Brazilian Journal of Psychiatry. 2019 Mar-Apr;41(2):168–178
Brazilian Psychiatric Association
Revista Brasileira de Psiquiatria
 I doi:10.1590/1516-4446-2018-0005

REVIEW ARTICLE

Anxiolytic properties of compounds that counteract oxidative stress, neuroinflammation, and glutamatergic dysfunction: a review

Patrícia Santos, Ana P. Herrmann, Elaine Elisabetsky, Angelo Piato

Programa de Pós-Graduação em Farmacologia e Terapêutica, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil.
APH https://orcid.org/0000-0002-0330-0741, EE https://orcid.org/0000-0002-9922-2863, AP https://orcid.org/0000-0001-5109-7306

Objective: Anxiety disorders are highly prevalent and the efficacy of the available anxiolytic drugs is less than desired. Adverse effects also compromise patient quality of life and adherence to treatment. Accumulating evidence shows that the pathophysiology of anxiety and related disorders is multifactorial, involving oxidative stress, neuroinflammation, and glutamatergic dysfunction. The aim of this review was to evaluate data from animal studies and clinical trials showing the anxiolytic effects of agents whose mechanisms of action target these multiple domains.
Methods: The PubMed database was searched for multitarget agents that had been evaluated in animal models of anxiety, as well as randomized double-blind placebo-controlled clinical trials of anxiety and/or anxiety related disorders.
Results: The main multitarget agents that have shown consistent anxiolytic effects in various animal models of anxiety, as well in clinical trials, are agomelatine, N-acetylcysteine (NAC), and omega-3 fatty acids. Data from clinical trials are preliminary at best, but reveal good safety profiles and tolerance to adverse effects.
Conclusion: Agomelatine, NAC and omega-3 fatty acids show beneficial effects in clinical conditions where mainstream treatments are ineffective. These three multitarget agents are considered promising candidates for innovative, effective, and better-tolerated anxiolytics.

Keywords: Anxiety; agomelatine; N-acetylcysteine; omega-3 fatty acids

Introduction

Anxiety has been defined as a state of high arousal and enhanced vigilance in the absence of immediate threat.[1] It is characterized by subjective experiences (such as persistent worry and tension) in addition to physiological changes (such as sweating and increased heart rate). Though healthy individuals may present sporadic anxiety, it becomes pathological if persistent, disruptive, and disproportionate.[2] Anxiety disorders have global lifetime prevalence rates as high as 28%,[3] and include social phobia, panic disorder, agoraphobia, and generalized anxiety disorder (GAD).[4] Though obsessive-compulsive disorders (OCD) and posttraumatic stress disorder (PTSD) present marked anxiety symptoms, the DSM-5 categorizes these conditions as obsessive-compulsive and related disorders and trauma and stressor-related disorders, respectively.

In addition to drug therapy, the current treatment of anxiety disorders involves lifestyle interventions, such as physical exercise and mindfulness-based stress reduction, as well as psychological interventions, such as cognitive behavioral therapy, which are difficult to implement. The main drug classes used to treat anxiety disorders are GABAergic or serotonergic agents, including benzodiazepines (BZD), 5-HT_{1A} serotonin receptor agonists, and selective serotonin reuptake inhibitors (SSRIs).[5] Unfortunately, however, not all patients respond to the available medications.[6] Moreover, BZDs and SSRIs are associated with unwanted adverse effects, including sedation, memory deficits, dependence, withdrawal syndrome, sexual dysfunction, and weight gain.[5] While these adverse effects decrease adherence to treatment, the better-tolerated 5-HT_{1A} agonist buspirone has the slowest onset of action and its efficacy is limited to GAD.[7,8]

Despite its high prevalence, few effective therapeutic targets have been identified for anxiety disorders. The expectation that highly selective agents acting on specific molecular targets would yield better and safer psychiatric drugs has not yet been met.[9] A newer approach involving multi-targeted agents [10,11] recognizes the complex pathophysiology underlying psychiatric disorders. In anxiety disorders, oxidative stress,[12-14] neuroinflammation,[15] and glutamatergic hyperactivity[16-18] are now recognized as key contributing factors.

Correspondence: Angelo Piato, Programa de Pós-Graduação em Farmacologia e Terapêutica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Av. Sarmento Leite, 500, sala 305, CEP 90050-170, Porto Alegre, RS, Brazil,
E-mail: angelopiato@ufrgs.br
Submitted Sep 25 2017, accepted Jan 31 2018, Epub Oct 11 2018.

How to cite this article: Santos P, Herrmann AP, Elisabetsky E, Piato A. Anxiolytic properties of compounds that counteract oxidative stress, neuroinflammation, and glutamatergic dysfunction: a review. Braz J Psychiatry. 2019;41:168-178. http://dx.doi.org/10.1590/1516-4446-2018-0005

Anxiety and neurochemical damage

Glutamatergic hyperactivity, a key feature in brain injuries, triggers a complex chain of events, including oxidative stress, mitochondrial dysfunction, and cellular signaling that result in inflammatory response and/or cell death.[19,20] Since glutamatergic hyperactivity is characteristic of anxiety,[17,18] oxidative stress and neuroinflammation are relevant.

Abnormalities in glutamate neurotransmission are among the biological mechanisms underlying stress response and anxiety disorders.[17] Anxiety disorders seem to result from a hyperactive glutamatergic system deregulating inhibitory/excitatory balance in the brain.[16,18] Metabotropic glutamatergic 2/3 ($mGlu_{2/3}$) receptors stand out as a potential target for anxiety-modulating drugs (Pitsikas).[16] Presynaptically located, $mGlu_{2/3}$ receptors are present in several brain areas where glutamate hyperactivity is associated with anxiety, including the cortex, thalamus, striatum, amygdala, and hippocampus.[21,22] The activation of $mGlu_{2/3}$ receptors limits neuronal glutamate release,[23] and agonists of such receptors show anxiolytic activity in diverse animal models of anxiety.[16]

An association between anxiety and oxidative stress has been documented in rodents and humans. Hovatta et al.[24] found a positive correlation between glyoxalase I and glutathione reductase I gene expression and anxiety phenotypes on stress-related behaviors in isogenic mice. Overexpression of the glyoxalase I gene has also been reported for naturally anxious mice.[25] Bouayed et al.[26] reported a positive correlation between markers of peripheral oxidative stress and anxious behavior in mice. Increased anxiety-like behavior accompanied by oxidative stress has been documented in rodents exposed to psychological stress,[27] chronic restraint stress,[28] and oxidative stress inducers.[29-31] Changes in antioxidant defenses and elevated lipid peroxidation products have been reported in GAD,[32-34] OCD,[35-39] panic disorder,[40] and social phobia.[41,42] Anxious women were found to have a lower total antioxidant capacity in the blood than controls.[43]

Associations between deregulation of the hypothalamic pituitary adrenal axis (HPA) and anxiety disorders are widely recognized, resulting in changes in the levels of pro- and anti-inflammatory cytokines and cortisol.[15,44] Inflammatory cytokines and immune cells can access the brain and alter behavior, including the synthesis, release, and reuptake of neurotransmitters such as glutamate, serotonin, and dopamine, which are affected by cytokines and their signaling pathways.[45] The kynurenine pathway is also activated by cytokines, generating neuroactive metabolites that influence dopamine and glutamate transmission and, by depleting tryptophan, regulate the synthesis of serotonin.[45]

Increased peripheral cytokine expression is associated with increased anxiety in mice.[46,47] Mice overexpressing interleukin (IL)-6 or tumor necrosis factor (TNF) exhibit an anxiogenic phenotype.[48,49] Several human studies have also shown a correlation between anxiety, neuroinflammation, and the immune system.[15,44] Injection of the immune activator lipopolysaccharide (LPS) induced anxiety symptoms in normal volunteers,[50] and a positive correlation between anxiety and increased levels of inflammatory markers (such as TNF-α and IL-6) has been repeatedly documented in anxiety disorders.[15,43,51,52]

Strategies to minimize and/or counteract the damage resulting from these accompanying neurochemical processes may lead to innovation in the field of anxiolytic drug research. As a key step in translational research is target validation, the aim of this study is to review drug candidates known to counteract oxidative stress, neuroinflammation, and glutamatergic hyperfunction that have undergone preclinical and clinical analyses relevant to anxiety disorders.

