

Available online at www.sciencedirect.com

PSYCHIATRY
RESEARCH

Psychiatry Research xx (2007) xxx–xxx

www.elsevier.com/locate/psychres

DNA damage in bipolar disorder

Ana Cristina Andreazza^{a,b}, Benicio Noronha Frey^{a,b}, Bernardo Erdtmann^c,
 Mirian Salvador^c, Fernanda Rombaldi^c, Aida Santin^b,
 Carlos Alberto Gonçalves^a, Flavio Kapczinski^{b,*}

^a Department of Biochemistry, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul. Rua Ramiro Barcelos, 2600/Anexo. Zip code: 90035-003. Porto Alegre, Brazil

^b Bipolar Disorders Program, Centro de Pesquisas, Hospital de Clínicas de Porto Alegre. Rua Ramiro Barcelos, 2350. Zip code: 90035-003. Porto Alegre, RS, Brazil

^c Institute of Biotechnology, Universidade de Caxias do Sul. Rua Francisco Getúlio Vargas, 1130. Zip code: 95070-560. Caxias do Sul, Brazil

Received 30 January 2006; accepted 20 March 2006

Abstract

Bipolar disorder (BD) is a prevalent, chronic, severe, and highly disabling psychiatric disorder that is associated with increased morbidity and mortality due to general medical conditions. There is an emerging body of evidence correlating chronic medical conditions with DNA damage. The present study was designed to assess DNA damage in BD patients using the comet assay (CA). Thirty-two bipolar-I outpatients diagnosed using the Structured Clinical Interview for DSM-IV were matched with 32 healthy volunteers. Manic and depressive symptoms were assessed using the Young Mania Rating Scale and the Hamilton Depression Rating Scale, respectively. Peripheral blood samples were collected and a standard protocol for CA preparation and analysis was performed. The present study showed that BD outpatients present an increased frequency of DNA damage relative to controls. The frequency of DNA damage correlated with the severity of symptoms of depression and mania.

© 2006 Elsevier Ireland Ltd. All rights reserved.

Keywords: Bipolar disorder; Comet assay; DNA damage; Oxidative stress; Mania; Depression

1. Introduction

Bipolar disorder (BD) is a prevalent, chronic, severe, and highly disabling psychiatric disorder (Belmaker, 2004). BD is considered one of the leading causes of disability amongst all medical and psychiatric conditions (Murray and Lopez, 1997), and studies have demonstrated that the annual costs of BD range between 1.8 and 45 billion US dollars, mainly reflecting indirect costs

attributable to work loss (Das Gupta and Guest, 2002; Hakkaart-van Roijen et al., 2004). Moreover, BD is associated with increased morbidity and mortality due to general medical conditions, such as cardiovascular disease, obesity and diabetes mellitus (Kupfer, 2005).

There is an emerging body of evidence correlating chronic medical conditions to DNA damage (Faust et al., 2004). The single cell gel electrophoresis technique, also called the Comet assay (CA), is a rapid method for assessing DNA damage quantitatively in single cells (Maluf and Erdtmann, 2000). Under alkaline conditions, the CA detects DNA double- and single-strand breaks and alkali-labile sites (Tice et al., 2000). The CA

* Corresponding author. Tel.: +55 51 32227309; fax: +55 51 21018846.

E-mail address: kapcz@terra.com.br (F. Kapczinski).

technique has been increasingly used for human biomonitoring studies (Kassie et al., 2000). As a biomarker of genotoxicity, the CA may identify groups of individuals at higher risk to develop degenerative diseases (Migliore et al., 2005).

Free radicals (especially OH^\cdot) may initiate DNA damage (Cooke et al., 2006; Culmsee and Mattson, 2005), which under certain circumstances may lead to DNA mutation. Multiple systems exist to prevent lesion formation, and should damage occur, ensure rapid lesion removal (Cooke et al., 2006). DNA damage may activate several intracellular signaling pathways such as the phosphorylation of p53 at the serine and threonine residues, leading to cell apoptosis (Evan and Littlewood, 1998; Culmsee and Mattson, 2005). In the only study to date that directly evaluated DNA fragmentation in BD subjects, no changes in single-stranded DNA breaks were found in the anterior cingulate cortex in a postmortem study (Benes et al., 2003). Benes et al. (2003) suggested that changes in the intracellular signaling pathways within the mitochondria may be associated with apoptotic cell death in response to oxidative stress.

