International Journal of Neuropsychopharmacology (2012), **15**, 1043–1050. © CINP 2011
doi:10.1017/S1461145711001532

ARTICLE

CINP

Peripheral oxidative damage in early-stage mood disorders: a nested population-based case-control study

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Abstract

Systemic toxicity is a relevant dimension of pathophysiology in bipolar disorder, and oxidative damage is one potential link between central and peripheral pathology. Although there is mounting evidence that chronic bipolar disorder is associated with oxidative stress, studies in the early stages of bipolar disorder are scarce, and heavily reliant on clinical *in lieu* of population studies. The objective of this study was to confirm leading hypotheses about the role of oxidative damage in bipolar disorder. To that end, we nested a case-control study in a population-based study of young adults aged 18–24 yr. After an initial psychopathology screen, all people with a lifetime history of (hypo)mania and matched controls underwent a structured diagnostic interview. This yielded a sample of 231 participants, in whom we measured serum protein carbonyl content (PCC) and thiobarbituric acid reactive substances (TBARS). People with bipolar disorder had higher PCC levels than healthy subjects. Those with major depression were not different from control subjects in either PCC or TBARS levels. Both bipolar disorder and major depression were associated with higher PCC levels in the *a priori* regression model controlling for possible confounders. These findings indicate that protein oxidative damage is present from early stages and can be seen as a sign of early illness activity in mood disorders.

Received 21 April 2011; Reviewed 8 June 2011; Revised 11 August 2011; Accepted 2 September 2011; First published online 19 October 2011

Key words: Bipolar disorder, major depression, oxidative stress, population-based study, protein carbonyl content, thiobarbituric acid reactive substances.

Introduction

There is growing interest in systemic pathophysiology as a relevant dimension of bipolar disorder (Kapczinski *et al.* 2008; Kupfer, 2005). This facet is thought of as mediating the frequently ensuing illness progression, resulting in medical comorbidity, cognitive deficits, functional impairment and, ultimately, premature mortality (Berk, 2009; Berk *et al.* 2010; Kapczinski *et al.* 2008). Recent research has indeed shown that several systemic markers are altered in patients with established bipolar disorder (Kapczinski *et al.* 2010, 2011). Oxidative stress biomarkers are prominent among these (Andreazza *et al.* 2008; Berk *et al.* 2011; Ng *et al.* 2008).

Allostasis has been a relevant paradigm for understanding how illness progression can be related to poor outcomes in mood disorders (Kapczinski *et al.* 2008; McEwen, 2003). Although allostasis promotes adaptation, when its mediators are not turned off or are overused by excessive challenge, the cumulative load leads to wear and tear of body and brain (McEwen & Gianaros, 2011). As such, several peripheral markers have been implicated in bipolar disorder as mediators of allostasis (Berk *et al.* 2011; Juster *et al.* 2010; Kapczinski *et al.* 2008).

Oxidative imbalance and damage have been repeatedly demonstrated in patients with bipolar disorder (Andreazza *et al.* 2008; Berk *et al.* 2011; Ng *et al.*

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2008). Available evidence points to extensive lipid, protein (Kapczinski *et al.* 2011) and DNA damage (Andreazza *et al.* 2007) in such patients compared to healthy control subjects. This is highly relevant, since pro-oxidant states may link central and peripheral pathophysiology (Gigante *et al.* 2010). Possibly by altering the permeability of the blood–brain barrier, peripheral oxidative stress has been demonstrated to effect significant brain toxicity (Chaudhary & Rao, 2010; Gilgun-Sherki *et al.* 2001).

Thus far, however, research has usually focused on chronic patients treated in tertiary centres. With a high cumulative illness burden, several biomarkers tend to be altered in a highly correlated manner (Kapczinski *et al.* 2010). This is concordant with the view of complexity in allostatic systems. Accordingly, studies in late-stage samples often reveal complex multivariate associations between disparate markers (Kapczinski *et al.* 2010, 2011). Although these studies confirm that late stage is associated with systemic toxicity and neuroprogression (Berk *et al.* 2011), primary and secondary pathology cannot be teased apart. As a corollary, early disease would be a more developmentally appropriate period to understand primary illness changes (Berk *et al.* 2009). Furthermore, current biomarker discovery in bipolar disorder relies heavily on clinical samples. Community samples avoid the selection bias that is inherent to studies in individuals who seek treatment (McDade *et al.* 2007).