Methods

The PubMed database was searched through March 2017. The search strategy used successive combinations of the following terms (compounds whose multi-target mechanisms of action have been well-established in the literature, including modulation of oxidative stress and/or neuroinflammation and/or glutamate hyperactivity): ascorbic acid, vitamin C, vitamin A, vitamin E, tocopherol, vitamin D, polyphenols, flavonoids, $mGlu_{2/3}$ modulator, melatonin, agomelatine, N-acetylcysteine, omega-3 fatty acids, omega-3 polyunsaturated fatty acids (PUFA) AND anxiety. The results were initially limited to clinical trials. The criterion for a compound's inclusion in this review was evidence of anxiolytic effects in both randomized double-blind placebo-controlled clinical trials and animal models. When no such studies were found for a given compound, it was excluded from further analysis. For compounds that had been tested in clinical trials, we also carried out searches for the compound AND each of these conditions (which are classified as anxiety disorders or have a strong relation with anxiety-related symptoms): generalized anxiety disorder, social phobia, specific phobia, panic disorder, obsessive-compulsive disorder, post-traumatic stress disorder, trichotillomania, nail biting, and excoriation (skin-picking) disorder. The publications were assessed for relevance to the selected topics. The search was limited to texts in English. To select articles for inclusion, all the abstracts found using the search criteria were read.

Results and discussion

We found that agomelatine, N-acetylcysteine (NAC), and omega-3 PUFA are the main agents fitting the inclusion criteria that have demonstrated antioxidant, anti-inflammatory, and glutamatergic effects.

Agomelatine

Agomelatine, a synthetic analog of melatonin, is a high-affinity agonist of MT_1 and MT_2 melatonin receptors.[53,54] Agomelatine antagonizes 5-HT_{2C} serotonin receptors, an effect thought to be involved in its anxiolytic effects.[55] Agomelatine also modulates glutamate neurotransmission in regions associated with mood and cognition, such as the prefrontal and frontal cortex,[55] the hippocampus,

and the amygdala.[56] In rats submitted to prenatal restraint stress, agomelatine blocked the stress-induced glutamate release in the prefrontal cortex[57] and regularized glutamate release and the expression of $mGlu_{2/3}$ receptor mRNA in the hippocampus.[58]

Agomelatine decreased lipid peroxidation levels and nitrite contents in the brains of mice submitted to chemically induced seizures[59] and protected cultured PC-12 neuronal cells from cytosolic reactive oxygen species production, as well as increased glutathione.[60]

Agomelatine was able to reduce LPS-induced upregulation of proinflammatory cytokines IL-6 and IL1-β both within and outside rat brains. These effects were accompanied by inhibition of nuclear factor kappa B (NF-κB) translocation and microglia activation.[61] Microglia are resident macrophages normally present in the healthy brain that perform active tissue scanning and can respond quickly to any microenvironment change.[62] Agomelatine also modified the expression of enzymes associated with the kynurenine pathway, possibly protecting the brain from the neurotoxic consequences of the conversion of kynurenine to quinolinic acid, an N-methyl-D-aspartate (NMDA) receptor agonist.[61]

Though the antidepressant properties of agomelatine have been better characterized,[63] its anxiolytic effects have been reported in different animal models[58,64-66] (Table 1). In most animal studies, agomelatine's anxiolytic effects were documented after acute administration. However, Morley-Fletcher et al. reported that agomelatine administered for 3 or 6 weeks prevented prenatal restraint stress (in the elevated plus-maze) as well as reversed the reduced hippocampal levels of $mGlu_{2/3}$ and $mGlu_5$ receptors in rats.[58] These effects were restricted to rats submitted to restraint stress, which suggests that agomelatine modulation of $mGlu_{2/3}$ receptors may be especially relevant in stressed subjects.

Most of the available clinical data on agomelatine as an anxiolytic refer to GAD patients and were published by the same group. The first clinical trial was published in 2008 (Table 2), in which GAD patients (comorbidity free) were randomized to receive agomelatine or placebo for 12 weeks.[65] This randomized double-blind placebo controlled trial (RDBCT) revealed that agomelatine (25-50 mg/day) was superior to placebo in the primary outcome (Hamilton Anxiety Rating Scale), as well as secondary outcome measures (clinical response, insomnia, and associated disability). In this study, agomelatine was well tolerated and discontinuation symptoms were lower in agomelatine than placebo patients.[80] An open-label study with agomelatine 25-50 mg/day for 16 weeks followed by a multicenter RDBCT (with the same doses of agomelatine) for 26 weeks was conducted to evaluate long-term tolerability to agomelatine and its efficacy in preventing relapse. The results showed that agomelatine was well tolerated and superior to placebo in preventing relapse.[81] A third trial compared agomelatine with escitalopram and placebo. The multicenter RDBCT showed that agomelatine and escitalopram were comparable regarding improved symptomatology, but escitalopram had a higher incidence of adverse events than placebo.[82] A recent trial evaluated the minimal effective optimal dose of agomelatine in GAD patients: the 12-week multicenter RDBCT showed that 10 and 25 mg/kg are better than placebo, and the best response was obtained with 25 mg.[83]

Data on other anxiety disorders are very limited and present too many confounding factors to allow meaningful conclusions.[99] Stein et al. reviewed data from three placebo-controlled short-term trials[100-102] and three comparative studies of agomelatine vs. the SSRI antidepressants venlafaxine,[103] fluoxetine,[104] and sertraline[105] in major depression patients with anxiety symptoms, finding that agomelatine had a greater effect on anxiety symptoms than placebo or antidepressants.[106]

Adverse events reported with agomelatine are mostly perceived as mild to moderate and include headache, dizziness, somnolence, fatigue, and gastrointestinal symptoms.[107] Elevation of liver transaminase levels and rare cases of hepatic failure were seen only with 50 mg/day.[107] The use of agomelatine was not associated with discontinuation symptoms,[108,109] a relevant aspect considering its beneficial effects on sleep disturbances observed in patients with depression and/or anxiety.[80,82]

NAC

NAC is a precursor of cysteine (required for the production of the primary endogenous antioxidant glutathione) and can directly sequester oxidants.[110] NAC supplementation results in additional cysteine, which activates the cystine/glutamate antiporter (also called x[c]-system), predominantly expressed by astrocytes in the brain. The cysteine dimer, cystine, is taken up by astrocytes and exchanged for glutamate, which activates $mGluR_{2/3}$ receptors on presynaptic neurons and reduces the synaptic release of glutamate.[110]

NAC has anti-inflammatory properties as result of multiple mechanisms. Through its direct antioxidant effect and as a glutathione (GSH) precursor, NAC inhibits the activation of the proinflammatory transcription factor NF-κB, which downregulates the expression of several proinflammatory genes.[111-113] Microglia inhibition also seems to be important in NAC's ability to reduce neuroinflammation.[114,115] Therefore, by stimulating GSH synthesis and regulating the cystine/glutamate antiporter, glutamate excitotoxicity, and oxidative stress, NAC inhibits microglia, macrophage activation, and the production of cytokines and oxidative species.[114,116]

The anti-inflammatory properties of NAC have been documented in animal models of ischemic and traumatic brain injury,[117-119] LPS-induced pulmonary edema,[120] and lethal endotoxemia.[121] In humans, NAC has reduced lung inflammation (Blackwell et al.[122]) decreased proinflammatory cytokines in burn[123] and dialysis patients,[124] and caused a reduction of proinflammatory cytokines, as well as shown antioxidant effects in cardiac injury after aortic aneurysm repair.[125]

Egashira et al. found that acute NAC (but not α-tocopherol) inhibited marble-burying behavior in mice (Table 1), suggesting that this anxiolytic-like effect is related to glutamate modulation rather than antioxidant effects.[67] Chen et al. showed that NAC reversed valproate-induced social interaction deficit and anxiety-like behavior in rats

