D2 dopamine receptor gene polymorphism: paroxetine and social functioning in posttraumatic stress disorder

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Abstract

This study examined whether allelic status of the D2 dopamine receptor (DRD2) gene was associated with response to a selective serotonin reuptake inhibitor, paroxetine, in the treatment of posttraumatic stress disorder (PTSD). Sixty-three Caucasian war veterans with combat-related PTSD were treated with paroxetine for 8 weeks. Patients were assessed at baseline and at follow-up using the General Health Questionnaire-28 (GHQ). TaqI A DRD2 alleles were determined by PCR. Before paroxetine treatment, patients with the DRD2 A1\textsuperscript{1} allele (A1A2 genotype) compared to those with the A1\textsuperscript{2} allele (A2A2 genotype) had higher total GHQ psychopathological scores ($P=0.040$) and higher GHQ subscale scores for anxiety/insomnia ($P=0.046$), social dysfunction ($P=0.033$) and depression ($P=0.011$). In an intention-to-treat analysis, paroxetine was associated with significant improvement in total GHQ scores ($P=0.014$) and in the factor scores of social dysfunction ($P=0.033$), anxiety ($P=0.009$) and depression ($P=0.026$). Furthermore, there was a significant allele by time interaction on the social dysfunction scale, with A1\textsuperscript{1} allelic patients showing significant improvement in social functioning compared to A1\textsuperscript{2} allelic patients ($P=0.031$), an effect independent of changes in depression or anxiety. This suggests changes in social functioning induced by paroxetine may be, in part, mediated via D2 dopamine receptors. The DRD2 A1 allele may prove to be a useful marker to assist clinicians in predicting which patients with PTSD are likely to obtain improvements in social functioning with paroxetine treatment.

Keywords: D2 dopamine receptor gene; A1 allele; PTSD; Paroxetine; Social functioning; Intention-to-treat

1. Introduction

Combat-related posttraumatic stress disorder (PTSD) is a highly debilitating condition with a chronic course. The quality of life of PTSD patients is frequently compromised by comorbid conditions such as social anxiety disorder, panic disorder, generalized anxiety disorder, dysthymia and major depressive disorder (Zatzick et al., 1997; O’Toole et al., 1998). Social, marital and vocational functioning are often impaired (Friedman et al., 1994).

Selective serotonin reuptake inhibitors (SSRIs) are an effective treatment for a wide variety of psychiatric disorders. These include PTSD, depression, social phobia, and mixed anxiety and depressive states. Indeed, SSRIs are generally accepted to be the first line pharmacotherapy for PTSD (Hidalgo and Davidson, 2000; Ballinger et al., 2000). Paroxetine is currently the most potent SSRI available. It is a weak inhibitor of noradrenaline uptake and has little affinity for dopaminergic systems (Bourin et al., 2001).

Paroxetine is approved for use in all five anxiety disorders as well as in major depression (Wagstaff et al.,
2. Experimental procedures

Sixty-three unrelated male Caucasian patients with the diagnosis of PTSD were recruited for study. All subjects were Vietnam combat veterans who had served in the Australian armed forces. None were being treated with psychotropic medication. Patients were excluded from the study if they had a diagnosis of psychosis, bipolar disorder, obsessive compulsive disorder, organic brain syndrome, glaucoma, cardiac disease, or were being treated with anticoagulants or drugs affecting hepatic metabolism (Aropax® (Paroxetine) product information, 1999).

Patients were assessed for PTSD by a consultant psychiatrist (BL) or a trainee psychiatrist (BK) using Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria. All met DSM-IV criteria for PTSD. Additionally PTSD caseness was confirmed with all patients exceeding the clinical cut-off score of 94 on the Mississippi Scale for combat-related PTSD (Zatzick et al., 1997). The validity and reliability of the Mississippi scale are well established in veterans (Keane et al., 1988). Patients then underwent clinical history taking by a psychiatrist (BL), a trainee psychiatrist (BK) or by a clinical nurse (EP). Demographic data and ethnic background were also obtained.

After initial assessment, patients were medically examined and then commenced on 20 mg/day of paroxetine for the initial 2 weeks of the study. This was followed by a 40-mg/day dose for the remaining 6 weeks. Response to paroxetine commences after 1 week of treatment but does not exceed placebo until 2 weeks of therapy have been completed (Aropax® (Paroxetine) product information, 1999).

The General Health Questionnaire-28 (GHQ) was administered at baseline (0–7 days on paroxetine) and at the end of treatment (8 weeks after commencement on paroxetine). This questionnaire measures four psychopathological factors particularly relevant to PTSD and its comorbid psychiatric conditions: somatic concerns (GHQ1), anxiety/insomnia (GHQ2), social dysfunction (GHQ3) and depression (GHQ4). The GHQ has been widely validated internationally as a means of detecting psychiatric caseness and is sensitive to change (Ormel et al., 1989). The GHQ has utility as a follow-up measure of veteran mental health following exposure to combat (Deahl et al., 1994) and is sensitive to changes in combat-related PTSD symptoms (Ward, 1997).

