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No effect of escitalopram versus placebo on brain-derived neurotrophic factor in healthy individuals: a randomised trial

Knorr U, Koefoed P, Soendergaard MHG, Vinberg M, Gether U, Gluud C, Wetterslev J, Winkel P, Kessing LV. No effect of escitalopram versus placebo on brain-derived neurotrophic factor in healthy individuals: a randomised trial.

Objective: Brain-derived neurotrophic factor (BDNF) seems to play an important role in the course of depression including the response to antidepressants in patients with depression. We aimed to study the effect of an antidepressant intervention on peripheral BDNF in healthy individuals with a family history of depression.
Methods: We measured changes in BDNF messenger RNA (mRNA) expression and whole-blood BDNF levels in 80 healthy first-degree relatives of patients with depression randomly allocated to receive daily tablets of escitalopram 10 mg versus placebo for 4 weeks.
Results: We found no statistically significant difference between the escitalopram and the placebo group in the change in BDNF mRNA expression and whole-blood BDNF levels. *Post hoc* analyses showed a statistically significant negative correlation between plasma escitalopram concentration and change in whole-blood BDNF levels in the escitalopram-treated group.
Conclusion: The results of this randomised trial suggest that escitalopram 10 mg has no effect on peripheral BDNF levels in healthy individuals.

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Keywords: affective disorders; brain-derived neurotrophic factor (BDNF); escitalopram healthy; trial

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Significant outcome

- In a stringently designed randomised, blinded clinical trial following best methodological standards we found no statistically significant differences of escitalopram 10 mg versus placebo for 4 weeks on brain-derived neurotrophic factor (BDNF) messenger RNA (mRNA) expression and whole-blood BDNF levels.

Limitations

- The doses of escitalopram may have been too low and the intervention period too short to fully affect BDNF gene expression and whole-blood concentrations.
- The sample size may have been too small to detect effects of escitalopram.
- The enzyme-linked immunosorbent assay kit we used recognised both proBDNF (precursor of BDNF) and mature BDNF in the blood; however, the kit cannot differentiate between the two forms.

Introduction

The compelling 'neurotrophin hypothesis' was introduced by Duman et al. in 1997 (1). It characterises major depressive disorder as being secondary to aberrant neurogenesis in brain regions that regulate emotion and memory. According to this hypothesis, depression may be associated with a disruption of mechanisms that govern cell survival and neural plasticity in the brain triggered by a complex neuropathological cascade (2). Several biomarkers have been suggested for major depression and among these BDNF seems to be a potential candidate (3,4). BDNF is a member of the neurotrophin family of growth factors and it plays a critical role in cell proliferation, cell differentiation, neuronal protection, and the regulation of synaptic function in the central nervous system (5). Mature BDNF is initially synthesised as a precursor, preproBDNF, which is converted to proBDNF. ProBDNF is cleaved by extracellular proteases to generate mature BDNF, which is able to cross the blood–brain barrier (6). ProBDNF may be associated with the activation of apoptosis (7), but the significance of proBDNF is still unclear.

Regarding studies of BDNF, a recent meta-analysis of 2384 antidepressant-free depressed patients relative to 2982 healthy controls and to 1249 antidepressant-treated depressed patients have confirmed abnormally low concentrations of serum BDNF concentrations in patients with major depression and normalisation of this by antidepressants (8). These findings are believed to reflect peripheral manifestations of the neurotrophin hypothesis, thus low serum BDNF being secondary to an altered expression of BDNF in the brain. However, the meta-analysis found no consistent associations between serum BDNF concentrations and the symptom severity of depression.

