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SHORT COMMUNICATION

A power analysis for future clinical trials on the potential adverse effects of SSRIs on amygdala reactivity

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Abstract **Treatment of adolescents with antidepressants may induce an increased risk for suicidality in this population. The activity of the amygdala during processing of emotional faces with functional Magnetic Resonance Imaging (fMRI) is a well-known measure of emotional dysregulation. Based upon data of our prematurely ended randomized clinical trial with fluoxetine (NTR3103) in anxious and or depressed girls (12–14 years of age) we calculated that with the found effect size of $r = 0.66$, compared to placebo, only 8 subjects are needed to demonstrate increased amygdala activity following 16 weeks of treatment with fluoxetine.**

Keywords amygdala reactivity, SSRI, adverse effects, anxiety, depression

Introduction

The safety of antidepressants in children and adolescents is a subject of concern, since in 2004 the Food and Drug Administration issued a black box warning regarding the use of antidepressants in children and adolescents (Hammad, 2004). Indeed, the neurobiological effects of antidepressants, such as selective serotonin reuptake inhibitors (SSRIs), on the developing neuronal circuitry of the human brain are largely unknown, as most imaging studies have been conducted in adults so far. However, SSRI studies in periadolescent animals, including non-human primates, show long-lasting effects on serotonergic outcome measures, such as an increase in the density of the serotonin (5-HT) transporters (Wegerer et al., 1999; Bouet et al., 2012; Shrestha et al., 2014) as well as anxious and depressive like behavior (Ansorge et al., 2004). In line with this, in adolescents SSRI prescription is associated with transient, age-delimited effects on suicidal ideation. To investigate the effects of SSRIs on brain regions and circuits critical to anxiety and depression in children, we designed a 16 week randomized clinical trial (ePOD-SSRI) with fluoxetine in medication naive girls suffering from major depressive disorder (MDD) and/or anxiety disorder (AD). We measured the activity of the amygdala during processing of emotional faces with functional Magnetic Resonance Imaging (fMRI) as it is a well-known measure of emotional dysregulation, i.e., depression. For instance, in adults fMRI has shown increased amygdala activity in patients suffering from MDD, which decreases after successful treatment with paroxetine (Ruhé et al., 2014). Only one previous study has been conducted with this technique in adolescents, and reported normalization of amygdala activity following 8 week treatment with fluoxetine (Tao et al., 2012). However, subjects were scanned during treatment, making it impossible to distinguish between effects of acute fluoxetine and the lasting effects of prolonged fluoxetine exposure on brain development. Here we report our preliminary findings on 7 patients who were included in the ePOD-SSRI trial with a wash out period of 3 weeks. Unfortunately, because of insufficient study inclusion (dental braces, insufficient study participation of primary care facilities) we had to end our clinical trial prematurely. Based on the findings of early SSRI treatment in animals discussed above, we hypothesized to find an increased responsiveness of the amygdala to fearful faces following chronic treatment with fluoxetine, when

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compared to placebo, reflecting hyperactivity of the affective neurocircuitry. The purpose of this brief report is to provide a power analysis for future clinical trials on the potential adverse effects of SSRIs on human brain development.

Materials and methods

ePOD-SSRI was a 16-week multicenter double blind, placebo-controlled trial (NTR3103) with a blinded end-point evaluation with fluoxetine in antidepressant naïve subjects suffering from MDD, and described in greater detail elsewhere (Bottelier et al., 2014). It was reviewed and approved by the Central Committee on Human Research in the Netherlands (CCMO) in the Hague. Written informed consent was obtained from patients and legal guardians before randomization. The effect of fluoxetine on emotional processing was assessed using fMRI in 7 girls suffering from MDD, before random assignment to either placebo or active treatment with fluoxetine (using a permuted block randomization scheme 1:1), and again 3 weeks after trial end (in week 19). Inclusion criteria were: female outpatients aged 12-14 years of age with a history of at least 2 weeks of moderate or severe MDD and or anxiety disorder (AD), as defined in the DSM-IV and as determined by a structured interview (Diagnostic Interview Schedule for Children fourth edition, DISC-IV) in need of pharmacotherapy with a score of >3 on the Clinical Global Impressions severity subscale (CGI-S), and a total score of >45 on the Depression Rating scale-Revised (CDRS-R). Exclusion criteria were co-morbid axis I psychiatric disorders requiring pharmacological treatment; IQ <80; current risk of suicide attempt and previous antidepressant use. According to standard criteria response to treatment was defined as a score of 1 or 2 on the CGI-S improvement item (indicating "very much improved" or "much improved") at trial end (week 19). Partial response was defined as a score of 3 and 4, and worsening of depression as a CGI-S improvement item score of 5, 6, or 7. The fMRI paradigm we used was a modified version of the event-related implicit emotion processing task (Demenescu et al., 2011) in which subjects viewed negative emotional faces and neutral stimuli (ellipses consisting of scrambled faces) alternated in a block design. fMRI data were acquired on a 3T Philips scanner (Best, the Netherlands) fitted with an 8-channel head-coil using the following scan parameters: TR/TE = 2300/30ms; GE-EPI readout; voxel size = $2.3\times2.3\times3$ mm; flip angle 80°. Data were preprocessed using FSL and a region of interest (ROI) analysis of amygdala activation was conducted, as the amygdala is an important relay for emotional processing between visual systems and modulatory responses. As there were no significant differences between the left and right amygdala at baseline and post-treatment, bilateral amygdala was used in subsequent analyses. One subject was excluded due to excessive motion. Due to the small sample size non-parametric statistical tests were used.