Table 1 Anxiolytic-like effects of mult-itarget compounds: preclinical studies

Compound/dose	Treatment duration	Species	Behavioral tests	Effects	Reference
Agomelatine					
2.5-80 mg/kg, i.p.	Acute	Rats	EPM , SI, UV, VCT	Anxiolytic	Millan et al.[65]
10-75 mg/kg, i.p.	Acute	Rats	Conditioned footshock-induced UV, EPM, VCT,	Anxiolytic	Papp et al.[66]
20-40 mg/kg, i.p.	Acute	Rats	EPM, NIH, PD, SSWS	Anxiolytic in the EPM	Loiseau et al.[64]
40-50 mg/kg, i.p.	Chronic	Rats	EPM, FST	Prevented prenatal restraint-induced anxiety-like behavior in the EPM	Morley-Fletcher et al.[58]
NAC					
50 mg/kg, i.p.,	Acute	Mice	MBB	Inhibited marble-burying behavior	Egashira et al.[67]
150 mg/kg, i.p.	10 days	Rats	EPM, OF, SI	Reversed valproate-induced anxiety-like behavior and social interaction deficit	Chen et al.[68]
30 or 60 mg/kg, i.p.	11 days	Mice	HB, SP	Prevented rhythm disruption-induced anxiety in the HB	Pilz et al.[69]
0.1, 1.0 and 10 mg/L of tank water	Acute	Zebrafish	L/D, NT	Anxiolytic in the L/D, prevented acute stressor-induced anxiety-like behavior in NT	Mocelin et al.[70]
60-150 mg/kg, i.p.	Acute and subacute (4 days)	Mice	ETM, HB, L/D, OF, SI, SIH	Anxiolytic (except at the elevated T-maze).	Santos et al.[71]
Omega-3					
Diet supplemented with DHA	Chronic	Mice	OF, L/D, MWM	Anxiolytic in the L/D	Carrié et al.[72]
Diet supplemented with different combinations of omega-3 PUFA	Chronic	Rats	EPM, OF	Attenuated i.c.v. IL-1 beta-induced anxiety.	Song et al.[73]
Diet supplemented with different proportions of ethyl-EPA	Chronic	Rats	EPM, OF	Attenuated the i.c.v. IL-1 beta-induced anxiety	Song et al.[74]
Diet supplemented with EPA + DHA	Chronic	Rats	EPM, modified FST, MWM	Counteracted restraint-induced anxiety	Ferraz et al.[75]
Diet supplemented with long-chain omega-3 PUFA	Chronic	Grey mouse lemur (*Microcebus murinus*)	OF	Anxiolytic	Vinot et al.[76]
Diet supplemented with EPA + DHA	Chronic	Rats	Avoidance conditioning, EPM	Prevented restraint stress-induced anxiety	Pérez et al.[77]
10:1 omega-6/omega-3 diet supplemented with DHA for three generations	Chronic	Mice	EPM, OF	Anxiolytic in third generation male offspring	Jašarević et al.[78]
Diet supplemented with long-chain omega-3 PUFA	Chronic	Grey mouse lemur (*Microcebus murinus*)	OF, Barnes maze	Anxiolytic	Pifferi et al.[79]

DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; EPM = elevated plus maze; ETM = elevated T-maze; FST = forced swim test; HB = hole-board; i.c.v = intracerebroventricular; IL = interleukin; i.p. = intraperitoneal; L/D = light/dark; MBB = marble-burying behavior; MWM = Morris water maze; NAC = N-acetylcysteine; NIH = novelty-induced hypophagia; NOR = novel object recognition test; NT = novel tank; OF = open field; PD = punished drinking test; PUFA = polyunsaturated fatty acid; SI = social interaction; SIH = stress-induced hyperthermia; SP = social preference; SSWS = safety signal withdrawal schedule (operant conflict procedure); UV = ultrasonic vocalization test; VCT = vogel conflict test.

Table 2 Anxiolytic effects of multitarget compounds: clinical trials

Compound/disorder	Study design	Study size	Daily dose and treatment duration	Main measures/ instruments	Results	Reference
Agomelatine						
GAD	RDBCT	121	25-50 mg, 12 weeks	CGI, HARS, LSEQ, SDS	Anxiolytic	Stein et al.[80]
GAD	Open-label treatment followed by a multicenter RDBCT	477	25-50 mg, 16 weeks (open-label) followed by 26 weeks (RDBCT)	CGI, DESS, HAD, HARS, LSEQ, SDS	Anxiolytic and well-tolerated in long-term treatment. Superior to placebo in preventing relapse.	Stein et al.[81]
GAD	Multicenter, RDBCT	412	25-50 mg, 12 weeks	CGI, HADS, LSEQ, SDS	Anxiolytic effect similar to escitalopram, with lower adverse events incidence.	Stein et al.[82]
GAD	RDBCT	412	10-25 mg, 12 weeks	HARS	Anxiolytic, placebo-agomelatine difference greater with the higher dose.	Stein et al.[83]
NAC						
TTM	RDBCT	50	1,200-2,400 mg, 12 weeks	CGI, HARS MGH-HPS, PITS	Reduced hair- pulling	Grant et al.[84]
OCD (refractory to SRI)	RDBCT	39	Initially 600 mg, doubling weekly to a maximum dose of 2,400 mg (add-on treatment to SRI), 12 weeks	CGI-S, Y-BOCS	Improved mean CGI-S and Y-BOCS scale scores	Afshar et al.[85]
Chronic nail biting	RDBCT	25	800 mg, 2 months	Nail length	Decreased nail biting over the short term	Ghanizadeh et al.[86]
OCD	RDBCT	44	3,000 mg (add-on treatment), 16 weeks	Y-BOCS	Decreased Y-BOCS score	Sarris et al.[87]
PTSD and SUD	RDBCT	35	2,400 mg, 8 weeks	CAPS, PCL-M, VAS	Improved PTSD and craving	Back et al.[88]
Skin-picking disorder	RDBCT	53	1,200-3,000 mg, 12 weeks	Measures of skin-picking severity: CGI-S and modified Y-BOCS	Decreased skin-picking	Grant et al.[89]
OCD	RDBCT	44	2,000 mg (add-on treatment to fluvoxamine), 10 weeks	Y-BOCS	Decreased scores in Y-BOCS	Paydary et al.[90]
Omega-3						
Test anxiety	Placebo controlled trial	126	90 mg of α-linolenic acid (omega-3) and 360 mg of linoleic acid (omega-6 fatty acid), 3 weeks	Standardized rating scale	Improved variables associated with test anxiety	Yehuda et al.[91]
SUD	RDBCT	24	3 g, 3 months	Modified version of the POMS (baseline and monthly)	Decreased anxiety scores progressively	Buydens-Branchey & Branchey[92]
SUD	RDBCT	22	3 g, 3 months	Modified version of POMS	Decreased anxiety scores	Buydens-Branchey et al.[93]
Healthy young adults	RDBCT	68	2.5 g, 12 weeks	BAI, CES-D	Decreased anxiety	Kiecolt-Glaser et al.[94]
Alcoholic patients	RDBCT	31	60 mg EPA + 252 mg DHA, 3 weeks	PSS	Decreased anxiety/stress	Barbadoro et al.[95]

Continued on next page

Table 2 (*continued*)

Compound/disorder	Study design	Study size	Daily dose and treatment duration	Main measures/ instruments	Results	Reference
Early postmyocardial infarction	RDBCT	52	1 g + standard pharmacotherapy, 1 month	BDI, ESQ, STAI-S, STAI-T, used at the baseline (3rd day of acute myocardial infarction) and after one month	Decreased anxiety (STAI-S)	Haberka et al.[96]
PMS	RDBCT	124	2 g, 3 months	VAS	Decreased anxiety severity and duration	Sohrabi et al.[97]
Japanese accident survivors (at risk for developing PTSD)	RDBCT	83	1,470 mg DHA + 147 mg EPA, 12 weeks	Monitoring of heart rate and skin conductance, script-driven imagery of their traumatic event	Decreased heart rate	Matsumura et al.[98]

BAI = Beck Anxiety Inventory; BDI = Beck Depression Inventory; CAPS = Clinician Administered PTSD Scale; CES-D = Center for Epidemiological Studies Depression Scale; CGI = Clinical Global Impression Scale; CGI-S = Clinical Global Impression - Severity of Illness; DESS = Discontinuation Emergent Signs and Symptoms checklist; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; ESQ = Emotional State Questionnaire; GAD = generalized anxiety disorder; HAD = Hospital Anxiety and Depression Scale; HARS = Hamilton Anxiety Rating Scale; LSEQ = Leeds Sleep Evaluation Questionnaire; MGH-HPS = Massachusetts General Hospital Hair Pulling Scale; NAC = N-acetylcysteine; OCD = obsessive-compulsive disorder; PCL-M = PTSD Checklist-Military; PITS = Psychiatric Institute Trichotillomania Scale; PMS = premenstrual syndrome; POMS = Profiles of Mood States; PSS = Perceived Stress Scale; PTSD = posttraumatic stress disorder; PUFA = polyunsaturated fatty acid; RDBCT = randomized double-blind placebo-controlled trial; SDS = Sheehan Disability Scale; SRI = serotonin reuptake inhibitor; STAI-S = State-Trait Anxiety Inventory in a Specific Situation; STAI-T = State-Trait Anxiety Inventory as a General Trait; SUD = substance use disorder; TTM = trichotillomania; VAS = Visual Analog Scale; Y-BOCS = Yale-Brown Obsessive-Compulsive Scale.