Regarding proBDNF, data are still sparse. A recent study showed that serum BDNF, but not serum proBDNF, were significantly lower in patients with depressive disorder than those of healthy controls (9). On the other hand, Zhou et al. (10) found that proBDNF was higher in depressive patients than in healthy controls and further that the balance between proBDNF and BDNF was deregulated in drug-free women with depression when compared with healthy female controls. Levels of BDNF have also been studied in patients with depression in subsequent remission. Thus, a recent study found no difference in BDNF levels between patients recruited from mental health care and primary care that had remitted from depression, and healthy controls (11). In contrast, data from our group showed decreased levels of BDNF in the remitted state of depression in patients recruited from psychiatric hospital care compared with healthy controls, suggesting that neurotrophic changes may exist beyond the depressive state in patients suffering from more severe unipolar depressive disorder (12).

Though the role of BDNF in the pathophysiology of affective disorders remains unclear, it is more clearly established that the efficacy of antidepressants is modulated by BDNF (8,13) and that increased levels of serum BDNF might even be regarded as a biomarker for successful treatment of depression (14). Antidepressants seemingly exert their therapeutic action through their ability to increase the synaptic content of monoamine neurotransmitters; however, the effects on neurotrophic factors, especially BDNF, also seem to play a role (2,15). A study recently investigated serum proBDNF/BDNF and response to the selective serotonin re-uptake inhibitor fluvoxamine in unmedicated first-episode patients with depression and found that no association between serum levels of proBDNF and BDNF during the treatment period (16). Thus, data are conflicting. Regarding peripheral mRNA BDNF, a successful antidepressant response in patients with depression ($n = 74$) was associated with an increase (+48%) in levels of leucocyte mRNA BDNF before and after 8 weeks of treatment with escitalopram or nortriptyline compared with healthy controls ($n = 34$) (17).

However, the potential causal relationship between the depressive state, antidepressant treatment, and BDNF mRNA expression/BDNF levels are still unclear. It is unknown whether antidepressants cause a direct increase in BDNF mRNA expression or BDNF levels resulting in improvement of depressive symptoms, or if improvement in depressive symptoms is simply leading to an increase in BDNF mRNA expression and BDNF levels and hence BDNF being a secondary response to improvement caused by the treatment.

The concept of endophenotypes has been created to facilitate the assessment of factors underlying psychiatric diseases (18). In this context, studies of biomarkers in healthy first-degree relatives to patients with a psychiatric disorder are central. Peripheral BDNF might be regarded a biomarker for the treatment of depression (14), but no prior studies has investigated the effect of antidepressants in a sample of healthy individuals. Thus, to examine the effect of antidepressants on peripheral BDNF levels, and excluding an effect on depression *per se*, we recruited healthy first-degree relatives of patients with depression for the AGENDA trial (associations between genepolymorphisms, endophenotypes for depression and antidepressant intervention) (19,20). The trial is the first to investigate the effect of a 4-week self-administered daily escitalopram versus placebo on peripheral BDNF and mRNA BDNF expression levels in healthy first-degree relatives of patients with

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depression. We tested the hypothesis that a 4-week-long intervention with escitalopram would increase both peripheral BDNF mRNA expression and protein BDNF concentration.

Materials and methods

The AGENDA trial was conducted at the Psychiatric Centre Copenhagen and the Laboratory of Neuropsychiatry, Rigshospitalet, Copenhagen University Hospital as part of the Centre for Pharmacogenomics, University of Copenhagen, Denmark. The trial protocol was published ahead of trial completion and registered at ClinicalTrials.gov: NCT 00386841 (AGENDA) (19). The trial was conducted and monitored in accordance with the Declaration of Helsinki and International Conference on Harmonization for Good Clinical Practice guidelines. The Local Ethics Committee (De Videnskabsetiske Komitéer for Københavns og Frederiksberg Kommuner, Københavns Kommune, H-KF 307413, and HA-2007-0077) and the Danish Data Agency (2006-41-6737 and 2007-41-0962) approved the trial. All participants signed informed consent.

Participants

Trial participants were healthy individuals with a family history of depression who were recruited as healthy adult children or siblings of patients (probands) diagnosed with major depression from psychiatric in- or out-patient hospital contact in Denmark who participated in ongoing studies at the Psychiatric Centre Copenhagen (former Department of Psychiatry, Rigshospitalet) (21). A total of 466 probands gave us permission to contact 359 first-degree relatives, who were the potential participants in the present trial. Of these, 80 individuals were included in the trial (see Fig. 1, CONSORT diagram).

