for ASD are largely symptomatic, and individually tailored according to subjects’ symptoms and needs. The most frequently prescribed medical treatments are those that help alleviate the most prominent and dramatic behavioural and neurological symptoms, and include, neuroleptic medication, anti-epileptic medication (frequently also used as a mood stabiliser), antidepressants, benzodiazepines etc. (reviewed in Leskovec et al, 2008, Mukaetova-Ladinska et al, 2011) However, the findings from a limited number of neuropathological (Perry et al, 2001, Nakamura et al, 2010, Azmitia et al, 2011), genetic (Guathakurta et al, 2008, Cubells et al, 2011, Kistner-Griffin et al, 2011) studies, as well as those conducted in blood and blood components (Janusonis et al, 2005, Hranilović et al, 2009, Kazek et al, 2010) and intestine (Kazek et al, 2010) in ASD subjects implement rather consistently the serotonergic (Guathakurta et al, 2008, Nakamura et al, 2010, Azmitia et al, 2011, Kistner-Griffin et al, 2011) and cholinergic (Perry et al, 2001, Martin-Ruiz et al, 2004, Ray et al, 2005, Cubells et al, 2011) neurotransmitter systems, and facilitate the use of more biologically oriented therapies to regulate both behavioural and cognitive symptoms in these subjects. Thus, the use of SSRIs (Selective Serotonin Re-uptake Inhibitors) appears to have some benefits to regulate obsessions, aggression and anxiety in adult ASD subjects (Williams et al, 2010), although conclusions about their usefulness in children with ASD cannot be made at this stage in the light of the non-conclusive findings from various studies (West et al, 2009, Williams et al, 2010).

The latest developments in the aetiology of ASD, especially those targeting the genetic causes of autism, may also result in novel development of pharmaco-genetically guided treatments for ASD. Interestingly the most recent work in this field has concentrated on the cholinergic neurotransmitter system (Cubells et al, 2011). Thus, this study explored the use of nicotinic cholinergic receptor (nAchRs) allosteric modulator and acetylcholinesterase inhibitor (ChEI) in the treatment of aggressive behaviour in an adult with ASD with a genetic deficit of α7-nAchRs-mediated neurotransmission arising from haploinsufficiency of the structural gene CHRNA7 due to the deletion (Cubells et al, 2011). This work further strengthens the role of acetylcholine receptors as pharmacological targets for ASD.

In the current review, we address the role and changes of the cholinergic neurotransmitter and associated receptors in generating cognitive and behavioural symptoms, characteristic for the ASD clinical phenotype and, in particular, the use of cholinesterase inhibitors and cholinomimetics in the treatment of cognitive and behavioural symptoms in ASD.

**CHOLINERGIC SYSTEM: ROLE IN BRAIN DEVELOPMENT AND ASD**

Acetylcholine is one of the major neurotransmitter systems and influences attention, executive function, learning and short-term memory, mood (such as depression and anxiety), reward, sensory processing, and sleep-waking cycle through modulatory influence on cortical neurons (reviewed in Pepeu and Giovannini, 2004, Miwa et al, 2011). It is released from growing axons and participates in regulating growth, differentiation, and plasticity of the developing central nervous system (Lauder and Schambra, 1999) and modulates neurite outgrowth in developing neurons (Tata et al, 2003). Acetylcholine also reduces the strength of
excitatory (glutamatergic) synapses, a finding that has implication for potential treatment of ASD. Thus, an increase in excitatory activity could be caused by genetic defects in the glutamate signalling pathway. However, the association between polymorphisms in the glutamate 6 receptor gene (chromosomal location 6q21) and autism has been somewhat non conclusive (Jamain et al, 2002, Shuang et al, 2004, Dutta et al, 2007), and further work is needed to elucidate the relationship between the cholinergic and glutamatergic neurotransmitter systems in ASD.

**Brain Cholinergic Receptors**

The cholinergic receptors in the brain consist of 2 classes: muscarinic and nicotinic, each including a range of subtypes.

**Muscarinic acetylcholine receptors:** The muscarinic acetylcholine receptors (mAChR) are implicated in learning and memory. They mediate most of the action of acetylcholine in the CNS and peripheral nervous system, as well as in the end organs of the parasym pathetic nerves, e.g. cardiac and smooth muscles, secretory glands etc. In mammals, five mAChR (M1-M5) have been identified, with each receptor subtype being the product of a different gene. M1 and M3 mAChR are most commonly postsynaptic, whereas M2, M3 and M4 are located presynaptically. M1 and M2 mAChR are predominant in the cerebral cortex and hippocampus, and may play a role in cognitive processing, i.e. working memory. M2 receptor is found both on cholinergic and noncholinergic terminals (reviewed in Nathanson, 2008).