2. Material and methods

2.1. Bipolar patients

This was a case-control study of 32 patients with BD, 18 years of age or older (43.28 ± 12.72), consecutively assessed from December 2003 to May 2005. All patients were recruited from the Bipolar Disorders Program-Federal University of Rio Grande do Sul, Porto Alegre, Brazil. Patients were non-smokers, did not present active medical conditions and were not on medication, apart from those prescribed for their psychiatric disorder. BD diagnoses were made using the Structured Clinical Interview for DSM-IV–Axis I (SCID-I) (First et al., 1998). Manic and depressive symptoms were assessed using the Young Mania Rating Scale (YMRS) (Young et al., 1978) and the Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960), respectively. This study was approved by the local ethics committee, and all subjects provided the informed consent before entering in the study.

2.2. Control group

The control group was composed of healthy subjects ($n=32$) who manifested interest in participating in the study. In addition, controls did not have history of major psychiatric disorders, dementia, mental retardation or cancer in their first-degree relatives. Control

subjects were non-smokers and were not on medication. All subjects were briefed about the procedure and gave written informed consent before entering in the study.

2.3. Comet assay

A standard protocol for CA preparation and analysis was followed (Singh et al., 1988; Tice et al., 2000). The slides were prepared by mixing 5 μl whole blood with 90 μl low melting point agarose (0.75%). The mixture (cells/agarose) was added to a fully frosted microscope slide coated with a layer of 300 μl of normal melting agarose (1%). After solidification, the cover slip was gently removed and the slides were placed in lyses solution (2.5 M NaCl, 100 mM EDTA and 10 mM Tris, pH 10.0–10.5, with freshly added 1% Triton X-100 and 10% dimethyl sulfoxide [DMSO]) for a minimum of 1 h and a maximum of 7 days. Subsequently, the slides were incubated in freshly prepared alkaline buffer (300 mM NaOH and 1 mM EDTA, pH 12.6) for 20 min. The DNA was electrophoresed for 20 min at 25 V (0.90 V/cm) and 300 mA; the buffer was neutralized with 0.4 M Tris (pH 7.5). Finally, the DNA was stained with nitrate of silver. The slides were coded for blind analysis.

Negative and positive controls were used for each electrophoresis assay to ensure the reliability of the procedure. Images of 100 randomly selected cells (50 cells from two replicated slides) were analyzed from each patient. Cells were also scored visually according to tail size into five classes, from no tails (0), to maximally (4), resulting in a single DNA damage score for each subject, and consequently for each study group. Therefore, a group damage index could range from 0 (all cells no tail, 100 cells \times 0) to 400 (all cells with maximally long tails, 100 cells \times 4) (Collins et al., 1996, 1997) (Fig. 1). The damage index was calculated based on the number of cells with tails vs. those without.

2.4. In vitro assay

An in vitro analysis was carried out to assess whether the drugs used by the patients could induce DNA damage. Drugs were incubated with peripheral blood (provided by a healthy volunteer) for 30 min, 37 °C. Drugs were diluted at therapeutic levels in water (lithium, valproate, amitriptyline, lamotrigine, sertraline, sulpiride, levomepromazine) or ethanol 0.1–0.5% (carbamazepine, fluoxetine, haloperidol, diazepam, clonazepam). The assay was carried out using the described therapeutic levels (British Pharmacopoeia, 1999). Peripheral blood was used as a negative control. DNA damage was evaluated using the