The objective of the current study was to confirm leading hypotheses about the role of oxidative stress in bipolar disorder. To that end, we nested a case-control study in a population-based study of young adults aged 18–24 yr. Every individual with a positive screen for bipolar disorder was invited to participate, as well as matched controls with only depressive episodes and without mood episodes; serum markers of oxidative damage to proteins and lipids were collected. In this manner, we could test whether oxidative damage is present in early disease stages in mood disorders with the advantage of a representative population-based sample.

Methods

This is a case-control study nested in a population-based cross-sectional study. Full details on the original study have been published elsewhere (Jansen *et al.* 2011). Briefly, the sample consisted of 1560 participants aged 18–24 yr living in urban Pelotas, Brazil. Sample selection was performed by clusters from August 2007 to December 2008, considering a population of 39 667 people in that age range in the current census of 448 sectors in the city. From these, 89 census-based sectors were systematically drawn. Individuals provided written informed consent and answered a questionnaire on socio-demographic data, drug misuse, and a diagnostic interview. The study was approved by the Ethics Committee of the Catholic University of Pelotas (UCPel).

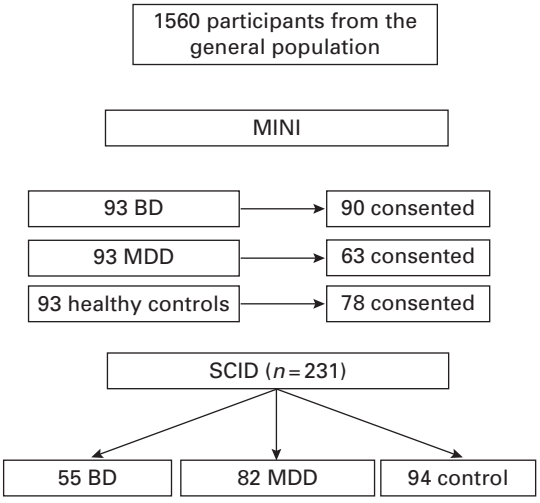

Fig. 1. Flow chart showing patient inclusion in the case-control study. MINI, Mini-International Neuropsychiatric Interview; BD, bipolar didorder; MDD, major depressive disorder; SCID, Structured Clinical Interview for DSM-IV.

As an initial psychopathology screen, the whole population underwent the Mini-International Neuropsychiatric Interview (Sheehan *et al.* 1998). For the purposes of the current study, cases were those with a past or current history of a manic episode from the population-based study. Ninety-three individuals met this criterion. Additionally, two groups of control subjects were recruited. People without any history of affective disorder were randomly selected and matched for sex, age and socioeconomic situation – i.e. a healthy control sample. Importantly, we did not exclude people from this control group on account of any other mental disorders or clinical morbidity. We also recruited a second control group, those with a current depression but no past history of (hypo)mania. This was, thus, an active control group. From these, 231 individuals provided informed consent (83% of the intended sample; see Fig. 1).

The whole case-control sample further underwent the Structured Clinical Interview for DSM-IV (SCID). This was performed to confirm diagnoses and improve reliability, and is the group-defining criterion for this study. The SCID interviews were undertaken by two master's-level psychologists at the laboratory

of the Health and Behavior Post-Graduate Program in Pelotas. They had intensive training in the specialist outpatient facilities at the Hospital de Clínicas de Porto Alegre under supervision of one of the senior investigators (F.K). After SCID diagnoses, the final sample for the case-control study consisted of 94 control subjects, 82 participants with major depression and 55 with bipolar disorder (33 type I and 22 type II).