**Nicotinic acetylcholine receptors (nAChRs):** One of the earliest neuroregulatory systems occurring in the developing brain is the nicotinic acetylcholine receptors (nAChRs). They play a role in neuronal development (e.g. neurogenesis, migration, differentiation, and synaptogenesis; reviewed in Dwyer et al, 2008), mediate neuronal pruning, regulate neural pattern formation by decreasing overproduction of cells and outgrowth, and guiding remaining neuritis to their targets. Besides their role in pre- and perinatal circuit formation, nAChRs play an important role in age-related cell degeneration. nAChRs start being established as early as the first half of the first trimester, and are followed by development of dopamine and norepinephrine neurons by the end of the first trimester, with the cholinergic fibers start entering the brain cortex much later, form 28-40 weeks of gestation.

nAChRs consist of different combinations of α and β subunits, eleven of which are found in the central nervous system (α2-α10 and β2-β4; reviewed in Gotti et al, 2006, Dwyer et al, 2008). The exact nAChR subtype composition in different brain parts is still not complete, and this is largely due to sensitivity of different methodological approaches that have been used, further complicated by species specific differences of distinct nAChR subunits. Thus α2β2 receptor is expressed in the primate and human cortex, but is absent in the rodent brain. Similarly there is no evidence that many of the possible nAChR subtypes are expressed in all brain areas of the mammalian brain. For example, α3β2 subunits are expressed in the visual pathway, whereas α4α6β2β3 receptor subtypes in visual and mesostriatal pathways (reviewed in Steinlein and Bertrand, 2008), α6β2 receptors are predominant in the mesolimbic pathway and α4α6β2 in the nigrostriatal pathway (Gotti et al, 2010). The α4β2 nAChR is the principal nicotinic AChR and is predominant in cortex, cerebellum, striatum, superior colliculus, and lateral geniculate nucleus. This receptor facilitates excitatory inputs when activated,
and is present very early in the developing brain, in various brain regions. The nAChR subtypes may also have different role, e.g. the α7 nAChR has neuroprotective function, and is highly expressed on hippocampal neurons and cholinergic projection neurons from the basal forebrain. Furthermore, the allosteric potentiation of α7 nAChR mediates the antipsychotic-like effect of the galantamine adjunctive treatment (Wiker et al, 2008). This provides further support for development of novel α7nAChR selective antipsychotic treatment that can be used in schizophrenia and other related psychiatric disorders, including ASD.

Brain Cholinergic Changes in ASD

Muscarinic receptor changes in ASD: Although muscarinic receptor changes have not been extensively investigated in autism, the findings are very similar to that of the nicotinic receptors (reviewed below). Thus, 30% decrease in M1 receptor binding is present in the cortical regions of autistic subjects (Perry et al, 2001). One of the explanation for the lower M1 receptor binding in autism may be the co-morbid epilepsy in these subjects (please note that up to 40% of autistic children to suffer from epilepsy; Minshew et al, 1997), since lower expression of M1 receptors is also characteristic of hippocampal sclerosis, and the latter is also linked with temporal lobe epilepsy (Pennell et al, 1999). Interestingly, Perry et al (2001) findings were not linked to epilepsy within their series of examined autism individuals, and may reflect a rather early neurochemical change in ASD, since the significant M1 loss in the frontal lobe is also present in young children with autism (Perry et al, unpublished). The findings from the latter study bear resemblance to those reported for schizophrenia, where one of the more consistent neurotransmitter abnormalities is loss of the various muscarinic receptors in different areas (Deng and Huang 2005). Further work on larger number of ASD individuals with and without epilepsy is required to determine the molecular changes in the muscarinic receptors in these subjects.

nAChRs changes in ASD: The nAChRs have been investigated in a number of post mortem studies in ASD, in both cortical and subcortical areas, including parietal and frontal cortex (Perry et al, 2001, Lee et al, 2002), cerebellum (Lee et al, 2002) and thalamus (Ray et al, 2005) (table 1). Substantial decrease (by 65-73%) in nAChRs was found in parietal and frontal cortices of autistic adult subjects in comparison to control counterparts (Perry et al, 2001). These abnormalities could be caused by abnormal cortical neuronal morphology (for example synaptic and dendritic abnormalities, Mukaetova-Ladinska et al, 2004, Feyder et al, 2010, Hutsler and Zhang, 2010, also reviewed in Penzes et al, 2011), similar to other developmental disorders (such as schizophrenia or epilepsy in neurodevelopmental disorders) and neurodegenerative disorders (e.g. Alzheimer’s disease and Parkinson’s disease) in which nicotinic receptors have also been implicated (reviewed in Miwa et al, 2011).