A 10-ml blood sample was drawn from each participant. Genomic DNA was extracted employing standard techniques and used as a template for determination of TaqI A DRD2 alleles by the polymerase chain reaction (Grandy et al., 1993). The amplification of DNA was carried out using a Perkin-Elmer GeneAmp 9600 thermocycler. Approximately 500 ng of amplified DNA were digested with five units of TaqI restriction enzyme (New England Biolabs) at 65 °C overnight. The resulting products were separated by electrophoresis in a 2.5% agarose gel containing ethidium bromide and visualized under ultraviolet light. Three genotypes are obtained: the A1A2 genotype is revealed by three fragments: 310, 180 and 130 bp. The A2A2 genotype by two fragments: 180 and 130 bp. The A1A1 genotype is shown by the uncleaved 310-bp fragment. A1 alleles are those that either have the A1A1 or A1A2 genotype; A1- allelic subjects have the A2A2 genotype only.

Patient assessments were conducted blind to their DRD2 allelic status. All adverse events were monitored and recorded. Drug compliance was checked by pill count. All participants provided written informed consent and were able to terminate treatment without prejudice. However, those terminating participation were asked to provide reasons for their withdrawal. Institutional ethics approval was obtained from the Greenslopes Private Hospital.

Information coded from the interviews, GHQ and genotyping results were entered into a computer database. Nominal data were analyzed by Yates corrected χ²-test and continuous data by ANOVA. P values ≤0.05 were considered statistically significant. P values >0.05 but <0.10 were considered to be approaching a significant level. An intention-to-treat analysis was used to evaluate the trial.
3. Results

The 63 subjects recruited were all males. They had the following genotypes: A1A2 (A1+ allele), n=25; and A2A2 (A1− allele), n=38. In the present sample there were no subjects with the A1A1 genotype. This genotype distribution did not deviate from Hardy–Weinberg equilibrium ($\chi^2=2.67, P=0.102$). The mean age±S.E. of the 59 patients for whom data were available was 51.4 years±0.7 years. There was no significant difference in the ages of A1+ (51.1±0.8 years) and A1− (51.6±1.0 years) allelic subjects ($F(1,57)=0.88, P=0.77$).

Of the 63 patients who entered the study, 18 (four A1+ and 14 A1− allele) discontinued paroxetine treatment for a variety of reasons. There was a trend for a greater number of A1− allelic subjects to discontinue treatment when compared to A1+ allelic participants ($\chi^2=3.21, P=0.064$). Amongst those who provided reasons for discontinuing the study, one A1+ and six A1− allelic subjects had adverse events relating to anxiety, insomnia, headache or tremor. In addition, one A1+ and two A1− allelic subjects experienced erectile dysfunction, decreased libido or delayed ejaculation.

The 45 subjects who completed treatment were 51.8±0.8 years old. They had the following alleles: A1+, n=22; A1−, n=23. There was no significant difference in the ages of these A1+ (51.4±0.9 years) and A1− (52.2±1.4 years) allelic subjects ($F(1,43)=0.27, P=0.61$).

As indicated earlier, 18 of the initial subjects dropped out of the study for a variety of reasons. The baseline GHQ total score of these subjects was 44.8±3.7 (mean±SD). In the remaining 45 subjects the GHQ total score was 48.0±2.5. There was no significant difference in the GHQ total score between those who dropped out and those who remained in the study ($F(1,63)=0.55, P=0.46$).

Fig. 1 shows the baseline GHQ total and subscale scores of the 63 patients who entered the paroxetine treatment study based on the presence or absence of the DRD2 A1 allele. GHQ total score was significantly higher in A1+ compared to A1− allelic patients ($F(1,62)=4.31, P=0.040$). No significant difference was found in GHQ1 (somatic concerns) subscale score between these two allelic groups ($F(1,62)=0.183, P=0.670$). However, in A1+ compared to A1− allelic subjects, significantly higher subscale scores were found in GHQ2 (anxiety/insomnia, $F(1,62)=4.15$, $P=0.046$), GHQ3 (social
dysfunction, $F(1.62)=4.77, P=0.033$) and GHQ4 (depression, $F(1.62)=6.91, P=0.011$).

Fig. 2 shows the intention-to-treat baseline and treatment GHQ total scores of the 63 patients. A significant improvement was found in the total patients during the course of paroxetine treatment ($F=6.35, P=0.014$). Fig. 2 also presents the baseline and treatment GHQ total scores of those patients based on their allelic status. The results showed no significant difference in improvement in the GHQ total score in A1+ patients compared to A1− subjects ($F=1.402, P=0.241$).