Immediately after the participants' blood was drawn, serum samples were obtained by centrifugation and kept frozen at −80 °C until biochemical assays were performed. Samples were assayed by laboratory technicians blinded to the clinical characteristics of participants. The levels of lipid peroxidation were measured using the thiobarbituric acid reactive substances (TBARS) method as previously described (Wills, 1966). TBARS consists of an acid-heating reaction of the lipid peroxidation end product, malondialdehyde, with thiobarbituric acid (TBA). The samples were mixed with 10% TCA and 0.67% TBA, and heated in a boiling water bath for 15 min. Butanol (2:1, v/v) was added and, after centrifugation (800 *g*, 5 min), TBARS was determined by monitoring the absorbance at 532 nm. Results were compared to a standard curve of TMP (1,1,3,3-tetramethoxypropane) and expressed as malondialdehyde (MDA) equivalents (nmol/mg protein) (Draper & Hadley, 1990). Oxidative damage to proteins was measured by the determination of carbonyl groups (protein carbonyl content; PCC) based on the reaction with dinitrophenylhydrazine (DNPH) (Levine *et al.* 1990). In this, proteins are precipitated by the addition of 20% TCA, re-solubilized in DNPH, and the absorbance read in a spectrophotometer at 370 nm. Values are expressed as nmol/mg protein. Both have been extensively performed previously at the Biochemistry Department of the Centre for Studies in Oxidative Stress (da Rocha *et al.* 2010; Oliveira *et al.* 2010; Pasquali *et al.* 2010; Rosemberg *et al.* 2010). These are traditional markers of oxidative damage that have been repeatedly employed in patients with mood disorders (Andreazza *et al.* 2008, 2009; Giustarini *et al.* 2009; Kapczinski *et al.* 2011). Of the 231 participants for whom a serum sample was available, in 11 samples TBARS levels could not be analysed and in 58 samples (19 controls, 32 major depression, 7 bipolar disorder) the same was true for PCC levels.

Socioeconomic status was evaluated according to the Brazilian Association of Research Companies (ABEP, 2008) classification, which is based on the total of material goods and the householder's schooling. It was further dichotomized into high (classes A, B, C) and low (classes D, E). Clinical illness was self-reported. Information on drug misuse was obtained with the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST), validated to Portuguese (Henrique *et al.* 2004).

Statistical analysis

One-way analysis of variance was used to test for between-group differences in continuous variables and χ^2 tests were used for differences between proportions.

We constructed fully-fledged *a priori* multivariate models to test for differences in PCC and TBARS. We preferred using a theoretical instead of a data-driven approach since it avoids overfitting (Babyak, 2004; Harrell *et al.* 1996). These should be seen as the main results of this study. In the models, we control for features of theoretical relevance or empirically associated with serum biomarker levels in previous studies. Specifically, in addition to diagnostic status, the model included sex and social class (Gianaros & Manuck, 2010; Hackman *et al.* 2010; Ortona *et al.* 2008), self-reported clinical illness (Kapczinski *et al.* 2008), smoking, alcohol or illicit substance abuse (Ng *et al.* 2008), current depression and mania (Kapczinski *et al.* 2010, 2011). Bipolar I and II disorder were placed in the same category mainly because of lack of power to investigate them separately. Diagnoses were entered in the model as 'dummy variables', with the control category as reference.

Linear regression with bias-corrected accelerated bootstrapping with 2000 resamples was used with the predictors mentioned above for the two models. Bootstrapping in this case has the advantage of being more robust when handling data for which the population distribution is unclear (Henderson, 2005) and for testing the validity of the model employed (Harrell *et al.* 1996).

Results

Subjects ($n=231$) were comparable regarding age and years of education, but women were underrepresented in the controls. Current use of psychiatric medications was very low, with only 9.6% of the sample reporting current use of any medications. Table 1 shows demographic and clinical information according to diagnosis.

PCC and TBARS serum levels were correlated in the sample ($\rho=0.19$, $p=0.017$). Those with bipolar disorder had higher PCC levels than healthy subjects ($F=3.95$, $p=0.049$). TBARS levels, however, did not significantly differ ($F=0.72$, $p=0.397$). Those with

Table 1. Sample demographic, clinical, and treatment characteristics according to SCID diagnosis

Characteristic	Healthy controls ($n=94$)	Major depression ($n=82$)	Bipolar disorder ($n=55$)
Age (yr)	22.4 ± 0.23	21.8 ± 0.22	21.7 ± 0.31
Female sex*	58%	77%	74%
Years of education	9.7 ± 3.1	8.9 ± 2.8	8.8 ± 3.6
Low socioeconomic status (D, E)	14%	22%	15%
Self-reported clinical illness	28%	33%	46%
Current medication			
Mood stabilizers	0%	3%	4%
Antipsychotics	0%	0%	4%
Antidepressants	1%	3%	6%
Benzodiazepines	0%	1%	4%
Any*	2%	12%	19%
Current depressive episode	0%	77%	76%
Current manic episode	0%	0%	20%
Age at onset (yr)	n.a.	16.9 ± 3.8	15.3 ± 4.5
Lifetime tobacco misuse*	25%	38%	46%
Lifetime alcohol misuse	27%	38%	38%
Lifetime illicit substance misuse	10%	23%	16%
Previous hospitalizations	1%	6%	9%
Protein carbonyl content	0.307 ± 0.031	0.388 ± 0.0436	0.416 ± 0.049
Thiobarbituric acid reactive substances	0.0159 ± 0.0002	0.0148 ± 0.0020	0.0190 ± 0.0031