The cerebellar nicotinic receptors are of particular interest in ASD, since most, if not all of them are present in cells within the cerebellum itself and not in extracerebellar afferents (Turner et al, 2011). This characteristic nAChRs localization per sé can be a potential drug target for disorders involving the cerebellum, such as ASD. Our own work on the cerebellar cortex (Lee et al, 2002) found 40-50% lower nicotinic receptors binding to the agonist epibatidine (α3, α4, β2) in the granule cell, Purkinje and molecular layers in autistic subjects than
controls. However, there was a three-fold increase in the nicotinic receptor binding the agonist α-bungarotoxin (α7 subunit) in the granule cell layer (Lee et al, 2002). This result in particular should be highlighted, as the gene which encodes the α7 subunit is found near to q11-15 on chromosome 15 (a region implicated in autism, Lamb et al, 2000). In contrast, there were no differences in the levels of choline acetyltransferase (ChAT) between autistic and

Table 1. Summary of post-mortem studies focusing on the cholinergic system. Please note that all of the published studies refer to adults with ASD. Over the last 6 years there have not been further studies exploring the cholinergic deficit in ASD.

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Main Findings</th>
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<tbody>
<tr>
<td>Perry et al., 2001</td>
<td>7 autistic adults (diagnosed by DSM-IV criteria), 6 MR adults, 3 adults with Down’s syndrome, 6 controls.</td>
<td>In autistic subjects compared to controls: Lower cortical M1 receptor binding (up to 30%) and decrease in nicotinic receptors by 65-73% present in the parietal and frontal cortices. Lower levels of α4 and β2 nicotinic receptor subunits were found in the parietal cortex, whereas BDNF expression was three-fold higher in the basal forebrain.</td>
</tr>
<tr>
<td>Lee et al., 2002</td>
<td>8 autistic MR adults (diagnosed by DSM-IV criteria), 11 non-autistic MR adults, 10 controls.</td>
<td>In autistic subjects compared to controls: A reduction of 40-50% nicotinic receptor binding to the agonist epibatidine (subunits α3, α4, β2) in the granule cell, Purkinje and molecular layers. A three-fold increase in the nicotinic receptor binding α-bungarotoxin (α7 subunit) in the granule cell layer. A decrease in α4 receptor subunits in Purkinje and other cell layers. A non significant increase in α7 subunit in the granule cell layer.</td>
</tr>
<tr>
<td>Martin-Ruiz et al., 2004</td>
<td>6 autistic adults (diagnosed with DSM-IV criteria), 8 controls.</td>
<td>In autistic subjects compared to controls: The parietal lobe had lower expression of α4 and β2 subunits mRNA levels, protein expression and receptor binding density were lower than controls. In contrast, in the cerebellum of autistic subjects, α4 subunit mRNA levels were higher than controls, and the protein expression and receptor density decreased. This was accompanied by non significant increases in α7 subunit mRNA and protein expression levels and significant increases in receptor binding density in the same region in the autistic subjects.</td>
</tr>
<tr>
<td>Ray et al., 2005</td>
<td>3 autistic adults (diagnosed by DSM-IV criteria), 3 controls.</td>
<td>In autistic subjects compared to controls: the paraventricular nucleus (PV) and nucleus reuniens exhibited decreased levels of α7 and β2 immunoreactive neurons. Immunoactivity tests to explore the co-expression of α7 and glutamic acid decarboxylase in the PV showed no difference between autistic and control subjects, suggesting that the loss of α7 subunits in autism is not due to a loss of GABA-ergic neurons.</td>
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</table>

DSM-IV: diagnostic and statistical manual of mental disorders; MR: mentally retarded; M1: muscarinic acetylcholine receptor M1; BDNF: brain derived neutrophic factor; PV: paraventricular nucleus
control subjects (Lee et al, 2002). These findings put together suggest that the presynaptic cholinergic system is not altered in autism.

In contrast, in the thalamus (a brain area involved in sensory perception and regulation of motor functions, consciousness, sleep, and alertness), there is a substantial decrease in nicotinic receptor subunits (α7 and β2 immunoreactive neurons) in the paraventricular nucleus and nucleus reuniens in autistic subjects (Ray et al, 2005). These deficits in nicotinic receptor subunits may reflect the inability the thalamus to correctly modulate sensory input, and contribute to the incorrect sensory processing characteristic of ASD.