Fig. 3 presents the intention-to-treat analysis results of the four GHQ subscale scores at baseline and after treatment. In the total patient group, there was a trend for GHQ1 (somatic concerns) subscale scores to improve over the course of treatment ($F=3.641, P=0.061$) but there was no difference between allele groups ($F=0.538, P=0.466$). GHQ2 (anxiety) subscale score was significantly reduced in the total patient group over the course of treatment ($F=7.314, P=0.009$). There was no significant difference between allele groups in changes in anxiety ($F=1.641, P=0.205$). There was also a significant improvement in GHQ3 (social dysfunction) subscale score in the total patient group over the course of treatment ($F=4.736, P=0.033$). However, there was a significant difference between allele groups with A1+ allelic subjects experiencing significantly greater improvement than A1− allelic patients ($F=4.903, P=0.031$). Finally, GHQ4 (depression) subscale score revealed a significant improvement in the total patient group during treatment ($F=5.191, P=0.026$).

No significant difference between A1+ allelic subjects and A1− subjects emerged ($F=1.059, P=0.307$).

Whereas, as shown in Fig. 1, significantly higher baseline GHQ total score and its three subscale scores (GHQ2, GHQ3, GHQ4) were found in the A1+ compared to the A1− allelic groups, at the end of paroxetine treatment, no significant differences were found between these allelic groups in any of the GHQ scores measured: GHQ total ($F(1,43)=0.001, P=0.99$); GHQ1 ($F(1,43)=1.5, P=0.23$); GHQ2 ($F(1,43)=0.14, P=0.71$); GHQ3 ($F(1,43)=1.1, P=0.30$); GHQ4 ($F(1,43)=2.5, P=0.12$).

4. Discussion

The present open label trial confirms the effectiveness of paroxetine treatment in reducing psychopathological symptoms in Vietnam veterans with combat-related PTSD (Marshall et al., 1998, 2001; Zygmunt et al., 1998; Brady et al., 2000). This reduction in total symptoms measured was unrelated to patients’ DRD2 allelic status.

At baseline, total psychopathology score was greater in DRD2 A1+ than in A1− allelic patients. In particular A1+ compared to A1− allelic individuals showed significantly higher levels of social dysfunction, anxiety and depression. After 8 weeks of paroxetine treatment three of the four GHQ-28 subscales showed a significant reduction. Furthermore, the social dysfunction subscale showed a significant allele by time interaction. Social dysfunction was significantly reduced in A1+ allelic patients compared
to those with A1− allelic status. At the conclusion of treatment there were no significant differences between these two allelic groups in any of the GHQ scores measured.

The potential mechanism of this reduction in social dysfunction may be related to D2 dopamine receptor physiology. An in vitro study using [3H]spiperone (Noble et al., 1991), as a D2 dopamine receptor ligand, found a significant decrease in the number of D2 dopamine receptors in the brains of subjects with the DRD2 A1+ allele compared to those with the A1− allele. An autoradiographic study (Thompson et al., 1997), using [3H]raclopride as a D2 dopamine receptor ligand, found significantly reduced D2 dopamine receptor binding in the brains of A1+ compared with A1− allelic subjects. In vivo positron emission tomography (PET) studies, using [11C]raclopride, found a significant reduction in brain D2 dopamine receptor density in healthy subjects with the A1+ allele than subjects with the A1− allele (Pohjalainen et al., 1998; Jönsson et al., 1999). Another study (Laruelle et al., 1998) determined D2 dopamine receptor binding potential in healthy controls and in schizophrenic patients using [123I]IBZM. No significant difference in this combined sample was found in the D2 dopamine receptor binding potential between A1+ and A1− allelic subjects. However, when the controls and schizophrenics were separately examined, a trend for a lower binding potential was found in A1+ allelic controls, whilst a trend for a higher binding potential was noted in A1+ allelic schizophrenics when compared to their respective A1− allelic subjects. Since two of the above studies (Pohjalainen et al., 1998; Laruelle et al., 1998) appeared in the same journal issue, an editorial (Hitzemann, 1998) reviewed their merits. It suggested that the study using [123I]IBZM (Laruelle et al., 1998) had insufficient power to detect a significant difference between A1+ and A1− allelic controls. Moreover, since schizophrenic patients showed a trend in the opposite direction than controls, the results on D2 dopamine receptor binding potential and allelic association in schizophrenic subjects may have been confounded by prior neuroleptic treatment. Indeed, in a recent PET study (Silvestri et al., 2000) using [11C]raclopride, increased D2 dopamine receptor binding was shown in schizophrenic patients subsequent to neuroleptic treatment.