(considered an experimental model of autism). The effects were that $mGlu_{2/3}$ receptor antagonist LY341495 blocked dependent $mGLU_{2/3}$ receptors. Accordingly, NAC also reduced enhanced presynaptic excitatory neurotransmission to normal levels in the amygdala of valproate-exposed rats.[68] NAC prevented rhythm disruption-induced anxiety in mice.[69] The anxiolytic effects of NAC have also been documented in the light/dark model and in stress-induced anxiety behavior in zebrafish,[70] as well as in the hole-board test, the light/dark model, the open field test, social interaction, and stress-induced hyperthermia in mice.[71] One report suggested that NAC may have anxiogenic properties, but this conclusion is questionable since it was based only on decreased time spent in the center of an open field, which was also accompanied by decreased locomotion.[84]

Very few studies have been designed to evaluate the specific effects of NAC in patients diagnosed with an anxiety disorder. Back et al. conducted a study with NAC in veterans diagnosed with comorbid PTSD and substance use disorder (SUD) in which the patients were treated with NAC or placebo, along with group cognitive-behavioral therapy. NAC decreased self-reported and clinician-rated PTSD symptoms, and the symptoms remained significantly lower after the drug was discontinued at one-month of follow-up. Patients receiving NAC also reported decreased cravings.[88]

NAC has been evaluated in obsessive-compulsive and related disorders where anxiety is a key component. An RDBCT conducted by Grant et al. revealed significant improvement in trichotillomania patients after 12 weeks of treatment[126]; this result was substantiated by a number of case reports.[127-129] In pediatric trichotillomania patients, an RDBCT found no significant reduction in hair pulling compared to placebo[130]; in this trial, the authors suggested that the improvement was associated with psychoeducation about trichotillomania rather than drug treatment, since significant improvement in several measures of hair pulling was observed regardless of treatment time. A series of case reports showed that NAC is effective against excoriation disorder,[127,131-133] and an RDBCT concluded that NAC significantly reduced skin-picking symptoms.[89] Berk et al. found a reduced nail biting frequency in three patients enrolled in a bipolar disorder treatment protocol with NAC.[134] Ghanizadeh et al.'s finding that NAC decreases nail biting in the short, but not the long term is somewhat questionable considering the lower dose and shorter treatment time used in their RDBCT compared to the case reports.[86]

It has been reported that NAC has beneficial effects in children, adolescents, and adults with OCD.[135,136] Afshar et al. conducted an RDBCT with NAC as an add-on treatment in OCD patients refractory to SSRIs, finding that NAC improved Yale-Brown Obsessive-Compulsive Scale (Y-BOCS) and Clinical Global Impression-Severity of Illness (CGI-S) scores, but not those on the Clinical Global Impression–Improvement Scale (CGI-I); full responders at the end of the study were significantly higher than placebo.[85] Sarris et al. conducted an RDBCT using NAC as add-on treatment (mainly to SSRIs) in OCD patients; the primary outcome measure was the Y-BOCS,

conducted every 4 weeks. At week 12 there was a significant reduction in Y-BOCS score, but the difference dissipated at week 16.[87] A third RDBCT was performed with moderate-to-severe OCD patients, randomized to receive fluvoxamine plus placebo or fluvoxamine plus NAC. NAC showed a significant effect on Y-BOCS score.[90]

Omega-3

Adequate dietary levels of PUFA, including omega-3 fatty acids, are essential for health since they are important components of cholesterol esters and phospholipids in the neuronal cell membrane. Changes in the composition of these membrane phospholipids can affect the regulation of neurotransmitter release, receptors, ion channels, and enzyme activity.[137,138] Omega-3 and omega-6 PUFAs are cleaved from membrane phospholipids and converted via different pathways to mediators that have opposing effects: arachidonic acid mediators are derived from omega-6 fatty acids and are proinflammatory, while mediators derived from omega-3 fatty acids have anti-inflammatory effects. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the two main types of omega-3 PUFA, and fish oil is their main dietary source. It has been suggested that EPA may play a role in brain function by counteracting arachidonic acid-mediated signaling, decreasing immune-inflammatory responses mediated by omega-6 derived eicosanoids, which have been linked to the pathophysiology of anxiety and other mental disorders.[92,139] Moreover, by inhibiting proinflammatory cytokine secretion, omega-3 may also decrease corticosteroid release from the adrenal gland, reducing the mood-altering effects associated with increased cortisol,[140] and hence reducing the impact of cortisol on anxiety.

Several studies have investigated the effects of omega-3 fatty acids in animal models of anxiety (Table 1). Most of the rodent studies involved long-term diet supplements with DHA or a combination of EPA and DHA. Carrié et al. used a DHA-supplemented diet in mice previously fed with a semisynthetic balanced diet or a diet deficient in alpha-linolenic acid (ALA) (another type of omega-3 fatty acid) until the age of 8 months. The supplemented diet showed anxiolytic effects, regardless of the previous diet condition, and restored water maze performance, which had been impaired in the ALA deficient diet group.[72] Jašarević et al. treated female mice for three generations with an omega-6/omega-3 supplemented diet and found that the male offspring of the third generation showed decreased anxiety-like behavior.[78] Rat diets supplemented with different combinations of PUFAs counteracted the anxiogenic effects of intracerebroventricular administered IL-1 beta[73,74] and restraint stress.[75,77] The anxiolytic effect of omega-3 supplementation has also been demonstrated in adult male grey mouse lemurs (*Microcebus murinus*), a nocturnal Malagasy prosimian primate.[76,79]

Low omega-3 levels in erythrocyte membranes have been observed in patients with anxiety disorders.[141-143] Nevertheless, most trials investigating omega-3 in anxiety focused on anxiety symptoms in different conditions rather than anxiety disorders themselves. In an RDBCT with healthy young adults, Kiecolt-Glaser et al. showed that EPA and DHA supplementation decreased anxiety symptoms and LPS-stimulated production of IL-6.[94] Yehuda et al. showed that a mixture of ALA and linolenic acid, given to university students experiencing significant anxiety associated with upcoming exams (test anxiety), improved variables associated with test anxiety (e.g., appetite, mood, concentration, fatigue, academic organization, sleep) and lowered cortisol levels.[91] The anxiolytic effects of omega-3 supplementation were found in patients with acute myocardial infarction[96] and women diagnosed with premenstrual syndrome (PMS).[97] In an RDBCT, Buydens-Branchey & Branchey investigated the effects of a mixture of EPA + DHA supplementation in patients with a history of substance abuse, finding that the supplementation progressively decreased anxiety scores, which remained decreased three months after treatment was discontinued.[138] In a subsequent similarly designed RDBCT, the same group showed that increases in circulating omega-3 levels paralleled decreases in anxiety scores.[93] Similar results were found with male alcoholic patients in a residential rehabilitation program: this small-sample RDBCT showed that fish oil (a source of omega-3 fatty acids) decreased stress/anxiety ratings and reduced basal levels of cortisol.[95] In a placebo-controlled crossover trial, Fux et al. showed that EPA is ineffective as an add-on treatment to SSRI in OCD patients, though the reliability of their results is questionable due to the small sample size and the high placebo response.[144] Matsuoka et al. reported that omega-3 supplementation was not superior to placebo for PTSD symptom prevention three months after accidental injury.[145] In a cohort of Japanese accident survivors at risk of developing PTSD, the same group reported that short-term supplementation with DHA and EPA lowered heart rates during script-driven imagery and/or resting, whereas the baseline heart rate did not differ from the placebo group.[98]

In addition to the compounds discussed above (agomelatine, NAC, and omega-3 fatty acids), we also found some evidence of anxiolytic effects in clinical trials and animal studies for ascorbic acid (vitamin C) and the $mGlu_{2/3}$ receptor agonist LY354740. Although ascorbic acid has presented anxiolytic effects in different animal models in rats,[146] mice,[147] and zebrafish,[148] evidence of its anxiolytic effects in humans is limited. Only one small randomized double-blind placeb-controlled clinical trial (n=42) with ascorbic acid conducted with normal volunteers was found: its results were that ascorbic acid decreased anxiety levels.[149] Although studies with LY354740 showed robust anxiolytic activity in several animal models, as well as in a few clinical trials, larger clinical trials were interrupted due to reports of seizures in animal studies.[16]

One limitation of our study is the likely existence of publication bias in this field. Despite the possibility that many negative results concerning this topic may have been deterred from publication, our main goal was to present the available data for compounds with a robust body of evidence.

Conclusion

We reviewed three compounds that may counteract key biochemical correlates of anxiety states. Despite a

reasonable body of evidence showing anxiolytic properties, the results show that the clinical data is deficient. Data from clinical trials are more indicative than conclusive, and larger trials specifically designed for anxiety disorders are needed. Nevertheless, the beneficial effect observed in clinical conditions where mainstream treatments are ineffective should not be overlooked.

Regarding safety and tolerability, clinical trials and toxicity studies have shown that agomelatine,[106,150] NAC,[111] and omega-3[151] were generally well tolerated and free from serious adverse effects. The most common side effects reported were headache, dizziness, somnolence, fatigue, and gastrointestinal symptoms for agomelatine,[150] gastrointestinal symptoms, with headache for NAC[111] and a fish aftertaste and nausea with omega-3.[140,151]

In conclusion, due to the prevalence and morbidity of anxiety disorders, the potential translational value of the biochemical basis of anxiety, and the safety profile of these compounds, investment in larger clinical trials seems justified.

Acknowledgements

The authors would like to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; EE, AP) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; PS) for fellowships.

Disclosure

The authors report no conflict of interest.

