Testing antidepressant compounds in a neuropsychological model of drug action

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Chapter 3

The Effects of ARA290, an Erythropoietin Analogue, on Resting State Networks Associated with Depression: a randomized placebo-controlled trial.

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In preparation

Abstract

Studies on the cognitive and neural effects of Erythropoietin (EPO) indicate that EPO may have antidepressant effects. ARA290 is an EPO-analogue peptide without dangerous hematopoietic side-effects, but it may still have neurotrophic and antidepressant effects. In a previous report, we found that ARA290 may modulate some aspects of cognitive and neural processing. The first aim of this study was to investigate whether ARA290 affects connectivity in the brain, in relation to eight standard networks. The primary networks of interest were the default mode network (DMN) and the executive salience network, known to be involved in MDD. The second aim of this study was to investigate whether ARA290 affects connectivity of the hippocampus, amygdala and/or the fusiform gyrus with other brain regions.

Healthy participants (N=36) received ARA290 (2 mg) or placebo in a double-blind, randomized, parallel-group design. Effects on functional connectivity (resting-state fMRI) were assessed one week after administration. Whole brain analysis revealed that ARA290 did not affect connectivity in relation to the eight standard networks, nor in relation to the seed regions (hippocampus/amygdala and fusiform gyrus) in the current sample of healthy volunteers.

We did not find evidence for an effect of ARA290 on MDD related networks, and thus could not confirm our hypothesis that the effects of ARA290 on functional connectivity contribute to its putative antidepressant effects. Future studies may benefit from higher dosage of ARA290 administration, a shorter time lag between administration and measurements, and a crossover design.

Introduction

The need for new pharmacological treatment options for depression has led to development of compounds that target different molecular pathways. One such compound is Erythropoietin (EPO), a glycoprotein that regulates erythropoiesis. EPO also crosses the blood brain barrier (BBB) and has neuroprotective and neurotrophic effects when delivered in high doses (Brines *et al.*, 2000; Shingo *et al.*, 2001; Gonzalez *et al.*, 2009). Studies have found beneficial effects of EPO on cognitive functions in patients with schizophrenia (Ehrenreich *et al.*, 2007a), multiple sclerosis (Ehrenreich *et al.*, 2007b) and depression (Miskowiak *et al.*, 2010).

The 11-amino acid, linear peptide ARA290 is an EPO analogue and exerts tissue-protective effects but is not a hematopoietic stimulant (Brines *et al.*, 2008). ARA290 exerts anti-inflammatory actions by acting on the innate repair receptor (IRR) (Niesters *et al.*, 2013). Specifically, activation of the IRR initiates multiple signalling pathways initiating tissue-protective actions, one of which is the inhibition of inflammation-induced apoptosis (Brines & Cerami 2012). More importantly, ARA290 does not stimulate erythropoiesis and does not initiate hematopoietic actions, even not after repeated administration (van Velzen *et al.*, 2014).

Given the beneficial effect of ARA290 on cell survival and tissue-protection and the absence of dangerous side effects, we investigated the potential antidepressant effects of ARA290 in a clinical trial in healthy volunteers (Cerit *et al.*, under review), using the neurocognitive model (Harmer *et al.*, 2009). This trial was modelled after the EPO studies and the effects of treatment were assessed one week after ARA290 administration. Some small effects were observed on attentional bias and on the BOLD response to emotional information, but not on the primary outcome measures. Furthermore, ARA290 elicited a larger BOLD response to fearful ***vs*** happy faces in the fusiform gyrus. This is one of the same regions that were sensitive to EPO administration (Miskowiak *et al.*, 2007a); however, this latter effect was not in the expected direction (Cerit *et al.*, under review).

Altered resting state-state connectivity is also suggested to contribute to the pathophysiology of MDD. Several pharmacological studies have investigated the effect of conventional antidepressant drugs on connectivity within the affective networks associated with MDD (Anand *et al.*, 2005; McCabe and Mishor, 2011; van Wingen *et al.*, 2014). These studies have mainly focussed on abnormalities in the cortico-limbic mood regulating circuit (MRC), the default-mode network (DMN) and the task-positive network (TPN) as these have been reported to be altered in depressed patients (see review by Wang *et al.*, 2012). Seven days of either citalopram, reboxetine or placebo was administered to healthy volunteers and a seed based connectivity analysis was conducted. Both citalopram and reboxetine reduced connectivity within the cortico-limbic network (McCabe and Mishor 2011). Two weeks of duloxetine administration in healthy volunteers resulted in reduced DMN and TPN connectivity (van Wingen *et al.*, 2014).