Trial design

The trial was conducted as a participant-, investigator-, observer-, and data-analyst-blinded trial and Copenhagen Trial Unit (CTU), Centre for Clinical Intervention Research, performed the centralised computerised randomisation 1 : 1 by telephone to secure adequate allocation sequence generation and allocation concealment. Randomisation was stratified in blocks of six, by age (18–31 and 32–60 years), and sex. Only the data manager knew the block size.

Interventions

During the trial the participants received either tablet escitalopram 10 mg versus placebo daily for a period of 4 weeks. The manufacturer provided escitalopram and placebo tablets. The tablets were identical in appearance, colour, smell, and solubility allowing for blinding of the assignment to intervention or placebo. An independent pharmacist packed the identically appearing blister packages containing escitalopram or placebo and then sealed and numbered the packages according to a randomisation list provided and concealed by the CTU. On completion of the 4 weeks of intervention, the participants entered a 5-day down-titration period to nil medication. Compliance to the protocol was sought by making weekly telephone calls to the enroled participants. The participants were asked at the end of the trial, if they had missed taking any tablets.

Assessment

Diagnoses were ascertained by the World Health Organization Schedules of Clinical Assessment in Neuropsychiatry interview (22) and The Structured Clinical Interview for DSM-IV Axis II Personality Disorders. Further assessment included information on family history of psychiatric disorders, ratings of mood using the 17-item Hamilton Depression Rating Scale (23), various sociodemographics, stressful life events (24), number of daily cigarettes, height, weight, and routine blood tests.

Blinding

All trial personnel and participants were blinded to the packaging of the trial drug, and blinding was maintained throughout monitoring, follow-up, assessment of outcomes, data management, data analyses, and drawing of conclusions.

Analyses of BDNF mRNA expression

Blood samples were obtained by venipuncture between 10.30 a.m. and 4.15 p.m. and processed for general health screening and total RNA purification. Blood samples for RNA purification were obtained in PAX-gene Blood RNA Tubes (Qiagen, Albertslund, Denmark) to prevent changes and degradation of the mRNA after sampling. Total RNA was purified using PAX-gene Blood RNA kit (Qiagen). The RNA (2 µl) was run on a 1% agarose gel to check for degradation of the sample. Quantification of RNA was calculated using Quant-iT™ RiboGreen® RNA Assay according to the manufacturer's manual (Invitrogen, Carlsbad, CA, USA). Total RNA (500 ng) was reverse transcribed using High Capacity RNA-to-cDNA Master Mix (Applied Biosystems, Foster City, CA, USA). All samples were run in triplex.

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Reverse transcription polymerase chain reaction (RT-PCR): gene of interest (*BDNF*) and reference genes (altogether eight genes and 14 primer sets) were optimised and validated. The gene of interest and five of the reference genes performed well with efficiencies in the range between 96.9% and 99.5%. Only one peak was seen in the melting curve analysis agreeing with only one product being produced. *GAPDH* and *RPLP0* were the less-regulated genes as well as having the best efficiency and dynamic range, thus they were used as reference genes in the analysis. The performance of our instrument was tested using the GAPDH primers and SybrGreen, and it performed within a SD of 0.25, as expected. RT-PCR was performed on complementary DNA (cDNA) in 96-well plates on the 7500HT Fast qPCR (Applied Biosystems) using the Power SYBR® Green PCR Master Mix (Applied Biosystems) according to the manufacturer's manual. The primer sets used were as follows: *BDNF* from RTprimerDB ID 352 (http://medgen.ugent.be/rtprimerdb/) generating a PCR fragment of 76 bp; *GAPDH* from RTprimerDB ID 2036 (http://medgen.ugent.be/rtprimerdb/) generating PCR products of 88 bp; and *RPLP0* from RTprimerDB ID 2507 generating PCR products of 95 bp.