References

1 Davis M, Walker DL, Miles L, Grillon C. Phasic vs sustained fear in rats and humans: role of the extended amygdala in fear vs anxiety. Neuropsychopharmacology. 2010;35:105-35.
2 Calhoon GG, Tye KM. Resolving the neural circuits of anxiety. Nat Neurosci. 2015;18:1394-404.
3 Baxter AJ, Scott KM, Vos T, Whiteford HA. Global prevalence of anxiety disorders: a systematic review and meta-regression. Psychol Med. 2013;43:897-910.
4 American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5). Arlington: American Psychiatric Publishing; 2013.
5 Stein MB, Craske MG. Treating anxiety in 2017: optimizing care to improve outcomes. JAMA. 2017;318:235-6.
6 Hamner MB, Robert S, Frueh BC. Treatment-resistant posttraumatic stress disorder: strategies for intervention. CNS Spectr. 2004;9:740-52.
7 Cryan JF, Sweeney FF. The age of anxiety: role of animal models of anxiolytic action in drug discovery. Br J Pharmacol. 2011;164:1129-61.
8 Loane C, Politis M. Buspirone: what is it all about? Brain Res. 2012;1461:111-8.
9 Griebel G, Holmes A. 50 years of hurdles and hope in anxiolytic drug discovery. Nat Rev Drug Discov. 2013;12:667-87.
10 Hopkins AL. Network pharmacology: the next paradigm in drug discovery. Nat Chem Biol. 2008;4:682-90.
11 Youdim MB, Buccafusco JJ. Multi-functional drugs for various CNS targets in the treatment of neurodegenerative disorders. Trends Pharmacol Sci. 2005;26:27-35.
12 Hassan W, Silva CE, Mohammadzai IU, da Rocha JB, Landeira-Fernandez J. Association of oxidative stress to the genesis of anxiety: implications for possible therapeutic interventions. Curr Neuropharmacol. 2014;12:120-39.
13 Ng F, Berk M, Dean O, Bush AI. Oxidative stress in psychiatric disorders: evidence base and therapeutic implications. Int J Neuropsychopharmacol. 2008;11:851-76.
14 Salim S. Oxidative stress and psychological disorders. Curr Neuropharmacol. 2014;12:140-7.
15 Furtado M, Katzman MA. Neuroinflammatory pathways in anxiety, posttraumatic stress, and obsessive compulsive disorders. Psychiatry Res. 2015;229:37-48.
16 Pitsikas N. The metabotropic glutamate receptors: potential drug targets for the treatment of anxiety disorders? Eur J Pharmacol. 2014;723:181-4.
17 Riaza Bermudo-Soriano C, Perez-Rodriguez MM, Vaquero-Lorenzo C, Baca-Garcia E. New perspectives in glutamate and anxiety. Pharmacol Biochem Behav. 2012;100:752-74.
18 Wierońska JM, Stachowicz K, Nowak G, Pilc A. The loss of glutamate-GABA harmony in anxiety disorders. In: Kalinin V, editor. Anxiety disorders. Rijeka: Intech; 2011. p. 135-58.
19 Sattler R, Tymianski M. Molecular mechanisms of glutamate receptor-mediated excitotoxic neuronal cell death. Mol Neurobiol. 2001;24:107-29.
20 Najjar S, Pearlman DM, Alper K, Najjar A, Devinsky O. Neuroinflammation and psychiatric illness. J Neuroinflammation. 2013;10:43.
21 Linden AM, Greene SJ, Bergeron M, Schoepp DD. Anxiolytic activity of the MGLU2/3 receptor agonist LY354740 on the elevated plus maze is associated with the suppression of stress-induced c-Fos in the hippocampus and increases in c-Fos induction in several other stress-sensitive brain regions. Neuropsychopharmacology. 2004;29:502-13.
22 Swanson CJ, Bures M, Johnson MP, Linden AM, Monn JA, Schoepp DD. Metabotropic glutamate receptors as novel targets for anxiety and stress disorders. Nat Rev Drug Discov. 2005;4:131-44.
23 Schoepp DD. Unveiling the functions of presynaptic metabotropic glutamate receptors in the central nervous system. J Pharmacol Exp Ther. 2001;299:12-20.
24 Hovatta I, Tennant RS, Helton R, Marr RA, Singer O, Redwine JM, et al. Glyoxalase 1 and glutathione reductase 1 regulate anxiety in mice. Nature. 2005;438:662-6.
25 Landgraf R, Kessler MS, Bunck M, Murgatroyd C, Spengler D, Zimbelmann M, et al. Candidate genes of anxiety-related behavior in HAB/LAB rats and mice: focus on vasopressin and glyoxalase-I. Neurosci Biobehav Rev. 2007;31:89-102.
26 Bouayed J, Rammal H, Younos C, Soulimani R. Positive correlation between peripheral blood granulocyte oxidative status and level of anxiety in mice. Eur J Pharmacol. 2007;564:146-9.
27 Li Q, Zhang M, Chen YJ, Wang YJ, Huang F, Liu J. Oxidative damage and HSP70 expression in masseter muscle induced by psychological stress in rats. Physiol Behav. 2011;104:365-72.
28 Noschang CG, Pettenuzzo LF, von Pozzer Toigo E, Andreazza AC, Krolow R, Fachin A, et al. Sex-specific differences on caffeine consumption and chronic stress-induced anxiety-like behavior and DNA breaks in the hippocampus. Pharmacol Biochem Behav. 2009;94:63-9.
29 de Oliveira MR, Silvestrin RB, Mello E Souza T, Moreira JC. Oxidative stress in the hippocampus, anxiety-like behavior and decreased locomotory and exploratory activity of adult rats: effects of sub acute vitamin A supplementation at therapeutic doses. Neurotoxicology. 2007;28:1191-9.
30 Salim S, Sarraj N, Taneja M, Saha K, Tejada-Simon MV, Chugh G. Moderate treadmill exercise prevents oxidative stress-induced anxiety-like behavior in rats. Behav Brain Res. 2010;208:545-52.
31 Salim S, Asghar M, Chugh G, Taneja M, Xia Z, Saha K. Oxidative stress: a potential recipe for anxiety, hypertension and insulin resistance. Brain Res. 2010;1359:178-85.
32 Bulut M, Selek S, Bez Y, Karababa IF, Kaya MC, Gunes M, et al. Reduced PON1 enzymatic activity and increased lipid hydroperoxide levels that point out oxidative stress in generalized anxiety disorder. J Affect Disord. 2013;150:829-33.
33 Kaya MC, Bez Y, Karababa IF, Emhan A, Aksoy N, Bulut M, et al. Decreased serum sulphydryl levels as a sign of increased oxidative stress in generalized anxiety disorder. Psychiatry Investig. 2013;10:281-5.
34 Emhan A, Selek S, Bayazıt H, Fatih Karababa İ, Katı M, Aksoy N. Evaluation of oxidative and antioxidative parameters in generalized anxiety disorder. Psychiatry Res. 2015;230:806-10.
35 Kuloglu M, Atmaca M, Tezcan E, Gecici O, Tunckol H, Ustundag B. Antioxidant enzyme activities and malondialdehyde levels in patients

