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Prefrontal grey and white matter neurometabolite changes after atomoxetine and methylphenidate in children with attention deficit/hyperactivity disorder: A ^{1}H magnetic resonance spectroscopy study

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ABSTRACT

Attention deficit/hyperactivity disorder (ADHD) is the most common neurobehavioral childhood disorder. Dysfunction of prefrontal neural circuits which are responsible for executive and attentional functions has been previously shown in ADHD. We investigated the neurometablite changes in areas included in dorsolateral prefrontal neural circuits after 2 months of long-acting methylphenidate or atomoxetine medication in children with ADHD who were responders to treatment. Twenty-one ADHD children were examined by single voxel ^{1}H-magnetic resonance spectroscopy (MRS) before and after 2 months of medication with OROS methylphenidate ($n=10$) or atomoxetine ($n=11$). The spectra were taken from the dorsolateral prefrontal cortex (DLPFC, 8 ml) and white matter behind the DLPFC (anterior semioval center, 7.5 ml), bilaterally. NAA and NAA/Cr (*N*-acetylaspartate/creatine) decreased in the left DLPFC and Cho/Cr (choline/creatine) increased in the right DLPFC after atomoxetine medication. Glu+Gln and Glu+Gln/Cr (glutamate/glutamine) increased in the left white matter after methylphenidate medication. We hypothesize that atomoxetine could decrease hyperactivation of DLPFC neurons and methylphenidate could lead to increased activation of cortical glutamatergic projections with the consequences of increased tonic dopamine release in the mesocortical system.

1. Introduction

Attention deficit/hyperactivity disorder (ADHD) is the most common neurobehavioral childhood disorder, with a worldwide prevalence 4–12% in children (American Association of Pediatrics, 2000). Diagnosis of ADHD includes three clusters of symptoms – hyperactivity, impulsivity, and inattention. Neurophysiological and imaging studies have shown that the abnormalities in frontal neural circuits that are responsible for executive and attentional functions are implicated in the disorder (Sagvolden and Sergeant, 1998; Rubia et al., 2001; Castellanos and Tannock, 2002; Max et al., 2005; Oades et al., 2005). The dorsolateral prefrontal cortex (DLPFC) plays an important role in sustaining attention, working memory, planning and organization of tasks (Posner and Petersen, 1990). White matter behind the DLPFC includes fibers leading to and from the DLPFC (Cihak, 1997) that form a part of the neural circuits responsible for motivation, executive function, and emotional responses (Gorelova et al., 2012).

The hypothesis of catecholamine deficiency in the prefrontal cortex and the basal ganglia is at the basis of the current forms of pharmacotherapy used in ADHD (Castellanos et al., 1994; Gainetdinov et al., 1999; Dresel et al., 2000; Madras et al., 2005). ADHD pharmacotherapy includes methylphenidate (MPH) and atomoxetine (ATX). MPH, a stimulant dopamine reuptake inhibitor (Volkow et al., 1999), has been found to increase dopamine in the prefrontal cortex, nucleus accumbens and striatum (Cusack et al., 1994). It has been shown that its behavioral effects are mediated by glutamatergic fibers from the prefrontal cortex to the ventral tegmental area and the nucleus accumbens (Wanchoo et al., 2009). ATX is connected with selective noradrenaline reuptake inhibition exclusively in the prefrontal cortex (Barton, 2005; Ledbetter, 2006). Clinical response to treatment has been found to occur at the end of

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the first week for long-acting MPH (Pelham et al., 2001; Wolraich et al., 2001) and after an 8-week period for ATX (Garnock-Jones and Keating, 2009).

Brain biochemistry in vivo can be assessed with ^{1}H magnetic resonance spectroscopy (MRS). Detectable brain metabolites include *N*-acetylaspartate (NAA), NAA-glutamate (NAAG), creatine (Cr), choline (Cho), Glu+Gln signal (a broad signal in the MR spectrum consisting of overlapping peaks of glutamate and glutamine), gamma-amino-butyric acid (GABA), myo-inositol (mI), lactate (Lac), and a few other metabolites. The creatine signal reflects creatine and phosphocreatine, generally referred to as tCr (total creatine). In the text below, we use the abbreviation Cr for simplification.