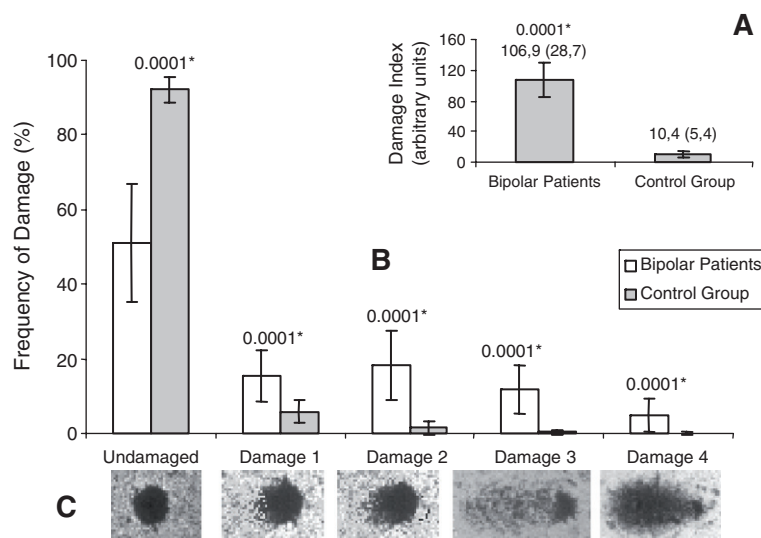


Fig. 1. DNA damage assessed using the single cell gel electrophoresis comet assay. * Mann–Whitney *U*-test, $P < 0.0001$. A: Index of DNA damage in patients and controls. B: Frequency of type of DNA damage in patients and controls. C: Evaluation of DNA damage using silver nitrate (200 \times). The cells were assessed visually and received scores from 0 (undamaged) to 4 (maximally damaged), according to the size and shape of the tail. Scores were obtained using the mean score of two independent, blind evaluators.

CA as described previously. The samples were analyzed in duplicate.

2.5. Statistical analysis

The analysis was carried out using Statistical Product and Service Solutions 12.0 Version (SPSS). The Kolmogorov–Smirnov Test was used to compare the observed cumulative distribution function to a theoretical cumulative distribution. Statistical differences between controls and treated samples were determined by the nonparametric Mann–Whitney *U*-test based on the score each subject received in the CA. Relationships between variables were assessed using Spearman’s product-moment correlation coefficient. Data are presented as means \pm S.D.

3. Results

BD patients and the control group did not differ in terms of demographic data (Table 1). The BD patients were currently medicated, mainly with lithium and/or valproate. DNA damage was markedly increased in BD patients. The frequency of DNA damage type-I, II, III and IV was higher in patients than controls, and the control group presented a higher frequency of undamaged DNA (Fig. 1). Gender did not influence the amount of DNA damage within the patient group (Mann–Whitney *U*-test Test=102.50, $Z = -0.042$, $P(2\text{-tailed}) = 0.967$). Depressive ($r = 0.45$, $P = 0.01$) and manic

($r = 0.62$, $P = 0.001$) symptoms correlated with the index of DNA damage in BD patients. A post hoc analysis showed that the index of DNA damage in BD

Table 1

Demographic factors of patients and controls

Variable	Bipolar patients (n=32)	Controls (n=32)	P
<i>Sex</i>			
Men	28.1%	40.6%	0.292 ^a
Women	71.9%	59.4%	
<i>Age (years)</i>			
Mean (S.D.)	43.28 (12.72)	43.47 (14.24)	0.956 ^b
<40	41.6%	42.0%	0.523 ^a
40–59	52.8%	51.5%	
≥ 60	5.6%	6.5%	
<i>Family income (US\$/month)</i>			
Median	610	538	0.994 ^c
1st tercile	16.2%	13.2%	0.854 ^a
2nd tercile	53.0%	50.0%	
3rd tercile	30.8%	36.8%	
<i>Years of schooling</i>			
Mean (S.D.)	9.15 (4.86)	9.16 (3.30)	0.992 ^b
<i>Hamilton Depression Rating Scale</i>			
Mean (S.D.)	6.31(7.38)	–	
<i>Young Mania Rating Scale</i>			
Mean (S.D.)	3.62 (3.01)	–	

^a Chi-square test.

^b *T*-test.

^c Kruskal–Wallis test.