SCID, Structured Clinical Interview for DSM-IV; n.a., not available.
Some patients did not recall the medication they were taking, hence 'any medication' figure is higher than sum of individual medications.
* $p<0.05$; continuous values displayed as mean ± S.E.M.

major depression were not different from control subjects in either PCC ($F=2.44$, $p=0.121$) or TBARS levels ($F=0.12$, $p=0.725$). Finally, the two mood disorder groups could not be differentiated by PCC ($F=0.18$, $p=0.669$) or TBARS ($F=1.43$, $p=0.235$) levels. Within the mood disorder groups, duration of illness did not correlate with either TBARS ($\rho=0.13$, $p=0.186$) or PCC ($\rho=-0.06$, $p=0.564$).

Serum PCC levels were further associated with a current manic episode ($F=4.43$, $p=0.036$), but not with a current depressive episode ($F=0.57$, $p=0.451$). Serum TBARS levels were not associated with mania ($F=0.18$, $p=0.671$) or depression ($F=0.61$, $p=0.434$). Current use of medication was not associated with PCC ($F=0.47$, $p=0.493$) or TBARS levels ($F=0.32$, $p=0.570$).

The *a priori* regression model kept both bipolar disorder ($\beta=0.199$, bias $=-0.005$, S.E. $=0.080$, $p=0.014$) and major depression ($\beta=0.200$, bias $=-0.004$, S.E. $=0.083$, $p=0.012$) as predictors of higher PCC levels. None of the variables in the model, however, was able to predict TBARS levels (Table 2).

Discussion

This study indicates that oxidative protein damage is present from the early stages in mood disorders. Young adults with bipolar disorder had higher serum levels of a marker of protein damage than participants free of mood disorders. The multivariate model also pointed to significantly increased damage to proteins in major depression.

These changes were independent of mood state at interview. The effects on protein damage were found for diagnosis, not for state (in fact, current depression was associated with lower PCC levels in the multivariate model). While we have previously reported oxidative damage to be associated with illness activity (Kapczinski *et al*. 2010, 2011), those findings were on clinical samples during severe mood episodes. This may be necessary to reveal state-related alterations (Magalhaes *et al.* 2011). The findings were also independent of illicit drug use. Misuse of any substance other than alcohol or nicotine was also related to less protein damage, and only in the multivariate model.

Table 2. Multivariate model predicting protein carbonyl content (PCC) and thiobarbituric acid reactive substances (TBARS) serum levels

	PCC		TBARS	
Variable	*B*	95% CI	*B*	95% CI
Sex	0.120*	0.04 to 0.20	−0.005	−0.011 to 0.001
Low socioeconomic status	0.070	−0.05 to 0.19	−0.002	−0.006 to 0.003
Smoking	−0.057	−0.15 to 0.03	0.003	−0.003 to 0.009
Alcohol abuse	0.028	−0.09 to 0.16	0.007	−0.003 to 0.013
Abuse of illicit drugs	−0.137*	−0.25 to −0.03	−0.002	−0.009 to 0.007
Clinical illness	0.024	−0.07 to 0.13	−0.002	−0.007 to 0.002
Current depression	−0.169*	−0.32 to 0.01	0.001	−0.011 to 0.010
Current mania	0.203	−0.07 to 0.47	0.001	−0.014 to 0.018
Major depression	0.200*	0.04 to 0.34	−0.003	−0.012 to 0.008
Bipolar disorder	0.199*	0.06 to 0.35	0.001	−0.009 to 0.013

CI, Confidence interval.
Linear regression with bias-corrected accelerated bootstrapping.
* $p < 0.05$.

The current study, however, was not designed to assess the effects of substance dependence, which were investigated as possible confounders. Substance abuse is generally thought to enhance oxidative imbalance (Ng *et al.* 2008), but there is very little literature on the effect of drug misuse on PCC levels in clinical populations.