with obsessive-compulsive disorder. Neuropsychobiology. 2002; 46:27-32.
36 Ersan S, Bakir S, Erdal Ersan E, Dogan O. Examination of free radical metabolism and antioxidant defence system elements in patients with obsessive-compulsive disorder. Prog Neuropsychopharmacol Biol Psychiatry. 2006;30:1039-42.
37 Chakraborty S, Singh OP, Dasgupta A, Mandal N, Nath Das H. Correlation between lipid peroxidation-induced TBARS level and disease severity in obsessive-compulsive disorder. Prog Neuropsychopharmacol Biol Psychiatry. 2009;33:363-6.
38 Behl A, Swami G, Sircar SS, Bhatia MS, Banerjee BD. Relationship of possible stress-related biochemical markers to oxidative/antioxidative status in obsessive-compulsive disorder. Neuropsychobiology. 2010;61:210-4.
39 Kandemir H, Abuhandan M, Aksoy N, Savik E, Kaya C. Oxidative imbalance in child and adolescent patients with obsessive compulsive disorder. J Psychiatr Res. 2013;47:1831-4.
40 Kuloglu M, Atmaca M, Tezcan E, Ustundag B, Bulut S. Antioxidant enzyme and malondialdehyde levels in patients with panic disorder. Neuropsychobiology. 2002;46:186-9.
41 Atmaca M, Tezcan E, Kuloglu M, Ustundag B, Tunckol H. Antioxidant enzyme and malondialdehyde values in social phobia before and after citalopram treatment. Eur Arch Psychiatry Clin Neurosci. 2004;254:231-5.
42 Atmaca M, Kuloglu M, Tezcan E, Ustundag B. Antioxidant enzyme and malondialdehyde levels in patients with social phobia. Psychiatry Res. 2008;159:95-100.
43 Arranz L, Guayerbas N, De la Fuente M. Impairment of several immune functions in anxious women. J Psychosom Res. 2007;62:1-8.
44 Salim S, Chugh G, Asghar M. Inflammation in anxiety. Adv Protein Chem Struct Biol. 2012;88:1-25.
45 Miller AH, Haroon E, Raison CL, Felger JC. Cytokine targets in the brain: impact on neurotransmitters and neurocircuits. Depress Anxiety. 2013;30:297-306.
46 Sakić B, Szechtman H, Talangbayan H, Denburg SD, Carbotte RM, Denburg JA. Disturbed emotionality in autoimmune MRL-lpr mice. Physiol Behav. 1994;56:609-17.
47 Schrott LM, Crnic LS. Increased anxiety behaviors in autoimmune mice. Behav Neurosci. 1996;110:492-502.
48 Connor TJ, Leonard BE. Depression, stress and immunological activation: the role of cytokines in depressive disorders. Life Sci. 1998;62:583-606.
49 Fiore M, Alleva E, Probert L, Kollias G, Angelucci F, Aloe L. Exploratory and displacement behavior in transgenic mice expressing high levels of brain TNF-alpha. Physiol Behav. 1998;63:571-6.
50 Reichenberg A, Yirmiya R, Schuld A, Kraus T, Haack M, Morag A, et al. Cytokine-associated emotional and cognitive disturbances in humans. Arch Gen Psychiatry. 2001;58:445-52.
51 Maes M, Song C, Lin A, De Jongh R, Van Gastel A, Kenis G, et al. The effects of psychological stress on humans: increased production of pro-inflammatory cytokines and a Th1-like response in stress-induced anxiety. Cytokine. 1998;10:313-8.
52 Pitsavos C, Panagiotakos DB, Papageorgiou C, Tsetsekou E, Soldatos C, Stefanadis C. Anxiety in relation to inflammation and coagulation markers, among healthy adults: the ATTICA study. Atherosclerosis. 2006;185:320-6.
53 Millan MJ, Gobert A, Lejeune F, Dekeyne A, Newman-Tancredi A, Pasteau V, et al. The novel melatonin agonist agomelatine (S20098) is an antagonist at 5-hydroxytryptamine2C receptors, blockade of which enhances the activity of frontocortical dopaminergic and adrenergic pathways. J Pharmacol Exp Ther. 2003;306:954-64.
54 San L, Arranz B. Agomelatine: a novel mechanism of antidepressant action involving the melatonergic and the serotonergic system. Eur Psychiatry. 2008;23:396-402.
55 Racagni G, Riva MA, Molteni R, Musazzi L, Calabrese F, Popoli M, et al. Mode of action of agomelatine: synergy between melatonergic and 5-HT2C receptors. World J Biol Psychiatry. 2011;12:574-87.
56 Reagan LP, Reznikov LR, Evans AN, Gabriel C, Mocaër E, Fadel JR. The antidepressant agomelatine inhibits stress-mediated changes in amino acid efflux in the rat hippocampus and amygdala. Brain Res. 2012;1466:91-8.
57 Tardito D, Milanese M, Bonifacino T, Musazzi L, Grilli M, Mallei A, et al. Blockade of stress-induced increase of glutamate release in the rat prefrontal/frontal cortex by agomelatine involves synergy between melatonergic and 5-HT2C receptor-dependent pathways. BMC Neurosci. 2010;11:68.
58 Morley-Fletcher S, Mairesse J, Soumier A, Banasr M, Fagioli F, Gabriel C, et al. Chronic agomelatine treatment corrects behavioral, cellular, and biochemical abnormalities induced by prenatal stress in rats. Psychopharmacology (Berl). 2011;217:301-13.
59 Aguiar CC, Almeida AB, Araújo PV, Vasconcelos GS, Chaves EM, do Vale OC, et al. Effects of agomelatine on oxidative stress in the brain of mice after chemically induced seizures. Cell Mol Neurobiol. 2013;33:825-35.
60 Akpinar A, Uğuz AC, Nazıroğlu M. Agomelatine and duloxetine synergistically modulates apoptotic pathway by inhibiting oxidative stress triggered intracellular calcium entry in neuronal PC12 cells: role of TRPM2 and voltage-gated calcium channels. J Membr Biol. 2014;247:451-9.
61 Molteni R, Macchi F, Zecchillo C, Dell'agli M, Colombo E, Calabrese F, et al. Modulation of the inflammatory response in rats chronically treated with the antidepressant agomelatine. Eur Neuropsychopharmacol. 2013;23:1645-55.
62 Hanisch UK, Kettenmann H. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat Neurosci. 2007; 10:1387-94.
63 de Bodinat C, Guardiola-Lemaitre B, Mocaër E, Renard P, Muñoz C, Millan MJ. Agomelatine, the first melatonergic antidepressant: discovery, characterization and development. Nat Rev Drug Discov. 2010;9:628-42.
64 Loiseau F, Le Bihan C, Hamon M, Thiébot MH. Effects of melatonin and agomelatine in anxiety-related procedures in rats: interaction with diazepam. Eur Neuropsychopharmacol. 2006;16:417-28.
65 Millan MJ, Brocco M, Gobert A, Dekeyne A. Anxiolytic properties of agomelatine, an antidepressant with melatoninergic and serotonergic properties: role of 5-HT2C receptor blockade. Psychopharmacology (Berl). 2005;177:448-58.
66 Papp M, Litwa E, Gruca P, Mocaër E. Anxiolytic-like activity of agomelatine and melatonin in three animal models of anxiety. Behav Pharmacol. 2006;17:9-18.
67 Egashira N, Shirakawa A, Abe M, Niki T, Mishima K, Iwasaki K, et al. N-acetyl-L-cysteine inhibits marble-burying behavior in mice. J Pharmacol Sci. 2012;119:97-101.
68 Chen YW, Lin HC, Ng MC, Hsiao YH, Wang CC, Gean PW, et al. Activation of mGluR2/3 underlies the effects of N-acetylcystein on amygdala-associated autism-like phenotypes in a valproate-induced rat model of autism. Front Behav Neurosci. 2014;8:219.
69 Pilz LK, Trojan Y, Quiles CL, Benvenutti R, Melo G, Levandovski R, et al. Effects of N-acetylcysteine and imipramine in a model of acute rhythm disruption in BALB/c mice. Chronobiol Int. 2015;32:248-54.
70 Mocelin R, Herrmann AP, Marcon M, Rambo CL, Rohden A, Bevilaqua F, et al. N-acetylcysteine prevents stress-induced anxiety behavior in zebrafish. Pharmacol Biochem Behav. 2015;139:121-6.
71 Santos P, Herrmann AP, Benvenutti R, Noetzold G, Giongo F, Gama CS, et al. Anxiolytic properties of N-acetylcysteine in mice. Behav Brain Res. 2017;317:461-9.
72 Carrié I, Smirnova M, Clément M, De JD, Francès H, Bourre JM. Docosahexaenoic acid-rich phospholipid supplementation: effect on behavior, learning ability, and retinal function in control and n-3 polyunsaturated fatty acid deficient old mice. Nutr Neurosci. 2002;5:43-52.
73 Song C, Li X, Leonard BE, Horrobin DF. Effects of dietary n-3 or n-6 fatty acids on interleukin-1beta-induced anxiety, stress, and inflammatory responses in rats. J Lipid Res. 2003;44:1984-91.
74 Song C, Leonard BE, Horrobin DF. Dietary ethyl-eicosapentaenoic acid but not soybean oil reverses central interleukin-1-induced changes in behavior, corticosterone and immune response in rats. Stress. 2004;7:43-54.
75 Ferraz AC, Delattre AM, Almendra RG, Sonagli M, Borges C, Araujo P, et al. Chronic ω-3 fatty acids supplementation promotes beneficial effects on anxiety, cognitive and depressive-like behaviors in rats subjected to a restraint stress protocol. Behav Brain Res. 2011;219:116-22.
76 Vinot N, Jouin M, Lhomme-Duchadeuil A, Guesnet P, Alessandri JM, Aujard F, et al. Omega-3 fatty acids from fish oil lower anxiety, improve cognitive functions and reduce spontaneous locomotor activity in a non-human primate. PloS One. 2011;6:e20491.
77 Pérez MÁ, Terreros G, Dagnino-Subiabre A. Long-term ω-3 fatty acid supplementation induces anti-stress effects and improves learning in rats. Behav Brain Funct. 2013;9:25.