To avoid interplate variation, all genes from each individual (*BDNF, GAPDH*, and *RPLP0*) were run on the same plate, likewise the baseline and the post-intervention samples from the same individual were run on the same plate. Water controls and genomic DNA controls (without the reverse transcriptase) were run simultaneously with the samples on all plates and did not differ significantly from the background. Amplification curves were visually inspected from each assay to set a suitable baseline and threshold level. The cycle threshold (Ct; i.e. the number of cycles necessary for the studied gene to be linearly expanded) for each sample was determined. The investigator was blinded for intervention group (escitalopram or placebo) during the assessment of the Ct value. Relative quantification was achieved by subtracting each Ct sample with the in-plate Ct of the two reference control genes (ΔCt). Finally, ΔCt was calculated for each individual as the post-intervention (placebo or escitalopram) values minus baseline values.

BDNF protein levels

Whole-blood samples were drawn in ethylenediaminetetraacetate-containing tubes, which were immediately frozen and stored at −80°C until assayed. A previous study has shown that whole-blood samples can safely be stored at −20°C for at least 5 years without the risk of a significant decrease in concentrations during the time span (25). The samples were processed with a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) measuring BDNF protein (ChemiKine™ BDNF Sandwich ELISA kit, Chemicon International, CA, USA; and CYT306, Millipore, Billerica, MA, USA) as described previously by our group (12). Processing according to the manufactures instructions was performed on all samples within 1 week by a laboratory technician, who was blinded to the intervention group. A standard curve was generated from the serial BDNF standard dilutions, and BDNF protein levels (ng/ml) in the samples were extrapolated directly from the standard curve BDNF protein levels in the samples and from the standard curve and from internal control samples included on every plate.

Measures of whole-blood BDNF levels in the present sample seem valid as we found no differences in BDNF levels measured in whole blood and in serum in a previously study from our group (26).

The BDNF protein levels were determined with a kit, which is not able to discriminate proBDNF and mature BDNF.

Plasma escitalopram concentration

The extraction and quantitation of escitalopram was carried out on an ASPEC XL combined with a high-pressure liquid chromatography system, both from Gilson, Villiers le Bel, France.

Lower and upper limits of quantitation were 10 and 3600 nmol/l. The interassay coefficients of variation ranged from 5.5% to 8.4% and trueness ranged from 93.2% to 103.0% within the measurement range.

Statistical analysis

Two regression analyses (using the general linear univariate model) were conducted, each including one of the Δ outcomes (Δ BDNF and Δ BDNF mRNA) as the dependent variables and the intervention indicator (escitalopram or placebo) as the independent variable. Two-sided significance test were done at the 0.05 level. Holm's adjustment for multiplicity was used (27).

These analyses were supplemented by exploratory analyses. First, the above regression analyses were repeated (if needed, see below) with one or more design variable(s) (see Table 1) included as additional independent variable(s). Preliminary analyses [each including the primary outcome of the AGENDA trial (20), the treatment indicator, and one design variable] were first done to select the design variables (if any) to be included in the final analysis (if any). If the analysis of a design variable had a p-value ≤ 0.1, the design variable was included

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Table 1. Demographic and clinical characteristics at baseline

Quantity	Escitalopram [$n = 41$, mean (SD)]	Placebo [$n = 39$, mean (SD)]
Age	32.0 (11.0)	31.1 (1.0)
Females (%)	15 (36.6)	14 (35.9)
Number of cigarettes per day	4.9 (8.2)	5.0 (9.4)
Hamilton's Depression Scale Score (baseline)	1.9 (2.0)	1.8 (1.7)
Number of stressful life events	2.5 (1.6)	2.3 (1.3)
Body mass index (baseline)	25.2 (3.7)	26.7 (4.8)
Length of blood storage (days)	427.2 (0.3)	427.2 (0.3)
Period from midnight (h)	12.0 (1.23)	12.3 (1.4)

in the final analysis. In addition to identifying candidate variables for the final analysis, the preliminary analyses also served to identify design variables with a regression coefficient corresponding to a p-value of 0.05 or less and thus hypothesised to be related to the outcome.