The substantial differences in nicotinic receptors between autistic and control subjects is further supported by studies investigating the levels of mRNA, protein expression and receptor binding densities of the various nicotinic receptor subunits. Thus, the decrease in nAChRs in the parietal cortices of autistic subjects is also accompanied by lower α4 mRNA levels, protein expression and receptor binding densities than in controls (Martin-Ruiz et al, 2004). Although the cerebellum α4 mRNA levels increased, the protein expression and receptor binding densities decreased. Levels of α7 receptor subunit binding density were also increased in the cerebellum, and also accompanied by non significant increases in mRNA levels and protein expression (Martin-Ruiz et al, 2004). These cholinergic system abnormalities could be related to cortical cell loss or dysfunction. Indeed, cellular migrational abnormalities are present in ASD cortex (Mukaetova-Ladinska et al, 2004, Korkmaz et al, 2007), and are accompanied by a lower protein expression of Bcl2 (an anti-apoptotic protein) in relation to p53 (an apoptotic regulator) in the neocortex (parietal cortex), possibly promoting apoptosis (Fatemi and Halt, 2001, Fatemi et al, et al, 2001). These findings are further supported by a recent diffusion imaging study (conducted on 17 high-functioning adolescents/adults with autism) that provided further evidence of neuronal fiber pathway abnormalities, especially in the hippocampal-temporal lobes (Conturo et al, 2008).

**From Neuropathological Studies to Clinics: Relevance of Brain Cholinergic Changes to Novel Pharmacological Treatments for ASD**

The findings from the neuropathological studies (as reviewed above) so far confirm significant and widespread loss of nicotinic receptors in various brain domains, including neocortical, cerebellar, thalamic and striatal regions in adult autistic individuals (Perry et al, 2001, Lee et al, 2002, Martin-Ruiz et al, 2004, Perry et al, unpublished). In ASD, both pre- and post-synaptically localised nAChRs (especially α4β2 nAChR, Martin-Ruiz et al, 2004) are affected, and this suggests widespread nicotinic receptor dysfunction and disconnectivity in ASD. Since nAChRs are known to modulate the release of other neurotransmitters, e.g. GABA and glutamate (Lavine et al, 1997, Baulac et al, 2001), the deficit of nicotinic receptors may reflect the early imbalance between the excitatory (glutamatergic) and inhibitory (GABA-ergic) interneurons in the autistic brain, and thus would increase the excitatory/inhibitory impulse ratio further, and consequently resulting in worsening of the clinical presentation of autism.

However, correlative clinico-neuroradiological and biochemical studies on ageing in ASD are lacking at the moment. Further progress in understanding the molecular mechanisms of ASD has been further hampered by the lack of larger ASD age-spanning cohorts to study.
the neuropathology and neurobiochemistry of ASD in greater detail, and relate it to the ASD clinical phenotype. The brain pathology studies in autism, as reviewed above, have all been conducted on relatively small numbers of children and adult individuals, and than in restricted brain areas, and not related to core or other ASD clinical symptoms. A key question is the extent to which the cholinergic receptor changes are central in the disorder, and whether they emerge at an early or late stage of ASD brain development, or they are subject to further changes in adulthood and beyond. Current advances in neuroimaging, based on markers for muscarinic and nicotinic PET or SPECT receptor imaging, may provide further insight regarding the cholinergic changes in ASD, and address both the temporal emergence of these alterations, and also their clinical relevance. There are recent reports of increased choline levels in autism (Hardan et al 2008; Gabis et al 2008, Vasconcelos et al 2008) using MRI proton spectroscopy imaging or MR SPECT, that may be relevant to cholinergic dysfunction. However, these findings are not conclusive, and similar choline levels have been described in both autistic boys aged 6-17 years and control subjects using the same methodology (DeVitto et al, 2007) or adults (Bernardi et al, 2011).

Modelling in mice is considered by some to be of value despite the vast species difference in cerebral complexity. Not surprisingly, the observed neuropathological and neurobiochemical changes of the cholinergic neurotransmitter system in ASD have been utilised in animal studies to develop animal models for autism (Walker et al, 2007). Thus, rats with neonatal basal forebrain cholinergic lesions have impaired cognitive function (Hohmann and Berger-Sweeney, 1998). Amongst models of autism, a β2 nicotinic receptor mutant has been reported to exhibit characteristics of the disorder (Granon et al 2003). The work on various models of cholinergic receptor knockout (KO) mouse models, including muscarinic acetylcholine receptor (mAChR) KO mice, nicotinic acetylcholine receptor (nAChR) KO mice, and acetylcholinesterase (AChE) KO mice, may also address individual subsets of symptoms of multifactorial disease, such as ASD, and also may lead to development of potential novel pharmacological interventions for ASD (reviewed in Zhang, 2006). Sadly, this work has not been followed by additional studies to address either the neuroanatomical and neuropathological mechanistics details of ASD, or relate them to cholinergic enhancing pharmacological interventions.