In order to gain a comprehensive understanding of the potential of ARA290 as an antidepressant, we investigated both its effects on cognitive and neural processing of emotional information (Cerit *et al.*, under review) and its effect on resting-state connectivity. This allows us to investigate whether the effects of ARA290 described in the previous report (Cerit *et al.*, under review) are task specific or rather a general effect of ARA290 on these regions. In summary, in the present study we examined the effect of ARA290 on resting state connectivity in the same healthy volunteers as in our previous report (Cerit *et al.*, under review). We employed a dual regression and a seed-based dual regression analysis using seed regions, known to be differentially activated in response to (emotional) stimuli following EPO and/or ARA290 administration compared to placebo (Miskowiak *et al.*, 2007a, 2007b, 2010; Cerit *et al.*, under review). Specifically, the following seeds were identified and compared over the groups: left and right hippocampus, left and right hippocampus-amygdala complex and bilateral fusiform gyrus. The following hypotheses were tested:

a) ARA290 affects connectivity in the brain (in relation to eight standard networks (Beckmann *et al.*, 2005), together covering around 80% of the brain) and specifically the default mode network (DMN) and the executive salience network, known to be involved in MDD.

b) ARA290 affects connectivity of the hippocampus, amygdala and/or the fusiform gyrus with other brain regions.

Methods

Participants

The data were acquired in the same participants as in a previous report, in which detailed information about the participants is outlined (Cerit *et al.*, under review). A total of 36 participants (18-35 years) were recruited and randomly assigned to either placebo or ARA290 condition. The groups did not differ in age, IQ, sex distribution and clinical characteristics (Cerit *et al.*, under review).

Design

We used a randomized, double-blind placebo-controlled, parallel-group design. Randomization was carried out in blocks of six, and was stratified for sex. The study was conducted at the LUMC and randomization was carried out by the LUMC pharmacy, Leiden. The study included two lab visits separated by 6 or 7 days. Participants came into the lab twice and were administered either placebo or ARA290 (2mg) on the first lab visit. During the second visit, which took place one week later, all participants underwent (functional) MRI (fMRI) scanning, both task-related (Cerit *et al.*, under review) and resting state fMRI scanning; both scans were part of one study and were acquired in one scanning session and in the same sample as reported in Cerit and colleagues (under review).

Image acquisition

Imaging data were acquired on a Philips 3.0-Tesla Achieva MRI scanner using a 32-channel SENSE head coil for radiofrequency transmission and reception (Philips Healthcare, Best, The Netherlands). RS-fMRI data were acquired using T2*-weighted gradient-echo echo-planar imaging with the following scan parameters: 200 whole-brain volumes; repetition time (TR) = 2.2 sec; echo time (TE)= 30 ms; flip angle= 80°; 38 axial slices scanned in ascending order; FOV = 220 x 220 mm; voxel size 2.75 x 2.75 x 2.75 mm, plus 10% interslice gap. For registration purposes and normalization to standard space, a high-resolution anatomical image (T1-weighted ultra-fast gradient-echo acquisition; TR=9.76 ms; TE= 4.59 ms; flip angle= 8°; 140 axial slices; FOV= 224 x 177.33 mm; in plane voxel resolution = 0.875 mm x 0.875 mm; slice thickness= 1.2 mm) and a high-resolution T2*- weighted gradient-echo EPI scan (TR= 2.2 sec; TE= 30 ms; flip angle=80°; 84 axial slices; FOV= 220 x 220 mm; in plane voxel resolution= 1.96 x 1.96 mm, slice thickness= 2 mm) were acquired for each participant. In accordance with the Leiden University Medical Center's policy, all anatomical MRI scans were screened by a radiologist to rule out incidental pathology.

Data analysis

Before statistical analysis, all MRI scans were submitted to a visual quality control check to ensure that no gross artefacts were present in the data. Data analysis was performed with Functional Magnetic Resonance Imaging of the Brain Software Library (FSL version 5.0.1, Oxford, United Kingdom, www.fmrib.ox.ac.uk/fslSmith *et al.*, 2004). Anatomical locations were determined using the Harvard-Oxford cortical and subcortical structures atlas integrated in FSL.