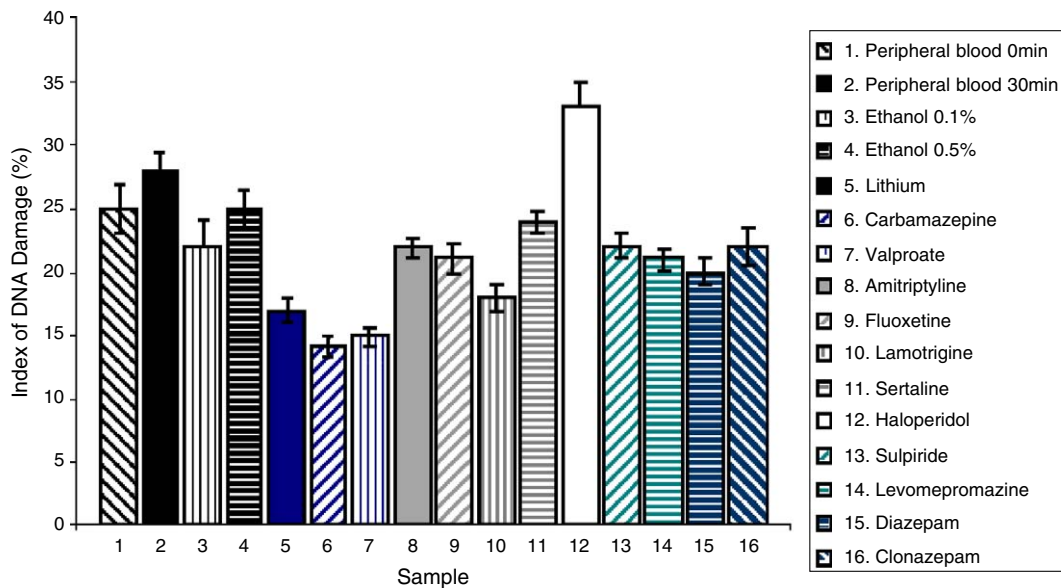


Fig. 2. DNA damage index by drug in peripheral blood.

patients did not correlate with the number of medications, number of hospitalizations, number of suicide attempts, length of illness or number of years undiagnosed. The patients were using an average of 2.65(1.15) medications. Drugs used included lithium, valproate, carbamazepine, fluoxetine, amitriptyline, sertraline, lamotrigine, haloperidol, levomepromazine, sulpiride, diazepam and clonazepam. These drugs did not induce DNA damage (ANOVA test; $F=0.789$; $P=0.425$) (Fig. 2).

4. Discussion

The present study showed that BD outpatients differ from controls in the frequency of DNA damage in peripheral cells. As far as we are aware, this is the first study to report these findings. These results add to the notion that BD patients are exposed to deleterious systemic changes (Kupfer, 2005).

Chronic illnesses such as obesity, diabetes mellitus and cancer have been shown to be associated with increased DNA damage (Faust et al., 2004). BD involves a combination of manic and depressive episodes, which can alternate or even occur simultaneously. In outpatient samples, along with euthymic subjects, an array of depressive and manic symptoms is likely to occur in a significant proportion of cases of BD (Belmaker, 2004). In the present study, the severity of manic and depressive symptoms was found to be associated with increased DNA damage. All patients studied were on medication, which may hamper the comparisons between patients and

controls. However, the correlation between the depressive and manic symptoms and the severity of DNA damage is unlikely to be solely explained by the use of medication. Moreover, the drugs used by patients did not induce DNA damage in the in vitro assay carried out to assess this issue. These findings were consistent with the literature (Frotschl et al., 2005; Singh et al., 2005; Gasiorowski and Brokos, 2001; Spanova et al., 1997; Saxena and Ahuja, 1988). In two studies using the CA method, chlorpromazine had no effects on DNA damage (Frotschl et al., 2005), whereas fluphenazine was found to enhance DNA repair after hydrogen peroxide damage (Gasiorowski and Brokos, 2001). Amitriptyline has been described as non-genotoxic as assessed by chromosome aberrations tests (Saxena and Ahuja, 1988), as well as the TUNEL test which measures apoptosis induced by DNA breaks (Spanova et al., 1997). Valproate has been found to exert an antiproliferative effect on certain cancer cell lines both in vitro and in vivo (Singh et al., 2005). Therefore, the findings of the present study are more likely to be related to the severity of symptoms than to be a by-product of the exposure to medication.