CHOLINERGIC THERAPIES FOR ASD

Cholinergic Therapies: An Overview

The latest developments in the dementia treatments, based on drugs that increase the rate at which acetylcholine is broken down, so more acetylcholine is present in the synaptic cleft, enables to address this in clinical setting. Thus, Acetylcholinesterase inhibitors (ChEIs), such as donepezil, galantamine and rivastigmine are all licensed for the treatment of Alzheimer’s disease (a neurodegenerative disease characterised by loss of neurons and cholinergic deficit). However, the ChEIs are also used in the treatment of other neuropsychiatric disorders such as Parkinson’s disease (please note that rivastigmine is also licensed for management of Parkinson’s disease, NICE Guidelines, 2006), Parkinson’s disease dementia or Lewy body dementia, Down syndrome, delirium, schizophrenia, depression, mania, traumatic brain injury, my-
asthenia gravis, attention deficit hyperactivity disorder etc. (reviewed in Yoo et al, 2007, Mehdiratta et al, 2011, Jones, 2011). In the case of dementia, ChEI have been shown to have significant benefit on both cognitive and behavioural changes, improve global function as well as activities of daily living (reviewed in Farlow et al, 2008), postpone institutionalization and reduce caregiver and economic burden (reviewed in Cappell et al, 2010). All of these studies suggest that the pharmacological improvement of the cholinergic deficit in elderly people with dementia by using ChEIs have far reaching benefits.

Cholinergic Therapies in Psychiatric Diseases Relevant to ASD

ChEIs and schizophrenia: In the light of cholinergic abnormalities apparent in adult autistic brain tissue, ChEIs maybe a useful treatment to counter one or more of the cognitive and non-cognitive symptoms of the condition. In that respect, the reports on in schizophrenic subjects with significant improvement of both the positive and negative symptoms (Keefe et al, 2008), depression (Risch et al, 2006), verbal learning (Erickson et al, 2005), and cognition (Lindenmayer and Khan, 2011) with treatment with donepezil are encouraging. However, benefits seem to be restricted to relatively younger schizophrenic subjects (Mazeh et al, 2006). Similar findings to the latter study were reported in respect to lack of improvement of neuro- and/or social cognition in stable community-treated schizophrenia treated with either donepezil (Freudenreich et al, 2005, Kohler et al, 2007) or rivastigmine (Sharma et al, 2006). However, longer (12 months) treatment with higher doses of rivastigmine (2x6mg/day) appear to have beneficial impact on cognitive function, learning and memory, as well as attention and quality of life in subjects with schizophrenia (Lenzi et al, 2003). Similar findings of improvement in processing speed and verbal memory have also been reported in galantamine treated subjects with schizophrenia (Buchanan et al, 2008), though these benefits appear to be diminished in non-smokers with schizophrenia (Dyer et al, 2008).

The findings regarding improvement of cognitive deficits in schizophrenics with memory problems are not conclusive. Thus, small to medium improvement in short- and long-term memory in schizophrenia subjects was reported compared to their baseline performance (reviewed in Stip et al, 2007, Lindenmayer and Khan, 2011). However, when used as an adjunctive therapy to treat schizophrenia and schizoaffective disorder (with other psychotropic drugs; Ribeiz et al, 2010), or in elderly with chronic schizophrenia further complicated with comorbid dementia (Mendelsohn et al, 2004), ChEIs improve cognitive (memory, motor speed and attention part of executive function), behavioural functioning and activities of daily living. These findings suggest that ChEIs may have potential beneficial effect on some of the core features of ASD, in particular the executive function and motor speed, as well as behaviour, and thus may improve the daily self-care activities of ASD subjects.

ChEIs and Down syndrome: Further support for the potential use of ChEI in ASD comes from clinical trials in subjects with learning disability, such as Down syndrome (DS). DS is characterised by substantial higher comorbidity of ASD than the general population (DiGuiseppi et al, 2010), with the expression of ASD symptomatology closely related to the extent of intellectual ability (reviewed in Moss and Howlin, 2009). In both children and adults with DS, both cognitive and behavioural changes appear to respond to ChEIs treatment. Thus,
smaller doses of ChEIs (e.g. 2.5mg/day and 5mg/day donepezil) in DS children provided significant improvements of expressive and receptive language performance (7 DS children, ag-

### Table 2. Review of studies using cholinesterase for treatment of ASD subjects. Includes all published studies on use of donepezil, rivastigmine and galantamine in ASD.

