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Red cell membrane omega-3 fatty acids are decreased in nondepressed patients with social anxiety disorder

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Abstract The "phospholipid hypothesis" attributes a pathophysiologic role to the polyunsaturated fatty acid (PUFA) composition of phospholipids in depression. The aim of the present study was to determine whether the hypothesis is relevant to social anxiety disorder (SAD). The study sample consisted of 27 untreated, nondepressed patients with SAD (DSM-IV) and 22 controls. Severity of SAD was assessed with the Liebowitz Social Anxiety Scale (LSAS). Erythrocyte PUFA concentrations were measured by gas–liquid chromatography. Concentrations of most n-3 PUFAs were lower in the patients: 18:3n-3 by 32% ($p<0.002$), 20:3n-3 by 34%, 20:5n-3 by 36% (all $p<0.001$) and 22:6n-3 by 18% ($p=0.002$). No significant differences were observed in other fatty acids. Significant inverse correlations were obtained between levels of n-3 PUFAs and LSAS scores. In conclusion, the phospholipid hypothesis may apply to SAD, thereby opening new therapeutic options. The robust relationship between low erythrocyte n-3 PUFA concentrations and SAD justifies exploration of relevant neuropathophysiological mechanisms.

1. Introduction

Researchers are focusing increased attention on the association of several psychiatric disorders with the fatty acid composition of membrane phospholipids. This has led to the proposal of the so-called "phospholipid hypothesis" of depression (Hibbeln and Salem, 1995) and schizophrenia (Horrobin, 1998). The plausibility of the hypothesis stems from several observations: (a) the two essential polyunsa-

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turated fatty acids (PUFAs), docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (AA, 20:4n-6), are major components of membrane phospholipids in brain tissue (Sastry, 1985); (b) cellular mechanisms implicated in psychiatric disorders, such as bipolar affective disorder (Manji and Lenox, 2000), schizophrenia (Manji et al., 2003), and anxiety disorders (Freeman et al., 2002) are affected by the tissue composition of essential fatty acids (EFAs) (Wainwright, 2002; Innis, 2000; Watanabe et al., 2003); and (c) the activity of some neurotransmitters involved in psychiatric disorders affects the metabolism of EFAs (Mills et al., 1994; Hayakawa et al., 2001).

DHA and AA are elongation—desaturation products of the parent fatty acids, α-linolenic acid (LnA) of the omega-3 (n-3) family (18:3n-3) and linoleic acid (LA) of the omega-6 (n-6) family (18:2n-6), respectively. LnA and LA are categorized as EFAs, because they cannot be synthesized de novo by animals and have to be obtained from plants by diet. They can, however, undergo a series of elongation—desaturation steps in the mammalian organism to form the longer chain derivatives (Sprecher, 2000). Both the absolute and the relative amounts of the dietary n-3 and n-6 EFAs are important for proper function of the organism at almost all levels examined (Holman, 1998; Innis, 1991). However, AA, the major n-6 PUFA in tissues, is relatively unaffected by dietary composition, whereas the DHA status is much more sensitive to changes in dietary EFAs (Fokkema et al., 2000). The importance of DHA, which is the major brain PUFA, has been amply demonstrated in epidemiological studies of food composition as well as in randomized controlled trials of dietary supplementation (Muskiet et al., 2004). It has recently become apparent that, at least in the Western world, a significant deficiency in dietary omega-3 fatty acid has developed, with a concomitant imbalance in the n-3/n-6 fatty acid ratio (Simopoulos, 1999). This imbalance has probably contributed to the increased prevalence of a series of chronic diseases, some affecting the central nervous system.

Several studies have documented a decrease in the n-3 PUFA content of red blood cell (RBC) membrane phospholipids in depressed patients (Edwards et al., 1998; Peet et al., 1998), and others reported a negative correlation between the level of RBC eicosapentaenoic acid (EPA, 20:5n-3) and severity of depression (Adams et al., 1996). In some clinical trials, the addition of ethyl-EPA (Nemets et al., 2002; Peet and Horrobin, 2002) or an n-3 PUFA preparation (Su et al., 2003) to antidepressant agents yielded beneficial effects. Interestingly, in a case report of treatment-resistant depression, the addition of EPA to conventional antidepressant treatment resulted not only in symptom remission but also in structural brain changes, as demonstrated by cerebral magnetic resonance scanning (Puri et al., 2001). Moreover, utilizing brain magnetic resonance imaging, increased membrane fluidity was postulated to occur in patients with bipolar disorder given varying doses of n-3 PUFA (Hirashima et al., 2004). In patients with schizophrenia, decreased n-3 PUFA levels were observed (Assies et al., 2001), but it has been argued that smoking status, gender and dietary intake might be responsible for some of the changes in PUFA levels (Hibbeln et al., 2003). Interestingly, some behavioral and endocrine indicators of psychological stress and anxiety were attenuated by n-3 PUFA administration in humans (Hamazaki et al., 1996; Sawazaki et al., 1999) and animals (Song et al., 2003).

Social anxiety disorder (SAD) is one of the most common psychiatric disorders (Pelissolo et al., 2000). It is characterized by an excessive, egodystonic fear and/or avoidance of situations in which the individual feels scrutinized by others and is fearful of a negative evaluation by others. The burden of SAD includes loss of income, increased suicide rate, underachievement, and high mental as well as physical health care expenditures.

Being the most recently defined anxiety disorder, SAD has received relatively little attention in basic biological research. The leading hypotheses implicate dopaminergic and serotonergic dysregulation (Stein et al., 2002; Schneier et al., 2000; Mathew et al., 2001). Selective serotonin reuptake inhibitor agents were found to be beneficial in the treatment of SAD (Stein et al., 1998). Since the neurotransmitter dysregulation in SAD shares common features with depression, which has been associated with the phospholipid hypothesis, we sought to examine the possibility that abnormalities in phospholipid membrane PUFA composition are also involved in SAD.

2. Experimental procedures

2.1. Subjects

The study group consisted of 27 patients with SAD referred to the Anxiety Disorders Clinic of the Geha Mental Health Center in Israel for cognitive behavioral group therapy. None had received either pharmacologic or psychotherapeutic treatment for at least 8 weeks before the study. Subjects with concurrent DSM-IV major depressive disorder, alcohol or drug abuse, or physical disease were excluded. Twenty-two healthy subjects served as controls. The Mini International Neuropsychiatric Interview (Sheehan et al., 1998) for DSM-IV diagnoses was completed for both patients and control subjects and was used to establish the diagnosis of SAD. The severity of SAD was assessed with the self-report Liebowitz Social Anxiety Scale (LSAS), Hebrew Version (Levin et al., 2002). Blood samples were drawn from all participants for fatty acid analysis, and from patients before commencing behavioral therapy.

The study was approved by the Geha Mental Health Center Review Board, and written consent was obtained from each participant after the nature of the study was fully explained.

2.2. Fatty acid analysis

Whole blood was drawn into EDTA-containing vacutainer tubes kept on ice. After centrifugation, the plasma and buffy coat were removed. The erythrocytes were washed 3 times in 0.9% NaCl and kept under N_2 at -70 °C until analysis. Lipid extraction was performed by homogenization of the cells in hexane/isopropanol (3:2 vol./vol.) containing 5 mg/100 ml butylated hydroxytoluene as an antioxidant and 5 mg/100 ml heneicosanoic acid (21:0) as an internal standard, as described by Hara and Radin (1978). Fatty acid analysis was performed essentially as described by Green and Yavin (1996). In brief, fatty acids were converted to