Resting-State functional connectivity analysis

Pre-processing of RS-fMRI images was carried out using FEAT (FMRI Expert Analysis Tool) Version 5.98, part of FSL. The following processing steps were applied: motion correction (Jenkinson *et al.*, 2002), brain extraction (Smith, 2002), spatial smoothing using a Gaussian kernel with a full width at a half maximum (FWHM) of 6 mm, grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor and a high-pass temporal filtering equivalent to 100 sec. Time series statistical analysis was carried out with local autocorrelation correction (Woolrich *et al.*, 2001). After pre-processing, the functional images were registered to the corresponding high-resolution echo planar images, which were registered to the T1-weighted images, which in turn were registered to the 2 mm isotropic MNI-152 standard space images (T1 standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada; Jenkinson and Smith, 2001; Jenkinson *et al.*, 2002). The registration parameters were combined to obtain the registration matrix from native space to MNI space and its inverse (from MNI space to native space).

The functional connectivity analysis was performed using the dual regression method of FSL, a technique that allows a voxel-wise comparison of resting-state functional connectivity (previously described in Filippini *et al.*, 2009). First, we performed a model-free analysis of eight standard resting state networks (Beckmann *et al.*, 2005), representing 80% of the total brain volume (Khalili-Mahani *et al.*, 2012). Previously, localized and drug-specific changes in functional brain connectivity have been shown using these networks in resting-state fMRI studies (Cole *et al.*, 2013; Khalili-Mahani *et al.*, 2012; Klumpers *et al.*, 2012; Niesters *et al.*, 2012). Secondly, we analysed the resting state connectivity changes induced by ARA290 in relation to two seeds, namely the amygdala-hippocampus complex (Miskowiak *et al.*, 2010), determined using the Harvard-Oxford cortical and subcortical structures atlas integrated in FSL, and the fusiform gyrus (functional seed, determined by the contrast: Fearful vs Happy in a previous report Cerit *et al., under review* and in line with Miskowiak *et al.*, 2007a).

In both analyses we used a dual regression analysis to create subject specific statistical maps: voxel-wise Z-scores of functional connectivity to each of the eight networks or to the seed regions. We accounted for non-specific and physiological variations by including nuisance variables, corresponding to fluctuations in the deep white matter and cerebrospinal fluid

(Birn, 2012). The statistical maps were then used for voxel-wise inference testing of the effect of ARA290 on functional connectivity with each of these networks or seed regions, using a General Linear Model (GLM) approach as implemented in FSL. Two statistical contrasts were made with regard to changes in functional connectivity between the two groups: ARA290>placebo and placebo>ARA290. Voxel-wise nonparametric permutation testing was performed using FSL-randomise (5000 permutations; Nichols and Holmes, 2001). All statistical maps were Family-Wise Error (FWE) corrected using $p < 0.05$, based on the Threshold-Free Cluster Enhancement (TFCE) statistic image (Smith and Nichols, 2009), applying a minimum cluster size of 80 mm^3.

Results

Voxel-wise comparison of each group of participants separately versus the eight predefined general resting-state networks (Beckmann *et al.*, 2005), using dual regression, revealed that the eight networks were significantly present in both groups of participants (Fig. 1). Figure 1 seems to show some differences between the two groups, for instance the contribution of the cingulate cortex to the auditory and somatosensory system (network c in Fig.1) in the placebo group is not significantly present in the ARA290 group. However, when directly comparing the two groups of participants, no significant differences in functional connectivity in any of the eight networks were found ($p<0.05$, threshold-free cluster enhancement corrected). Thus, the apparent difference between the groups in cingulate cortex contribution to network c (Fig. 1) did not reach significance (it was present in the uncorrected data $p<0.05$). Similarly, when comparing the two groups of participants, we did not find significant differences in functional connectivity in relation to the seed regions, anywhere in the brain.