<table>
<thead>
<tr>
<th>ChEI</th>
<th>Study</th>
<th>Trial/Subjects</th>
<th>Criteria for Diagnosis</th>
<th>Main Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donepezil</td>
<td>Hardan and Haden, 2002</td>
<td>8 autistic children and adolescents (7-19 years) Open trial</td>
<td>DSM-IV</td>
<td>50% of subjects had improvement on ABC and Clinical Global impression scale.</td>
</tr>
<tr>
<td></td>
<td>Chez et al, 2003</td>
<td>43 children with autism or pervasive developmental disorder. Double blind study over first 6 weeks, followed by open labelled 6 weeks study</td>
<td>DSM-IV</td>
<td>Improvements in receptive and expressive language scores, using CARS</td>
</tr>
<tr>
<td></td>
<td>Hetzman, 2003</td>
<td>1 adult treated with 5 mg/day donepezil</td>
<td>DSM-IV</td>
<td>Verbal and behavioural regression after 1 month treatment.</td>
</tr>
<tr>
<td></td>
<td>Henden et al, 2011</td>
<td>34 children (8-17 years) with autism (IQ&gt;75), treated with 5-10mg donepezil. Double-blind, placebo-controlled trial over 10 weeks, followed by a 10-week open label trial for placebo non-responders.</td>
<td>DSM-IV</td>
<td>Improvement in a number of executive function measures, but no statistically significant between-group differences were found</td>
</tr>
<tr>
<td>Rivastigmine</td>
<td>Chez et al, 2004</td>
<td>Open labelled study. 32 children (unspecified age)</td>
<td>DSM-IV</td>
<td>Improvements in autistic behaviour, particularly verbalization. Assessments done using Childhood Autistic Rating Scale, Gardner's Expressive and Receptive One-Word Picture Vocabulary tests, and the Conners' Parent Rating Scale.</td>
</tr>
<tr>
<td>Galantamine</td>
<td>Niederhofer et al, 2002</td>
<td>20 boys (mean age 7.4 years). Dose and duration of treatment not specified.</td>
<td>ICD-10</td>
<td>Improvements in hyperactivity, inadequate eye contact and inappropriate speech. ABC used.</td>
</tr>
<tr>
<td></td>
<td>Hertzman, 2003</td>
<td>3 autistic adults treated with 4mg/day galantamine, the dose increased to 12mg/day after 2 months.</td>
<td>DSM-IV</td>
<td>Improvement in expressive language and communication</td>
</tr>
<tr>
<td></td>
<td>Nicolson et al, 2006</td>
<td>13 children (mean age 8.8 years). 12 weeks open-label trial</td>
<td>DSM-IV</td>
<td>61.5% children responded. Improvements in irritability, social withdrawal, emotional lability, inattention and aggression. ABC, Conner’s Parent Rating Scale-Revised, Children’s Psychiatric Rating Scale and Clinical Global Impressions Scale used.</td>
</tr>
<tr>
<td></td>
<td>Cubells et al, 2011</td>
<td>39 year male with 15q13.3 deletion syndrome (IQ=55). Galantamine initiated 4mg daily, and dose titrated over 2 weeks to 12mg bd, and followed over 10 months period.</td>
<td>DSM-IV</td>
<td>Dramatic decline in frequency and intensity of rage outbursts.</td>
</tr>
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</table>
es 8-13 years; Heller et al, 2004) and some improvement on measures of memory and sustained attention (7 DS children, age 8-13 years; Spiridigllozzi et al, 2007). Overall, donepezil was well tolerated, though some of the participants had increased irritability and/or assertiveness. Similar results of improvement of adaptive function, attention, memory and language have been reported for rivastigmine in 11 young DS subjects (ages10-17 years; Heller et al, 2006). However, a most recent randomized, double-blind, placebo-controlled multicenter study, conducted on a substantially larger DS cohort (129 participants) failed to demonstrated any significant benefits of donepezil in relation to cognitive and behavioural improvements in DS children and adolescents (age 10-17 years; Kishnani et al, 2010). Although the latter study included tests covering performance across multiple psychological domains, including functional ability of the DS subjects, it was with a relatively short duration (over 10 weeks). Further studies on similarly larger cohorts are now needed to address the benefits of ChEIs on the cognitive and behavioural performance in children and younger adults, and preferably they will need to be done over a longer period of time, rather than 2-3 months period.

Similar benefits were also found in DS adults treated with ChEIs. Thus, DS adults with no cognitive impairment treated with donepezil have improvement in verbal and written communication, as well as quality of life (Johnson et al, 2003, Kondoh et al, 2005), cognition and behaviour (Kishnani et al, 2009), whereas in DS adults with dementia, ChEIs (e.g. rivastigmine and donepezil) treatment results in slower decline in both global functioning and adaptive behaviour (Prasher et al, 2005), and improve in abilities in daily lives (Kondoh et al, 2011) over 24 weeks compared to the untreated group, respectively. Another study conducted on long-term use of rivastigmine (over 38 months) in adults with DS did not report any major improvements in respect to their cognition and language performance (Heller et al, 2010). The Cochran review (Mohan et al, 2009) also reported non-statistically significant trend in favor of people with Down syndrome and co-morbid Alzheimer's dementia who are able to tolerate donepezil.