Results

Prior to randomization, the fluoxetine and placebo group did not differ on demographics nor clinical characteristics (Table 1). Baseline amygdala activation also did not differ between the two groups. As shown in Fig. 1, fluoxetine treatment increased amygdala activation to threatening faces compared to baseline (mean = 11.67, sd = 14.47, $p = 0.14$, + 40.94%), whereas placebo treatment resulted in equal or less amygdala activation (mean = − 5.88, sd = 8.37, $p = 0.11$, − 36.36%). The interaction term between treatment x time showed a trend ($U = 1.0$, $p = 0.11$) and an effect size of $r = 0.66$. A power calculation indicated that in future clinical trials only 8 subjects are needed, 4 in each arm, to detect a significant effect (with 80% power and $\alpha = 0.05$) of fluoxetine treatment on amygdala activation. At trial end, all girls in the placebo group had shown a 'partial response' or 'much improved' (CGI-S improvement score <4), whereas in the 3 girls that had received active treatment with fluoxetine a mixed response was observed (CGI –S improvement was 2, 4 or 6).

Table 1

Subject No.	Age	DISC diagnosis	Treatment	CDRS* children *Baseline*	CDRS* parents *Baseline*	CGI score *Baseline*	CGI score 8 *weeks*	CGI change 8 *weeks*	CGI score 19 *weeks*	CGI change 19 *weeks*
1	14	MDD	Placebo	>85	84	5	3	2	4	3
2	14	MDD, PD	Placebo	>85	84	5	5	4	4	4
3	13	MDD, SoP, PD	Placebo	>85	66	5	5	5	4	3
4	14	MDD	Placebo	>85	73	4	2	2	2	2
5	12	SoP, GAD	Fluoxetine	85	63	5	4	2	3	2
6	14	MDD	Fluoxetine	>85	61	5	4	3	5	4
7	14	MDD, PD, OCD, SpP	Fluoxetine	>85	85	5	4	3	5	6

* Adjusted score.
MDD = Major depressive disorder; SoP = Social phobia; GAD = Generalized anxiety disorder; PD = Panic disorder; OCD = Obsessive compulsive disorder; SpP = Specific phobia.

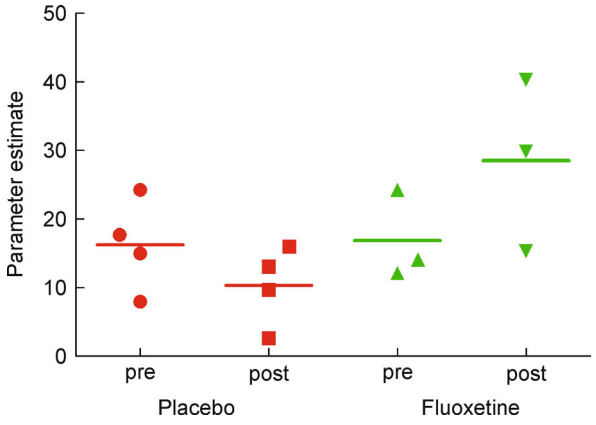

Figure 1 Amygdala activation before and after randomization for 16-week treatment with placebo or fluoxetine in girls suffering from severe MDD and/or AD. Y axis shows mean BOLD response in bilateral amygdala (anatomical ROI). Fluoxetine treatment increases amygdala activation to threatening faces compared to baseline (on average + 40.94%), whereas placebo treatment resulted in equal or less amygdala activation (on average −36.36%).

Discussion

In this first RCT on the effects of SSRIs on brain development, we found a trend of increased amygdala activity for fluoxetine-, vs. placebo treated girls suffering from MDD and AD. As the fMRI paradigm we used is strongly associated with emotional dysregulation, our preliminary findings suggest that treatment with fluoxetine may aggravate emotional dysregulation in these young/adolescent girls with MDD and PD.

Our findings are in concordance with preclinical studies measuring the effect of fluoxetine on the developing brain. In fact, in juvenile rats chronically treated with fluoxetine we also observed an increased brain response to an acute challenge with fluoxetine, whereas this response was decreased in adult treated rats (Klomp et al., 2012). An increase in 5-HT transporter density following SSRI treatment in early, but not in adult, rats and non-human primates, may underlie this increased reactivity of the juvenile brain following antidepressant treatment. Indeed, evidence is gradually emerging that the long-term effects of drug exposure are delayed and come to expression once the vulnerable system reaches maturation (i.e., typically during adulthood). This phenomenon is known as neuronal imprinting and occurs when the effects of drug exposure outlast the drug itself (Andersen and Navalta, 2004). As the prefrontal cortex is still developing in adolescents, we hypothesize that children and adolescents lack the 'regulatory' prefrontal decreasing activity of antidepressant action to negative stimuli (Ma, 2015) thereby inducing different effects in children and adolescents vs. adults.

Our results are in contrast with a previous clinical study in depressed girls which found a decrease in amygdala activity after 8 weeks of open label treatment with fluoxetine (Tao et al., 2012). However, subjects in that study were scanned during treatment, thereby making it difficult to disentangle acute and long-term effects and together with the lack of a placebo group this likely explains the discrepancy between this study and ours.

In sum, in view of the trend we observed on adverse effects of fluoxetine treatment on amygdala activity in female adolescents, we concluded that: 1) it is important to replicate this highly relevant finding in a larger sample, as this may underlie the increased risk for suicidal behavior in children and adolescents treated with antidepressants, 2) we have shown that such a study does not need to be very large, as the effect size of 0.66 is large.

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Compliance with ethics guidelines

Marco Bottelier declares that he received research funding from a private fund Suffugium. Anouk Schrantee, Guido van Wingen, Eric Ruhe, Michiel de Ruiter and Liesbeth Reneman declare that they have no conflict of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study.

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