There is a lack of evidence about the influence of medication used for ADHD treatment on neurometabolism in prefrontal gray and white matter. To our best knowledge, only four studies investigated post-medication changes in neurometabolites in the prefrontal cortex. The first one was a series of four case reports, two patients were medicated with ATX and two with MPH for 14–18 weeks. A decrease of Glu+Gln/Cr was found only in the right prefrontal cortex in ATX-treated patients (Carrey et al., 2002). No differences in the right prefrontal cortex after 13 weeks of treatment with various medications (MPH, ATX, dexedrine) were found; however, the study was not focused on the effects of distinct drugs on neurometabolite levels (Carrey et al., 2003). Similarly, after 8 weeks of IR (immediate-release) MPH medication exclusively, no differences were found in the right anterior cingulate cortex in 13 ADHD children (Carrey et al., 2007). However, after 12 weeks of long-acting MPH administration in 21 children with ADHD, NAA/Cr significantly increased both in the right and the left prefrontal cortex, Glu/Cr and Cho/Cr significantly decreased in the right and the left prefrontal cortex, and ml/Cr significantly decreased in the left prefrontal cortex (Wiguna et al., 2012). This difference in the influence of MPH on neurometabolite levels could be the consequence of the different formulation of MPH medication, the treatment duration, or both. Although long-acting MPH administered once a day and MPH administered t.i.d. (three times daily) have been shown to have similar effects on ADHD symptoms in short-term studies (Pelham et al., 2001; Wolraich et al., 2001; Biederman et al., 2007), it would be of interest to evaluate the effects of long-acting MPH on neurometabolite levels after 8 weeks, the period in which IR MPH was not shown to have effects on neurometabolites in prefrontal cortex (Carrey et al., 2007).

To our best knowledge, no study has evaluated neurometabolite changes in prefrontal white matter behind the DLPFC despite the fact that the activity of fibers leading from the PFC, particularly glutamatergic afferents, could play a key role in ADHD symptoms (Prieto-Gomez et al., 2005; Wanchoo et al., 2009).

Our aim was to examine treatment-related neurometablite changes in areas included in prefrontal neural circuits (dorsolateral prefrontal gray and white matter) in children with ADHD after 2 months of long-acting MPH or ATX medication separately. We chose the 8-week medication period due to the development of ATX's clinical effect (Garnock-Jones and Keating, 2009) and evaluation of OROS MPH's effects on neurometabolites after 8 weeks for reasons described above. Based on the findings of higher Glu (Courvoisie et al., 2004; MacMaster et al., 2003) and Cho (Courvoisie et al., 2004) in the prefrontal cortex and of NAA in gray (Yeo et al., 2003; Courvoisie et al., 2004) and white (Fayed et al., 2007) matter of prefrontal regions in children with ADHD compared with healthy controls, we previously hypothesized that increased glutamate levels potentially leading to ADHD symptoms could increase mitochondrial (represented by NAA (Mason and Krystal, 2006; Rigotti et al., 2007)) and membrane (represented by Cho (Mason and Krystal, 2006)) metabolism of prefrontal circuits (Husarova et al., 2010). Thus, medication could be effective in ameliorating ADHD symptoms via the decrease of Glu levels that has been shown both after ATX (Carrey et al., 2002) and long-acting MPH (Wiguna et al., 2012).

Based on the aforementioned studies of differences in neurometabolite levels between ADHD and healthy children, neurometabolite changes before and after medication in ADHD children and studies of the molecular effects of MPH and ATX, we aimed to elucidate the following hypotheses:

- Both ATX and MPH decrease Glu in gray matter in the DLPFC.
- ATX and MPH affect NAA in gray matter in the DLPFC.
- ATX and MPH decrease Cho in gray matter in the DLPFC.
- Only MPH affects neurometabolite levels in the white matter behind the DLPFC; it increases Glu, potentially reflecting activity of PFC glutamatergic afferents within white matter, from which Glu can be released during propagation of action potentials (Kukley et al., 2007; Ziskin et al., 2007).