**ChEI Treatment in ASD**

Most of the studies on ChEI treatment in ASD are conducted in children, and so far there have been only few studies assessing the value of treating ASD subjects with donepezil, rivastigmine and/or galantamine (summarised in table 2). Nevertheless, ChEIs appear to be well tolerated by the ASD participants, with no major side effects reported in any of the studies, although gastrointestinal problems, a mild increase in irritability (Hardan and Haden, 2002) and headaches (Nicolson et al, 2006) have been noted in two of the studies. Donepezil has been found to improve irritability and hyperactivity (8 children and adolescents, 7-19 years age; Hardan and Haden, 2002), receptive and expressive language score (43 children with ASD or pervasive developmental disorder, 2-10 years of age; Chez et al, 2003) and some executive function measures (34 children, 8-17 years of age; Handen et al, 2011). So far there is only one open label study involving rivastigmine and ASD subjects that has been published (Chez et al, 2004; table 2). In this study, 32 autistic children (unspecified ages) were recruited and assessed using the Childhood Autistic Rating Scale, Gardner's Expressive and Receptive One-Word Picture Vocabulary tests, and the Conners’ Parent Rating Scale. Improvements in expressive speech and overall behaviour were noted in the treated ASD subjects.
Galantamine is of particular interest for the treatment in ASD since, in addition to an AChE inhibitory activity, it also has an allosteric nicotinic receptor modulation which could be particularly relevant in autism in the view of nicotinic receptor pathology. A placebo controlled, double blind crossover, randomized controlled trial with galantamine showed improvements in hyperactivity, inadequate eye contact and inappropriate speech in the treated ASD subjects (20 boys, mean age 7.4 years; Niederhofer et al, 2002). Similarly, improvements in irritability, social withdrawal, emotional lability, inattention and aggression were documented in 60% (8/13) of galantamine treated children (Nicolson et al, 2006) and in a 39 years old adult (Cubells et al, 2011). Similarly, improved verbal skills were noted in 3 autistic adults treated with galantamine, and this was also accompanied by improvement in social behaviour in some of them (Hertzman, 2003).

The reviewed studies above provide evidence that donepezil, rivastigmine and galantamine are overall well tolerated and effective in treating cognitive and behavioural changes seen in children, adolescents and even adults with ASD. However, the results are not conclusive and more double blind randomised control studies need to be carried out.

**Cholinomimetic Agents: Nicotinic Agonists**

Cholinomimetic agents are chemicals that can act by either directly stimulating the nicotinic or muscarinic receptors, or promote acetylcholine release. One group of these agents is nicotinic agonists that enhance the action at the nicotinic acetylcholine receptor (nAChR). Examples of such drugs include: nicotine, acetylcholine, choline, epibatidine, lobeline and varenclline, although not all of them have beneficial effect upon mental health (Campbell and Anderson, 2010). Interestingly, the expression of some of these cholinomimetic agents can be regulated by hypothalamus induced agents and/or drugs, e.g. digoxin (an endogenous Na+-K+ ATPase inhibitor) that, in specific, upregulates nicotine. This is of particular interest since autistic children (10-15 years old, all nonsmokers) have higher levels of serum digoxin than their control counterparts (Kurup and Kurup, 2003). The authors speculated that changes in the plasma nicotine levels in the ASD group in the latter study may be due to the increase in tryptophan (a precursor of nicotine) and its catabolites, and the reduction in tyrosine and its catabolites in ASD serum. However, to date these findings have not been replicated.

Nicotine stimulates the release of several neurotransmitters in various brain areas, and the nAChRs stimulation by nicotine or the endogenous agonist, acetylcholine, induces a significant increase of glutamate in the layer V of the frontal cortex, via the involvement of α4β2 nAChRs (Lambe et al, 2003), receptors that are significantly depleted in autism. Hippocampus-dependent learning is particularly sensitive to the effects of nicotine. However, the effects of nicotine on hippocampus-dependent learning vary with the nicotine doses and whether nicotine is administered acutely, chronically, or withdrawn after chronic nicotine treatment (reviewed in Kenney and Gould, 2008).