78 Jašarević E, Hecht PM, Fritsche KL, Beversdorf DQ, Geary DC. Dissociable effects of dorsal and ventral hippocampal DHA content on spatial learning and anxiety-like behavior. Neurobiol Learn Mem. 2014;116:59-68.
79 Pifferi F, Dorieux O, Castellano CA, Croteau E, Masson M, Guillermier M, et al. Long-chain n-3 PUFAs from fish oil enhance resting state brain glucose utilization and reduce anxiety in an adult nonhuman primate, the grey mouse lemur. J Lipid Res. 2015;56:1511-8.
80 Stein DJ, Ahokas AA, de Bodinat C. Efficacy of agomelatine in generalized anxiety disorder: a randomized, double-blind, placebo-controlled study. J Clin Psychopharmacol. 2008;28:561-6.
81 Stein DJ, Ahokas A, Albarran C, Olivier V, Allgulander C. Agomelatine prevents relapse in generalized anxiety disorder: a 6-month randomized, double-blind, placebo-controlled discontinuation study. J Clin Psychiatry. 2012;73:1002-8.
82 Stein DJ, Ahokas A, Márquez MS, Höschl C, Oh KS, Jarema M, et al. Agomelatine in generalized anxiety disorder: an active comparator and placebo-controlled study. J Clin Psychiatry. 2014;75: 362-8.
83 Stein DJ, Ahokas A, Jarema M, Avedisova AS, Vavrusova L, Chaban O, et al. Efficacy and safety of agomelatine (10 or 25 mg/day) in non-depressed out-patients with generalized anxiety disorder: a 12-week, double-blind, placebo-controlled study. Eur Neuropsychopharmacol. 2017;27:526-37.
84 Durieux AM, Fernandes C, Murphy D, Labouesse MA, Giovanoli S, Meyer U, et al. Targeting glia with N-acetylcysteine modulates brain glutamate and behaviors relevant to neurodevelopmental disorders in C57BL/6J mice. Front Behav Neurosci. 2015;9:343.
85 Afshar H, Roohafza H, Mohammad-Beigi H, Haghighi M, Jahangard L, Shokouh P, et al. N-acetylcysteine add-on treatment in refractory obsessive-compulsive disorder: a randomized, double-blind, placebo-controlled trial. J Clin Psychopharmacol. 2012;32:797-803.
86 Ghanizadeh A, Derakhshan N, Berk M. N-acetylcysteine versus placebo for treating nail biting, a double blind randomized placebo controlled clinical trial. Antiinflamm Antiallergy Agents Med Chem. 2013;12:223-8.
87 Sarris J, Oliver G, Camfield DA, Dean OM, Dowling N, Smith DJ, et al. N-acetyl cysteine (nac) in the treatment of obsessive-compulsive disorder: a 16-week, double-blind, randomised, placebo-controlled study. CNS Drugs. 2015;29:801-9.
88 Back SE, McCauley JL, Korte KJ, Gros DF, Leavitt V, Gray KM, et al. A double-blind, randomized, controlled pilot trial of N-acetylcysteine in veterans with posttraumatic stress disorder and substance use disorders. J Clin Psychiatry. 2016;77:e1439-e46.
89 Grant JE, Chamberlain SR, Redden SA, Leppink EW, Odlaug BL, Kim SW. N-acetylcysteine in the treatment of excoriation disorder: a randomized clinical trial. JAMA Psychiatry. 2016;73:490-6.
90 Paydary K, Akamaloo A, Ahmadipour A, Pishgar F, Emamzadehfard S, Akhondzadeh S. N-acetylcysteine augmentation therapy for moderate-to-severe obsessive-compulsive disorder: randomized, double-blind, placebo-controlled trial. J Clin Pharm Ther. 2016; 41:214-9.
91 Yehuda S, Rabinovitz S, Mostofsky DI. Mixture of essential fatty acids lowers test anxiety. Nutr Neurosci. 2005;8:265-7.
92 Buydens-Branchey L, Branchey M. n-3 polyunsaturated fatty acids decrease anxiety feelings in a population of substance abusers. J Clin Psychopharmacol. 2006;26:661-5.
93 Buydens-Branchey L, Branchey M, Hibbeln JR. Associations between increases in plasma n-3 polyunsaturated fatty acids following supplementation and decreases in anger and anxiety in substance abusers. Prog Neuropsychopharmacol Biol Psychiatry. 2008;32:568-75.
94 Kiecolt-Glaser JK, Belury MA, Andridge R, Malarkey WB, Glaser R. Omega-3 supplementation lowers inflammation and anxiety in medical students: a randomized controlled trial. Brain Behav Immun. 2011;25:1725-34.
95 Barbadoro P, Annino I, Ponzio E, Romanelli RM, D'Errico MM, Prospero E, et al. Fish oil supplementation reduces cortisol basal levels and perceived stress: a randomized, placebo-controlled trial in abstinent alcoholics. Mol Nutr Food Res. 2013;57:1110-4.
96 Haberka M, Mizia-Stec K, Mizia M, Gieszczyk K, Chmiel A, Sitnik-Warchulska K, et al. Effects of n-3 polyunsaturated fatty acids on depressive symptoms, anxiety and emotional state in patients with acute myocardial infarction. Pharmacol Rep. 2013;65:59-68.
97 Sohrabi N, Kashanian M, Ghafoori SS, Malakouti SK. Evaluation of the effect of omega-3 fatty acids in the treatment of premenstrual syndrome: "a pilot trial." Complement Ther Med. 2013;21:141-6.
98 Matsumura K, Noguchi H, Nishi D, Hamazaki K, Hamazaki T, Matsuoka YJ. Effects of omega-3 polyunsaturated fatty acids on psychophysiological symptoms of posttraumatic stress disorder in accident survivors: a randomized, double-blind, placebo-controlled trial. J Affect Disord. 2017;224:27-31.
99 Huijbregts KM, Batelaan NM, Schonenberg J, Veen G, van Balkom AJ. Agomelatine as a novel treatment option in panic disorder, results from an 8-week open-label trial. J Clin Psychopharmacol. 2015;35:336-8.
100 Lôo H, Hale A, D'haenen H. Determination of the dose of agomelatine, a melatoninergic agonist and selective 5-HT(2C) antagonist, in the treatment of major depressive disorder: a placebo-controlled dose range study. Int Clin Psychopharmacol. 2002;17:239-47.
101 Olié JP, Kasper S. Efficacy of agomelatine, a MT1/MT2 receptor agonist with 5-HT2C antagonistic properties, in major depressive disorder. Int J Neuropsychopharmacol. 2007;10:661-73.
102 Kennedy SH, Emsley R. Placebo-controlled trial of agomelatine in the treatment of major depressive disorder. Eur Neuropsychopharmacol. 2006;16:93-100.
103 Lemoine P, Guilleminault C, Alvarez E. Improvement in subjective sleep in major depressive disorder with a novel antidepressant, agomelatine: randomized, double-blind comparison with venlafaxine. J Clin Psychiatry. 2007;68:1723-32.
104 Hale A, Corral RM, Mencacci C, Ruiz JS, Severo CA, Gentil V. Superior antidepressant efficacy results of agomelatine versus fluoxetine in severe MDD patients: a randomized, double-blind study. Int Clin Psychopharmacol. 2010;25:305-14.
105 Kasper S, Hajak G, Wulff K, Hoogendijk WJ, Montejo AL, Smeraldi E, et al. Efficacy of the novel antidepressant agomelatine on the circadian rest-activity cycle and depressive and anxiety symptoms in patients with major depressive disorder: a randomized, double-blind comparison with sertraline. J Clin Psychiatry. 2010;71:109-20.
106 Stein DJ, Picarel-Blanchot F, Kennedy SH. Efficacy of the novel antidepressant agomelatine for anxiety symptoms in major depression. Hum Psychopharmacol. 2013;28:151-9.
107 Goodwin GM, Emsley R, Rembry S, Rouillon F; Agomelatine Study Group. Agomelatine prevents relapse in patients with major depressive disorder without evidence of a discontinuation syndrome: a 24-week randomized, double-blind, placebo-controlled trial. J Clin Psychiatry. 2009;70:1128-37.
108 De Berardis D, Di Iorio G, Acciavatti T, Conti C, Serroni N, Olivieri L, et al. The emerging role of melatonin agonists in the treatment of major depression: focus on agomelatine. CNS Neurol Disord Drug Targets. 2011;10:119-32.
109 Green B. Focus on agomelatine. Curr Med Res Opin. 2011;27:745-9.
110 Dean O, Giorlando F, Berk M. N-acetylcysteine in psychiatry: current therapeutic evidence and potential mechanisms of action. J Psychiatry Neurosci. 2011;36:78-86.
111 Deepmala, Slattery J, Kumar N, Delhey L, Berk M, Dean O, et al. Clinical trials of N-acetylcysteine in psychiatry and neurology: a systematic review. Neurosci Biobehav Rev. 2015;55:294-321.
112 Palacio JR, Markert UR, Martínez P. Anti-inflammatory properties of N-acetylcysteine on lipopolysaccharide-activated macrophages. Inflamm Res. 2011;60:695-704.
113 Yang R, Gallo DJ, Baust JJ, Watkins SK, Delude RL, Fink MP. Effect of hemorrhagic shock on gut barrier function and expression of stress-related genes in normal and gnotobiotic mice. Am J Physiol Regul Integr Comp Physiol. 2002;283:R1263-74.
114 Berk M, Malhi GS, Gray LJ, Dean OM. The promise of N-acetylcysteine in neuropsychiatry. Trends Pharmacol Sci. 2013;34:167-77.
115 Tsai GY, Cui JZ, Syed H, Xia Z, Ozerdem U, McNeill JH, et al. Effect of N-acetylcysteine on the early expression of inflammatory markers in the retina and plasma of diabetic rats. Clin Exp Ophthalmol. 2009;37:223-31.
116 Kigerl KA, Ankeny DP, Garg SK, Wei P, Guan Z, Lai W, et al. System xc- regulates microglia and macrophage glutamate excitotoxicity in vivo. Exp Neurol. 2012;233:333-41.
117 Chen G, Shi J, Hu Z, Hang C. Inhibitory effect on cerebral inflammatory response following traumatic brain injury in rats: a potential neuroprotective mechanism of N-acetylcysteine. Mediators Inflamm. 2008;2008:716458.