In the regression analyses the residuals followed a Gaussian distribution with reasonable approximation. The distributions were reasonably symmetric but several of them deviated significantly from a Gaussian one as judged from the Shapiro–Wilks test. P of Levene's test of variance inhomogeneity was always well above 0.05.

In a *post hoc* analysis confined to the group receiving escitalopram, we examined if each outcome was related to the logarithm of the plasma escitalopram level with a p-value of 0.05 or less. If neither of the two marginal distributions differed significantly from the Gaussian distribution, Pearson's r was used otherwise Spearman's ρ was used as a test quantity. The value of the plasma level was log transformed to normalise the distribution.

Results

A total of 80 participants were randomised. The mean and SD of the continuous baseline variables and the distributions of the protocol-specified stratification variables (sex and age group) in each intervention group are shown in Table 1. The randomisation seems successful. Further, the reasons for non-participation are presented in the CONSORT diagram (Fig. 1). No severe adverse reactions or serious adverse events were observed during the study.

As can be seen from the CONSORT diagram, the data set was not complete since at 4 weeks BDNF was not obtainable for three participants in the escitalopram group and for one participant in the placebo group. Regarding BDNF mRNA, the sample was complete except for two participants in the escitalopram group who only provided baseline blood samples.

At baseline, whole-blood BDNF was 31.9 ng/ml (mean) (SD 15.0) and mean plasma escitalopram at 4 weeks was 50 nmol/l (SD 29).

Effects on peripheral BDNF

Table 2 shows for each intervention group the number of observations, the mean, and the SD of the Δ quantities for BDNF mRNA and BDNF protein concentrations and p of the difference between the intervention groups. There was no statistically significant difference between the escitalopram versus the placebo group in the change of BDNF mRNA expression and whole-blood BDNF levels. The exploratory analysis showed that adding selected baseline variables as covariates in the analyses did not change the results noticeably (see Table 2). The analyses, including one baseline variable at a time as a covariate, showed that sex was significantly related to Δ BDNF protein concentrations ($p = 0.039$, regression coefficient of females with men as reference −4.22, 95% CI −8.2 to −0.21).

A *post hoc* analysis showed that Ln (plasma escitalopram) as measured in the experimental intervention group was significantly correlated with change in whole-blood BDNF protein levels (Pearson's $r = -0.36$, $p = 0.035$).

Discussion

This is the first trial to investigate the effect of an antidepressant on levels of peripheral BDNF in healthy individuals. In contrast with our hypothesis, in this stringently designed randomised, blinded clinical trial we found no statistically significant difference between the escitalopram versus the placebo group regarding the change in BDNF mRNA expression and whole-blood BDNF protein levels. *Post hoc* analysis confined to the escitalopram group showed that increasing plasma escitalopram levels were significantly correlated with decreasing change in BDNF protein levels. These results are thus in direct contrast with our hypothesis that a 4-week-long intervention with escitalopram as compared with placebo would increase both peripheral protein BDNF and BDNF mRNA expression. Further, our *post hoc* analysis showed a negative correlation between plasma escitalopram concentration and change in whole-blood BDNF protein levels. This contrast the findings of a positive correlation between plasma paroxetine levels and BDNF levels in 45 patients treated for major depression (28). We cannot exclude that our finding may be a chance finding.