In clinical studies, nicotine has been documented to ameliorate some cognitive and behavioural (notably negative) symptoms in schizophrenia (reviewed in Winterer, 2010), and even delay the onset of the schizophrenia symptomatology (Ma et al, 2010). Thus, the high rates of tobacco smoking in schizophrenia have been thought to be a self-medication attempt for their cholinergic neurotransmission impairment, and the nicotine itself may ameliorate some of their cognitive deficits, especially working memory and attention (reviewed in D’Souza and Markou, 2011).
Nicotine-agonists (e.g. alpha4beta 2 agonists) have been shown in animal studies to improve speed of processing, working memory, visual learning and memory, and social cognition (Radek et al, 2010). Furthermore, nicotine agonists (Gaynor and Handley, 2001), and nicotine patches improve attention and reduce complex tics in autistic Tourette’s syndrome subjects (Howson et al, 2004). Interestingly, even the use of a single nicotine patch produced a significant reduction of the symptomatology with an average duration of 1-2 weeks post-application (Shytle et al, 1998). However, to date there are no studies to support either the short-term or long-term efficacy of nicotine patches in treatment of ASD symptomatology.

Our own experience (please see appended Case Report) suggests that transdermal nicotine therapy can provide long-term remediation of drug-resistant behavioral dysfunction (including violence and aggression) in ASD adults. In the instance to which we refer, continuing use of the patch allowed for discontinuation of the subject’s other medications, while side-effects appear to have been limited to an initial period of nausea. Taken together, these observations suggest that transdermal nicotine therapy may be a safe and efficacious alternative to conventional medicines.

CONCLUSIONS

Since etiological factors in ASD remain unknown, treatment of the disturbing behavioural and cognitive profile of the affected individuals still largely remains symptomatic. The cholinergic deficit in autism and the promising results of cholinergic drug trials so far indicate that further testing of ChEI to regulate the cognitive and associated behavioural changes in ASD subjects is needed. The use of ChEI (donepezil, galantamine and rivastigmine) in open label trials have been reported to improve core symptoms, but double blind placebo trials still remain to be conducted to provide more accurate information. In addition to the findings from clinical studies in ASD, animal studies also suggest that ChEIs may have an impact on regulating auditory sensory gating (Hohnadel et al, 2007), warranting further studies on similar deficits in ASD.

Another therapeutic approach that can be implemented very early on is modifying the neurotransmission via change in diet or regulation of inflammatory responses (reviewed in Jyunouchi, 2009), and avoiding anticholinergic drugs (including neuroleptics, antidepressants, King et al, 2003; Kolevzon et al, 2006). Thus, regulating the intake of tryptophan, tyrosine and choline can be useful to regulate sleep and mood (Zeisel, 1986). Similarly, numerous plants used in medicine have cholinesterase inhibitory activity (Houghton et al 2006, reviewed in Howes and Perry, 2011). Some of these phytochemicals such as sage (Salvia officinalis) and lemonbalm (Melissa officinalis) have been shown to improve cognition and mood in normal adults (Kennedy et al 2003, Tildesley et al, 2003, 2005; Scholey et al, 2008). Since they are without adverse side effects at standard doses, such agents may also be worth considering in autism spectrum disorders.

The cholinergic abnormalities found in autistic patients post mortem, combined with the (partial) success of ChEI, may indicate a role for acetylcholine in the aetiopathology of autism. Although the case report studies have been largely conducted on adult autistic subjects, novel drug treatments utilising the current knowledge may prove to be applicable for young autistic children, targeting core disease mechanisms. In this respect, exploring the use of cho-
linomimetic agents, may find a place in the treatment of some of the symptoms of the autistic spectrum disorders. Thus, a Russian study (Krasnoperova et al, 2004) found improvement in 89% of the children (n=20, aged 3-8years, with mild to moderate severity autism) with choline alfoscerate (CA; 400mg/day over 8 weeks, alongside with maintenance therapy with neuroleptics. The clinical benefits were: general improvement of behaviour, development of social and communicative skills, reduction of marked speech disturbances, enhancement of learning activity and productivity. The findings of this study, alongside with the presented case report in this review suggest that cholinomimetic agents are an effective and safe medicine for treatment of cognitive and behavioural disturbances in both children and adults with autism, and can be used safely combined with additional neuroleptic therapy. Since nicotine is likely to be used off-label in the future, we recommend that its use be restricted to adult subjects manifesting with severe behavioural dysfunction refractory to conventional pharmacologials. Further clinical trials need to be initiated in near future for larger ASD cohorts for both cholinergic and cholinomimetic drugs, and determine their long-term benefits, so that they can be employed in routine clinical setting for treatment of both cognitive and behavioural problems in ASD subjects.

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REFERENCES


of single doses of Melissa officinalis (Lemon balm) with human CNS nicotinic and muscarinic receptor-binding properties. *Neuropsychopharmacology* 28:1871-1881.