Low D2 dopamine receptor binding is associated with the personality feature of harm avoidance (Yasuno et al.,...
and patients with social phobia have low D2 dopamine receptor binding potential (Schneider et al., 2000). Moreover, low D2 dopamine receptor density is also associated with depressive personality features (Kessler et al., 2000). Animal studies have shown that subordinate female cynomolgus monkeys who are fearful and disengaged in social events have decreased D2 dopamine receptor binding and demonstrate pathological behaviors suggestive of underlying anxiety (Shively et al., 1997). These human and animal studies are consistent with the baseline characteristics of those with the A1 allele reporting higher anxiety, social dysfunction and depression.

SSRIs have a significant and complex impact on dopaminergic function but generally have a marked inhibitory effect on dopamine release. While stimulation of 5-HT 1A and 5-HT 1B receptors facilitate dopamine release, stimulation of 5-HT 2A receptors inhibit dopamine release (Ng et al., 1999; Rollema et al., 2000; Gobert et al., 2000). Paroxetine down-regulates 5 HT 2A receptors in young depressed patients indicating significant agonist stimulation (Meyer et al., 2001). In rat studies, fluoxetine administration produces a 60–70% decrease in extracellular dopamine levels in the caudate putamen, and nucleus accumbens (Clark et al., 1996) as well as a 41% reduction in the rat striatum (Yamato et al., 2001). Decreased mesolimbic dopamine release in subjects taking SSR1 antidepressants has been linked to the commonly found adverse effects of decreased libido and anorgasmia (Hull et al., 1999). SSR1 induced increase in extrapyramidal serotonin levels and consequent inhibition of the dopaminergic pathways controlling movement has been hypothesized to cause SSR1 induced bruxism (Bostwick and Jaffee, 1999). Similarly diminished striatal extracellular levels of dopamine and its metabolites have been linked to a parkinsonian syndrome induced by sertraline (Di Rocco et al., 1998). Other extrapyramidal reactions associated with SSRIs include dystonias, dyskinesias, akathisia, exacerbation of Parkinson’s disease and possibly neuroleptic malignant syndrome (Caley, 1997). Other effects due to diminished dopaminergic neurotransmission include hyperprolactinemia (Goodnick et al., 2000), galactorrhea (Bonin et al., 1997) as well as breast tenderness and enlargement (Hall, 1994).

Subjects with low D2 dopamine receptor binding are likely to respond to paroxetine via upregulation of mesolimbic D2 dopamine receptors as a consequence of inhibition of dopamine release. Therapeutic actions of drugs and their adverse effects are due to neurotransmitters activating genes in target neurons (Stahl, 1999). For example, fluoxetine causes an increase in nucleus accumbens shell D2 dopamine receptor mRNA resulting in increased D2 dopamine receptor postsynaptic binding in the nucleus accumbens (Ainsworth et al., 1998). Similarly, the SSRI citalopram increases D2 dopamine receptor mRNA in the striatum and nucleus accumbens (Dziedzic-Wasylewska et al., 1997). This effect of citalopram on increased transcription of the D2 dopamine receptor gene is mediated by increased serotonin levels induced by SSRIs, as 5-hydroxytryptophan also increases D2 dopamine receptor mRNA transcription (Kameda et al., 2000).

Increased D2 dopamine receptor gene expression and consequent upregulation of D2 receptors is likely to be the mechanism by which paroxetine improves social dysfunction. Low pretreatment D2 dopamine receptor density may be a requirement for effective treatment. Conversely high pretreatment D2 dopamine receptor density may result in a lack of improvement in social dysfunction. In a PET study of depressed subjects [123I]IBZM binding was significantly lower in treatment responders at baseline and increased over time. Amongst non-responders, baseline IBZM binding was significantly higher (Klimke et al., 1999).

There are some limitations to this study. The study was an open label trial and hence the influence of both patient and clinician expectancy cannot be controlled. However, as both patient and clinician were blind to the patient’s DRD2 allelic status, the results are unlikely to be biased by expectancy effects. Laboratory staff were also blind to behavioral data until after allelic determination for all subjects was completed. The sample size is modest, however, the significance of the results indicates a large effect size. The subjects were all males and the relevance of the findings to females remains unknown. Equally, these results may also generalize to the treatment of other disorders characterised by social dysfunction, such as recurrent major depression, social phobia and avoidant personality disorder.

In sum, PTSD patients with the DRD2 A1 allele, in contrast to those without this allele, showed a significant positive response to paroxetine treatment with particular respect to social dysfunction. The study suggests that the A1 allele may be a useful marker to assist clinicians in determining which patients with PTSD are likely to experience improved social functioning with paroxetine.

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