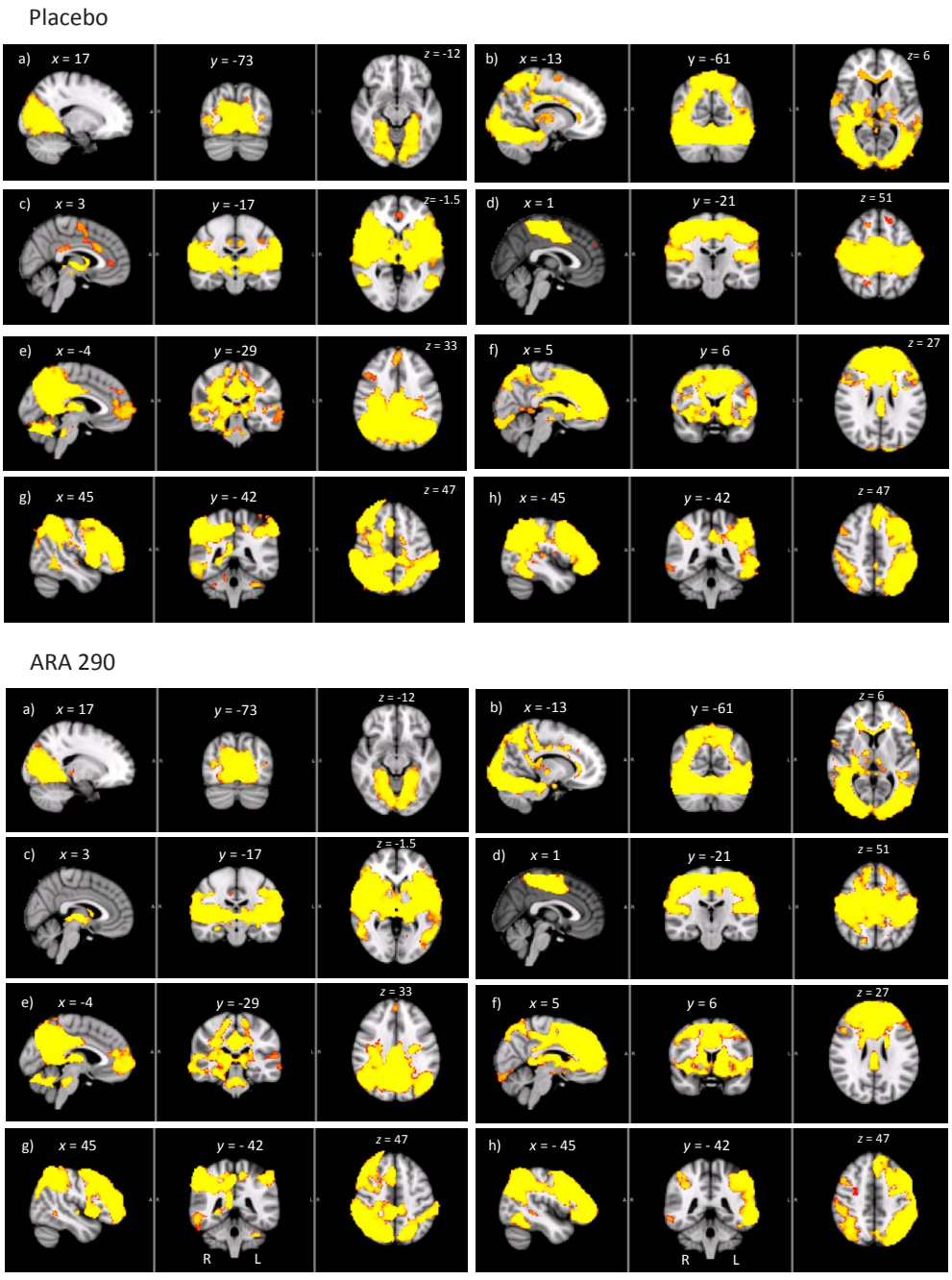

Figure 1. Statistical connectivity maps (P < 0.05, threshold-free cluster enhancement corrected) of the resting-state network connectivity in relation to eight template networks (Beckmann *et al.*, 2005): medial and lateral visual systems (networks a and b respectively), auditory and somatosensory system (network c), sensorimotor system (network d), the default mode network (network e), executive salience network (network f) and visual-spatial and working memory networks (networks g and h), separately for the placebo-group (at the top) and for the ARA290-group (at the bottom).

Discussion

The present study examined the effect of ARA290, an EPO analogue, on resting state connectivity in healthy volunteers using a randomized, double-blind, placebo-controlled, parallel group design. We did not find evidence that ARA290 affects functional connectivity. Specifically, ARA290 did not affect functional connectivity with brain regions that showed effects in the EPO studies, nor with the networks typically affected by antidepressants. Our results indicate that the differential BOLD response of the bilateral fusiform gyrus in response to fearful ***vs*** happy faces after ARA290 administration (Cerit *et al.*, under review), is due to task specific activation rather than a general function of the fusiform gyrus as part of a coherent network. The other seeds included the hippocampus and hippocampus-amygdala complex and were based on ROIs which were found to be differentially activated after EPO administration. Although ARA290 did not have an effect on the hippocampus (or amygdala) during task related BOLD response (Cerit *et al.*, under review), these seeds were of interest since ARA290 was expected to elicit similar effects as EPO (Miskowiak *et al.*, 2007b; 2010). Overall, our findings indicate that the currently used dosage of ARA290 does not seem to exert antidepressant-like effects on resting state connectivity.