2. Methods

2.1. Subjects

Patients were recruited from the Clinic of Psychiatry, Jessenius Faculty of Medicine, Martin University Hospital in Martin, Slovakia, and had MRI performed at the same place. Twenty-one medication-naive children participated in the study. All subjects were in-patients of the Children's Department. Patients were hospitalized twice – as medication-naïve and after 2 months of medication for evaluation of the treatment effects. Patients switched to other forms of therapy, as described below, were hospitalized three times. All patients were right-handed. Written informed assent/consent was obtained from the participating children and their parents. The protocol was approved by the Ethics Committee of Martin University Hospital. The study conformed to the code of ethics stated in the Declaration of Helsinki.

The mean age of the children was 12.3 years (range 6.1–16.8 years). Seventeen patients were males ($n_{ATX}=9$, $n_{MPH}=8$) and four females ($n_{ATX}=2$, $n_{MPH}=2$). The clinical diagnosis of ADHD was made by an experienced senior consultant psychiatrist based on a clinical observation during the first 2 days at the Children's Department of the Clinic of Psychiatry and after an extensive interview with the patient's parents including questions about hyperactivity, impulsivity, and inattention based on DSM-IV criteria. The symptoms were quantified by The ADHD-RS-IV (DuPaul et al., 1998), administered and scored by clinicians based on clinical observation and a semi-structured interview with the patients' parents. The scale consists of 18 items, with 1 item for each of the 18 symptoms contained in the DSM-IV diagnostic criteria. Each item is scored on a 4-point scale (0 – never or rarely; 1 – sometimes; 2 – often; 3 – very often) (DuPaul et al., 1998; Chang et al., 2009). ADHD-RS-IV has been demonstrated to have good cross-cultural factorial validity and internal consistency in a sample of European children (Dopfner et al., 2006). All patients had at least six symptoms of inattention and at least six symptoms of hyperactivity/impulsivity; thus, all patients fulfilled the criteria for ADHD, combined subtype. Additionally, six patients fulfilled the criteria for oppositional defiant disorder. After completion of the diagnostic process, patients were randomized to receive OROS MPH ($n=10$) or ATX ($n=12$). The titration of OROS MPH started at the dose of 18 mg/day in the morning, the effect was evaluated after 5 days, and the dose was increased to 36 or 54 mg/day according to the clinical effect. Atomoxetine was titrated from 0.5 mg/kg/day in the morning, and the dose was gradually increased after 4–5 days to 1.2–1.4 mg/kg/day. The titration of drugs followed the prescribing regulations according to The State Institute for Drug Control. The effect of medication was evaluated after 2 months during the second hospitalization. A reduction of at least 30% in the ADHD-RS-IV score administered by clinicians was evaluated as a clinical response. This cutoff point (or a lower one) has been previously used as a measure of clinical response in both ATX and stimulant studies (Chang et al., 2009; Jain et al., 2011; Mattingly et al., 2013; Soutullo et al., 2013). Only responders to medication were included to the second MRS examination process. Two patients taking OROS MPH and three patients taking ATX were non-responders to medication and were switched to the opposite medication after a wash-out period at least 1 week. These children were hospitalized three times, the third time after the next 8 weeks to evaluate the clinical effect of the second medication. Responders were included in the second MRS scanning. One patient was a non-responder to both medications and was excluded from the study. At the end of the study, the clinical characteristics of10 patients on MPH and 11 patients on ATX had been measured twice – at baseline, thus with the manifestation of ADHD symptoms, and after 2 months of medication.

Exclusion criteria included a current major depressive episode or a diagnosis of recurrent depressive disorder in the past, anxiety disorder, current or past pharmacotherapy for ADHD, current or past bipolar or psychotic disorder, serious