In the AGENDA trial, we investigated a line of putative biomarkers for depression. As the primary outcome we chose the cortisol response in the

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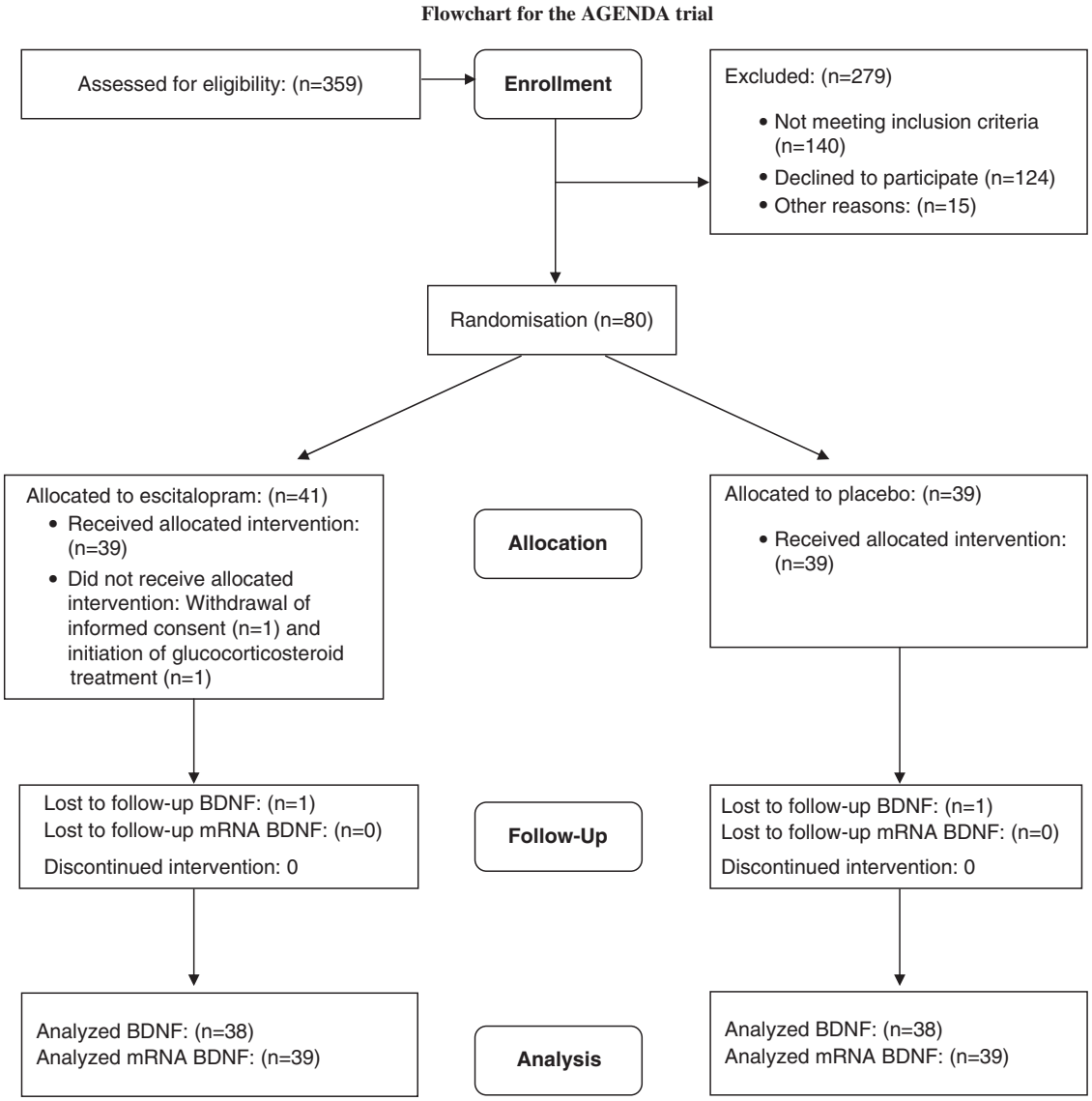

Fig. 1. CONSORT diagram for the associations between genepolymorphisms, endophenotypes for depression and antidepressant intervention (AGENDA) trial. BDNF, brain-derived neurotrophic factor; mRNA, messenger RNA.