A strength of our study is that we had a relatively large sample size and power. Furthermore, participants were matched for age, sex and IQ. We conducted a model-free analysis of eight standard resting state networks (Beckmann *et al.*, 2005) which encompasses approx. 80% of the total brain volume (Khalili-Mahani *et al.*, 2012) and, therefore, ensures an thoroughgoing assessment of the possible effects of ARA290 on functional connectivity. However, this study has some limitations which need to be addressed.

We did not apply retrospective correction on our data in order to exclude the noise originating from physiological confounders (e.g., heartbeat, breathing related chest movement, respiration rates). Since physiological noise influences fluctuations in the MRI signal and as a consequence the estimates of functional connectivity (Birn, 2012), it is conceivable that a possible effect of ARA290 has been drown out by physiological noise.

The lack of effect may have been due to several reasons which may need attention in future studies investigating the effect of ARA290 on resting state connectivity. Since this is the first study assessing the antidepressant properties of ARA290 in humans, selecting the right dose and treatment duration was based on previous studies with pain patients. A single dose of 2 mg might have been too low to exert an antidepressant-like effect in healthy participants and/or the effect of ARA290 may have lasted shorter than one week. We might not have been able to examine the effect of ARA290 with the highest effective dose and the time point on which ARA290 is the most effective. Administration of this dose (2 mg i.v.) to somatic patients (i.e., neuropathic pain patients) raised no safety concerns (Heij *et al.*, 2012; Niesters *et al.*, 2013).

Within this context some pharmacological differences should also be mentioned. EPO was administered as 40 000 IU (equal to 336 micrograms) and has a half-life of approx. 5 hours (Miskowiak *et al.*, 2007a; Eckardt *et al.*, 1989; McMahon *et al.*, 1990) whereas, ARA290 was administered as 2000 micrograms to healthy volunteers and has a plasma half-life of 2 minutes (Niesters *et al.*, 2013). Once the IRR receptor is activated by ARA290 it initiates multiple signalling pathways resulting in tissue protective and anti-inflammatory actions. It is possible though that these effects are not enduring as the effects of EPO, since ARA290 and EPO differ in half-life and the exact signalling pathways they activate.

The antidepressant effects of six week sertraline treatment in MDD patients increased corticolimbic connectivity compared to baseline (at which the connectivity was reduced) (Anand *et al.*, 2005), therefore, it is conceivable that the effect of ARA290 may not have been detected due to its assessment in healthy volunteers i.e., in order to detect an antidepressant effect on connectivity associated with affective networks one may need to asses these specific effects in a patient group. On the other hand, administration of conventional antidepressant drug for a period of one to two weeks in healthy volunteers did elicit an difference between intervention and placebo groups as reduced connectivity in healthy volunteers was reported (McCabe and Mishor 2011; van Wingen *et al.*, 2014). Similar to pharmacological resting-state studies carried out with antidepressant drugs (i.e. including daily administration for at least one week), future resting-state studies with ARA290 may also benefit from repetitive administration (Anand *et al.*, 2005; McCabe and Mishor, 2011; van Wingen *et al.*, 2014) and from a baseline assessment in order to exclude intersubject variability (Anand *et al.*, 2005).

The fMRI study with ARA290 administration in the same healthy population yielded modest to small effects on neural and behavioural measures of emotional processing which was measured by means of the neurophysiological model of drug action (Cerit *et al.*, under review). The finding that ARA290 does not affect functional connectivity in the same healthy population does strengthen the possibility that the effect of ARA290 on task related activity was not affected by randomization failure. Although the neuropsychological model of drug action is validated and assesses depression related emotional processes both at neural as well as behavioural level, the emerging view is that instead of different brain regions activated in response to stimuli, alterations in specific neural circuits may underlie depressive symptoms (Wang *et al.*, 2012). Our finding of ARA290 not eliciting an effect on depression related networks may be interpreted as ARA290 lacking the strength to affect functional connectivity in a way that could contribute to observable/detectable antidepressant effects. Nonetheless, a future resting-state study would benefit from designs used in previous pharmacological resting state studies including baseline measurements (Anand *et al.*, 2005), administration of treatment for at least one week and a crossover design (van Wingen *et al.*, 2014). This may provide us with a better understanding of the effects of ARA290 on functional connectivity in healthy volunteers.

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