118 Jatana M, Singh I, Singh AK, Jenkins D. Combination of systemic hypothermia and N-acetylcysteine attenuates hypoxic-ischemic brain injury in neonatal rats. Pediatr Res. 2006;59:684-9.
119 Khan M, Sekhon B, Jatana M, Giri S, Gilg AG, Sekhon C, et al. Administration of N-acetylcysteine after focal cerebral ischemia protects brain and reduces inflammation in a rat model of experimental stroke. J Neurosci Res. 2004;76:519-27.
120 Gatti S, Faggioni R, Echtenacher B, Ghezzi P. Role of tumour necrosis factor and reactive oxygen intermediates in lipopolysaccharide-induced pulmonary oedema and lethality. Clin Exp Immunol. 1993;91:456-61.
121 Victor VM, Rocha M, De la Fuente M. N-acetylcysteine protects mice from lethal endotoxemia by regulating the redox state of immune cells. Free Radic Res. 2003;37:919-29.
122 Blackwell TS, Blackwell TR, Holden EP, Christman BW, Christman JW. In vivo antioxidant treatment suppresses nuclear factor-kappa B activation and neutrophilic lung inflammation. J Immunol. 1996; 157:1630-7.
123 Csontos C, Rezman B, Foldi V, Bogar L, Drenkovics L, Röth E, et al. Effect of N-acetylcysteine treatment on oxidative stress and inflammation after severe burn. Burns. 2012;38:428-37.
124 Nascimento MM, Suliman ME, Silva M, Chinaglia T, Marchioro J, Hayashi SY, et al. Effect of oral N-acetylcysteine treatment on plasma inflammatory and oxidative stress markers in peritoneal dialysis patients: a placebo-controlled study. Perit Dial Int. 2010;30:336-42.
125 Mahmoud KM, Ammar AS. Effect of N-acetylcysteine on cardiac injury and oxidative stress after abdominal aortic aneurysm repair: a randomized controlled trial. Acta Anaesthesiol Scand. 2011;55: 1015-21.
126 Grant JE, Odlaug BL, Kim SW. N-acetylcysteine, a glutamate modulator, in the treatment of trichotillomania: a double-blind, placebo-controlled study. Arch Gen Psychiatry. 2009;66:756-63.
127 Odlaug BL, Grant JE. N-acetyl cysteine in the treatment of grooming disorders. J Clin Psychopharmacol. 2007;27:227-9.
128 Rodrigues-Barata AR, Tosti A, Rodríguez-Pichardo A, Camacho-Martínez F. N-acetylcysteine in the treatment of trichotillomania. Int J Trichology. 2012;4:176-8.
129 Taylor M, Bhagwandas K. N-acetylcysteine in trichotillomania: a panacea for compulsive skin disorders? Br J Dermatol. 2014;171:1253-5.
130 Bloch MH, Panza KE, Grant JE, Pittenger C, Leckman JF. N-Acetylcysteine in the treatment of pediatric trichotillomania: a randomized, double-blind, placebo-controlled add-on trial. J Am Acad Child Adolesc Psychiatry. 2013;52:231-40.
131 Grant JE, Odlaug BL, Chamberlain SR, Keuthen NJ, Lochner C, Stein DJ. Skin picking disorder. Am J Psychiatry. 2012;169:1143-9.
132 Percinel I, Yazici KU. Glutamatergic dysfunction in skin-picking disorder: treatment of a pediatric patient with N-acetylcysteine. J Clin Psychopharmacol. 2014;34:772-4.
133 Silva-Netto R, Jesus G, Nogueira M, Tavares H. N-acetylcysteine in the treatment of skin-picking disorder. Rev Bras Psiquiatr. 2014; 36:101.
134 Berk M, Jeavons S, Dean OM, Dodd S, Moss K, Gama CS, et al. Nail-biting stuff? The effect of N-acetyl cysteine on nail-biting. CNS Spectr. 2009;14:357-60.
135 Yazici KU, Percinel I. N-acetylcysteine augmentation in children and adolescents diagnosed with treatment-resistant obsessive-compulsive disorder: case series. J Clin Psychopharmacol. 2015;35:486-9.
136 Lafleur DL, Pittenger C, Kelmendi B, Gardner T, Wasylink S, Malison RT, et al. N-acetylcysteine augmentation in serotonin reuptake inhibitor refractory obsessive-compulsive disorder. Psychopharmacology (Berl). 2006;184:254-6.
137 Politi P, Rocchetti M, Emanuele E, Rondanelli M, Barale F. Randomized placebo-controlled trials of omega-3 polyunsaturated fatty acids in psychiatric disorders: a review of the current literature. Curr Drug Discov Technol. 2013;10:245-53.
138 Prior PL, Galduróz JC. (N-3) Fatty acids: molecular role and clinical uses in psychiatric disorders. Adv Nutr. 2012;3:257-65.
139 Layé S. Polyunsaturated fatty acids, neuroinflammation and well being. Prostaglandins Leukot Essent Fatty Acids. 2010;82:295-303.
140 Mischoulon D, Freeman MP. Omega-3 fatty acids in psychiatry. Psychiatr Clin North Am. 2013;36:15-23.
141 Green P, Hermesh H, Monselise A, Marom S, Presburger G, Weizman A. Red cell membrane omega-3 fatty acids are decreased in nondepressed patients with social anxiety disorder. Eur Neuropsychopharmacol. 2006;16:107-13.
142 Liu JJ, Galfalvy HC, Cooper TB, Oquendo MA, Grunebaum MF, Mann JJ, et al. Omega-3 polyunsaturated fatty acid (PUFA) status in major depressive disorder with comorbid anxiety disorders. J Clin Psychiatry. 2013;74:732-8.
143 Ross BM. Omega-3 polyunsaturated fatty acids and anxiety disorders. Prostaglandins Leukot Essent Fatty Acids. 2009;81:309-12.
144 Fux M, Benjamin J, Nemets B. A placebo-controlled cross-over trial of adjunctive EPA in OCD. J Psychiatr Res. 2004;38:323-5.
145 Matsuoka Y, Nishi D, Hamazaki K, Yonemoto N, Matsumura K, Noguchi H, et al. Docosahexaenoic acid for selective prevention of posttraumatic stress disorder among severely injured patients: a randomized, placebo-controlled trial. J Clin Psychiatry. 2015;76: e1015-22.
146 Hughes RN, Lowther CL, van Nobelen M. Prolonged treatment with vitamins C and E separately and together decreases anxiety-related open-field behavior and acoustic startle in hooded rats. Pharmacol Biochem Behav. 2011;97:494-9.
147 Angrini MA, Leslie JC. Vitamin C attenuates the physiological and behavioural changes induced by long-term exposure to noise. Behav Pharmacol. 2012;23:119-25.
148 Puty B, Maximino C, Brasil A, da Silva WL, Gouveia A Jr, Oliveira KR, et al. Ascorbic acid protects against anxiogenic-like effect induced by methylmercury in zebrafish: action on the serotonergic system. Zebrafish. 2014;11:365-70.
149 de Oliveira IJ, de Souza VV, Motta V, Da-Silva SL. Effects of oral vitamin C supplementation on anxiety in students: a double-blind, randomized, placebo-controlled trial. Pak J Biol Sci. 2015;18:11-8.
150 Levitan MN, Papelbaum M, Nardi AE. Profile of agomelatine and its potential in the treatment of generalized anxiety disorder. Neuropsychiatr Dis Treat. 2015;11:1149-55.
151 Bozzatello P, Brignolo E, De Grandi E, Bellino S. Supplementation with omega-3 fatty acids in psychiatric disorders: a review of literature data. J Clin Med. 2016;5(8): E67. doi: 10.3390/jcm5080067.