Table 2. Comparison of change in brain-derived neurotrophic factor (BDNF) messenger RNA (mRNA) expression and whole-blood BDNF level in healthy individuals treated with escitalopram versus placebo

Quantity	Escitalopram [mean (SD), range]	Placebo [mean (SD), range]	*p* of difference*	Covariates with $p < 0.1$†	*p* of difference with covariates included
Δ BDNF ($n = 38$)	0.10 (8.40), 40.1	−1.04 (8.75), 58.0	0.56	Number of stressful life events, sex	0.48
Δ BDNF mRNA ($n = 39$)	0.038 (1.95), 12.0	−0.042 (1.62), 8.1	0.84	None	–

* Only the intervention indicator was included in the analysis as an independent variable.

† The covariates included were those that were significant ($p \leq 0.1$) in an univariate general linear analysis, including covariate and the intervention indicator as the only two independent variables.

dexamethasone corticotropin-releasing hormone (DEX-CRH) test in healthy first-degree relatives to patients with depression (29). The primary outcome was the intervention difference in the change of the total area under the curve ($CorAUC_{total}$) for plasma cortisol in the DEX-CRH test at entry to after 4 weeks

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of intervention. Change in $CorAUC_{total}$ showed no statistically significant difference between the escitalopram and the placebo group ($p = 0.47$). Overall, the conclusion was that the present trial did not support an effect of escitalopram 10 mg daily compared with placebo on the hypothalamic–pituitary–adrenal (HPA) axis in healthy first-degree relatives to patients with depression (20). In contrast, we found that a long-term escitalopram administration to healthy participants resulted in a decrease in the HPA activity measured by salivary cortisol compared with inert placebo (30). Furthermore, we found no effect of escitalopram versus placebo on inflammatory outcomes in healthy individuals (31).

A new hypothesis regarding depression was presented by Cai et al. (2) in their recent review who state that it is important to have an integrated view of the mechanisms underlying depression. Genetic and stress vulnerabilities interplay to initiate a cascade of neurobiological changes that disrupt a dynamic system. In short, the authors state that stress factors trigger extensive activation of the HPA axis and that the up-regulation of glucocorticoid release suppresses BDNF expression, leading to hypofunction of BDNF. Further, glucocorticoid stimulate the macrophage migration inhibitory factor, which has been found to be a key intermediate that links the activities of inflammatory cytokines and the HPA axis (32). The participants of the AGENDA trial had an increased hereditary risk for depression as they had a first-degree relative with depression. They were, however, fully healthy with very low ratings of depressive symptoms. Their putative hereditary vulnerability was affected by escitalopram regarding salivary cortisol but not regarding the other examined potential endophenotypes for depression. This may suggest that an over-activation of the HPA axis could be a primary biological step in the pathway to depression. Furthermore, as we observed no anti-inflammatory effect and no effect on peripheral BDNF, the immune system activation and BDNF attenuation may be occurring during the final pathways towards depression. Our data support that levels of BDNF are affected by antidepressants only during the presence pathology hence, during depression.

Limitations

It is possible that the used doses of escitalopram have been too low and that the intervention period has been too short to see the full effect on BDNF gene and protein levels in our trial. However, a daily dose of 10 mg escitalopram during a 4-week-period of treatment would be expected to have an effect in patients with depression (33). Furthermore, the sample size may have been too limited for detecting differences in BDNF outcomes. However, as we did not find any tendency towards a difference it is less likely that a greater sample size would have changed our results. Finally, we used the ELISA kit for measurement of BDNF protein. This ELISA kit recognised both proBDNF (precursor of BDNF) and mature BDNF in blood (34) and we cannot provide data that differentiate between the two forms and unfortunately the results of this trial cannot add information regarding the interplay between proBDNF and mature BDNF.

In conclusion, the results of this randomised trial suggest that escitalopram 10 mg has no effect on peripheral BDNF levels in healthy individuals.

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Conflicts of Interest

U.K. has been a speaker for Servier and a consultant for AstraZeneca. M.V. has been a consultant for Eli Lilly, Lundbeck, and Servier. L.K. has been a consultant for Lundbeck and AstraZeneca during the recent 3 years.

Ethical Standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 2008.

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