One attractive hypothesis that links neuroplasticity and oxidative stress to neuroprogression and medical burden is that of mitochondrial dysfunction (Chen *et al.* 2010; Kato & Kato, 2000). Aberrations in the mitochondrial electron chain have been demonstrated in bipolar disorder, centrally and in the periphery, in patients and animal models (Andreazza *et al.* 2010; Cataldo *et al.* 2010; Frey *et al.* 2006; Valvassori *et al.* 2010). In one study, bipolar disorder was associated with decreased complex I activity in the prefrontal cortex (Andreazza *et al.* 2010). This was, in turn, correlated with protein carbonylation, providing a basis for the protein damage observed in bipolar disorder. This pathology is probably part of an intricate and complex network of dysfunctional regulatory systems operating in bipolar disorder (Juster *et al.* 2010; Kapczinski *et al.* 2008). Hyperdopaminergic states, one of the salient features of the condition, induce oxidative damage in the central nervous system (Berk *et al.* 2007). Oxidative stress and inflammation have further reciprocal relations that can lead to neuronal toxicity and damage (Yamamoto & Raudensky, 2008).

Protein carbonyl derivatives are broad markers of oxidation. They are usually considered markers of protein dysfunction, not only oxidative stress (Dalle-Donne *et al.* 2003). Protein damage can have a myriad of downstream consequences, from loss of functional properties to apoptosis and necrosis (Aldini *et al.* 2007). Elevated protein carbonyl levels have been demonstrated to predict adverse clinical outcomes in diverse samples. This includes persistence of illness activity in lupus, an association with colorectal cancer and a greater risk of mortality in elderly women (Morgan *et al.* 2009; Semba *et al.* 2007; Yeh *et al.* 2010). In the central nervous system, protein damage is probably one of the allostatic mechanisms leading to cognitive dysfunction and illness progression (Berk *et al.* 2011; Kapczinski *et al.* 2008).

It is unclear at this time why we detected protein but not lipid damage. The method of Wills (1966) has been criticized for having specificity difficulties when applied to body fluids (Halliwell & Chirico, 1993). This may partly explain the absence of a significant difference in this sample. Nevertheless, elevated levels of TBARS are among the most consistently replicated findings in the peripheral pathophysiology of people with mood disorders (Andreazza *et al.* 2009; Maes *et al.* 2010*b*). In treated patients, TBARS levels have been repeatedly shown to be elevated when measured in serum (Andreazza *et al.* 2007; Machado-Vieira *et al.* 2007; Ozcan *et al.* 2004). One possibility is that protein carbonyl derivatives are more sensitive to early oxidative damage because they circulate for longer periods than lipid peroxidation products (Dalle-Donne *et al.* 2003). Another possibility is that these markers of damage are related to an impaired

antioxidant defence system (Schwedhelm *et al.* 2003). As demonstrated recently, the cell has evolved mechanisms to distinctively deal with different classes of reactive oxygen species (Sedlak *et al.* 2009). Thus, damage to lipids and protein could follow impairment in different pathways. Again, most previous studies are heavily weighted towards late-stage patients. This might suggest that lipid damage is characteristic of neuroprogression, but a comparative design is necessary to establish that. In the one previous comparative study, systemic changes in early-stage patients were indeed more subtle than those at late-stage (Andreazza *et al.* 2009; Kauer-Sant'Anna *et al.* 2009).

In spite of the obvious advantages of population samples of young adults, this is a report on cross-sectional data. Therefore if it is possible to assert that oxidative damage is associated with early-stage mood disorders, longitudinal research is necessary to establish causality. This study may also have been underpowered to detect subtle state-related changes in biomarkers of oxidative damage. As this study was designed to detect oxidative damage in bipolar disorder, it is difficult to evaluate the meaning of protein damage in major depression. Hypotheses regarding the role of oxidative stress in this condition have been put forward, but far fewer studies are available (Maes *et al.* 2010*a*).

These differences in the level of protein damage can be seen as a sign of early illness activity in mood disorders. The findings here reinforce an already very consistent body of work indicating that oxidative imbalance is a prominent node in the chain of events leading to disease progression in bipolar disorder.

Acknowledgements

This work was awarded the Cyro Martins prize by the Associação de Psiquiatria do Rio Grande do Sul. Dr Jansen was supported by a post-doctoral scholarship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil. Professors Pinheiro and Silva were supported by scholarships from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Statement of Interest

Professor Klamt has received grant support from PRONEX/FAPERGS. Professor Kapczinski has received grant/research support from AstraZeneca, Eli Lilly, Janssen-Cilag, Servier, CNPq, CAPES, NARSAD and the Stanley Medical Research Institute; he has been a member of the speakers' boards for AstraZeneca, Eli Lilly, Janssen and Servier; and has served as a consultant for Servier.

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