Actually, mood stabilizers seem to act in the opposite way. A recent study demonstrated that lithium and valproate decreased glutamate-induced lipid peroxidation and protein carbonyl oxidation (markers of oxidative stress) and inhibited glutamate-induced DNA fragmentation in cerebral cortical neurons (Shao et al., 2005). Moreover, both mood stabilizers increased glutathione *S*-transferase expression in cultured neurons

(Wang et al., 2004). Interestingly, King and Jope (2005) demonstrated that lithium decreased caspase-3 activity induced by rotenone and H₂O₂ and may promote up-regulation in Bcl-2, which may be related to its neuroprotective actions (Mora et al., 1999, 2002; Chuang, 2004). The use of valproate or lithium (chronic administration) can stimulate bcl-2 expression as well as inhibit GSK-3 β activity, which renders a cell less susceptible to apoptosis. Thus, mood stabilizers may act to restore the balance among aberrant signaling pathways in specific areas of the brain and prevent degeneration (Brunello, 2004). Moreover, the neuroprotective role of mood stabilizers has been reported to be related to the inhibition of *N*-methyl-D-aspartate receptors and activation of PI3 Kinase/AKT signaling pathways (Chuang, 2005). Using a CA protocol, Psimadas et al. (2004) showed that schizophrenic patients and matched controls were not different regarding DNA damage and repair capacity. They also demonstrated that high levels of antipsychotics did not increase DNA damage in peripheral cells (Psimadas et al., 2004).

The role of DNA damage in BD may be related to the fact that these patients present increased morbidity and mortality due to general medical conditions. Chronic medical disorders such as cardiovascular disease (Demirbag et al., 2005), obesity (Gao et al., 2004) and diabetes mellitus (Blasiak et al., 2004) are associated with increased rates of DNA damage. Such disorders are more prevalent in BD patients than in the general population (Kupfer, 2005). It has been suggested that DNA fragmentation detected using the CA is caused by increased oxidative stress (Migliore et al., 2005; Faust et al., 2004). In a recent study, lower levels of two antioxidant enzymes (superoxide dismutase and catalase) and elevated lipid peroxidation (TBARS) were demonstrated in patients with BD, indicating an increased oxidative stress status (Ranjekar et al., 2003).

Some limitations of the present study should be taken into consideration, such as the fact that the CA provides information about recent DNA fragmentation, but does not tell about mechanisms of DNA repair. Studies assessing the DNA repair capacity in patients with BD are warranted to further investigate the impact of BD on DNA strand breaks. The comet assay has been put forward as a biomarker in populations exposed to hazardous conditions (e.g. radiation and pollutants) (Faust et al., 2004). However, results of the CA can be influenced by non-specific factors such diet, use of medication and lifestyle. At the present stage, it is not possible to determine whether DNA damage in BD patients is a state- or trait-related finding. The present findings may be used to augment strategies tailored to prevent DNA damage in BD patients.

Acknowledgements

This work was supported by Fundação de Amparo a Pesquisa do Rio Grande do Sul (FAPERGS), Conselho Nacional de Pesquisa (CNPq), Grupo de Pesquisa e Pós-Graduação from Hospital da Clinicas de Porto Alegre, Department of Biochemistry of the Federal University of the Rio Grande do Sul (UFRGS) and Laboratory of Oxidative Stress and Antioxidants of the University of Caxias do Sul (UCS).

References

- Belmaker, R.H., 2004. Medical progress: bipolar disorder. *New England Journal of Medicine* 351, 476–486.
- Benes, F.M., Walsh, J., Bhattacharyya, S., Sheth, A., Berretta, S., 2003. DNA fragmentation decreased in schizophrenia but not bipolar disorder. *Archives of General Psychiatry* 60, 359–364.
- Blasiak, J., Arabski, M., Krupa, R., Wozniak, K., Zadzorny, M., Kasznicki, J., Zurawska, M., Drzewoski, J., 2004. DNA damage and repair in type 2 diabetes mellitus. *Mutation Research* 554, 297–304.
- British Pharmacopoeia, 1999. Her Majesty's Stationery Office, London.
- Brunello, N., 2004. Mood stabilizers: protecting the mood...protecting the brain. *Journal of Affective Disorders* 79, 15–20.
- Chuang, D.M., 2004. Neuroprotective and neurotrophic actions of the mood stabilizer lithium: can it be used to treat neurodegenerative diseases? *Critical Reviews in Neurobiology* 16, 83–90.
- Chuang, D.M., 2005. The antiapoptotic actions of mood stabilizers: molecular mechanisms and therapeutic potentials. *Annals of the New York Academy of Sciences* 1053, 195–204.
- Collins, A.R., Dusinska, M., Gedik, C.M., Stetina, R., 1996. Oxidative damage to DNA: do we have a reliable biomarker? *Environmental Health Perspectives* 104, 465–469.
- Collins, A., Dusinska, M., Franklin, M., Somorovska, M., Petrovska, H., Duthie, S., Fillion, L., Panayiotidis, M., Raslova, K., Vaughan, N., 1997. Comet assay in human biomonitoring studies: reliability, validation, and applications. *Environmental Molecular Mutagenic* 30, 139–146.
- Cooke, M.S., Olinski, R., Evans, M.D., 2006. Does measurement of oxidative damage to DNA have clinical significance? *Clinica Chimica Acta* 365, 30–49.
- Culmsee, C., Mattson, M.P., 2005. p53 in neuronal apoptosis. *Biochemical and Biophysical Research Communications* 331, 761–777.
- Das Gupta, R., Guest, J.F., 2002. A model to estimate the cost benefit of an occupational vaccination programme for influenza in the UK. *Pharmacoeconomics* 20, 475–484.
- Demirbag, R., Yilmaz, R., Koçyigit, A., 2005. Relationship between DNA damage, total antioxidant capacity and coronary artery disease. *Mutation Research* 570, 197–203.
- Evan, G., Littlewood, T., 1998. A matter of life and cell death. *Science* 281, 1317–1322.
- Faust, F., Kassie, F., Knasmüller, S., Boedecker, R.H., Mann, M., Mersch-Sundermann, V., 2004. The use of the alkaline comet assay with lymphocytes in human biomonitoring studies. *Mutation Research* 566, 209–229.
- First, M.B., Spitzer, R.L., Gibbon, M., Williams, J.B., 1998. Structured Clinical Interview for DSM-IV (SCID-I). Biomedics Research Department, New York.

- Frotschl, R., Weickardt, S., Staszewski, S., Kaufmann, G., Kasper, P., 2005. Effects of chlorpromazine with and without UV irradiation on gene expression of HepG2 cells. *Mutation Research* 575, 47–60.
- Gao, D., Wei, C., Chen, L., Huang, J., Yang, S., Diehl, A.M., 2004. Oxidative DNA damage and DNA repair enzyme expression are inversely related in murine models of fatty liver disease. *American Journal of Physiology: Gastrointestinal and Liver Physiology* 5, 1070–1077.
- Gasiorowski, K., Brokos, B., 2001. DNA repair of hydrogen peroxide-induced damage in human lymphocytes in the presence of four antimutagens. A study with alkaline single cell gel electrophoresis (comet assay). *Cellular & Molecular Biology Letters* 6, 897–911.
- Hakkaart-van Roijen, L., Hoesienbos, M.B., Regeer, E.J., Tem-have, M., Nolen, W.A., Veraart, C.P., Rutten, F.F., 2004. The societal costs and quality of life of patients suffering from bipolar disorder in the Netherlands. *Acta Psychiatrica Scandinavica* 110, 383–392.
- Hamilton, M., 1960. A rating scale for depression. *Journal of Neurology, Neurosurgery and Psychiatry* 23, 56–62.
- Kassie, F., Parzefall, W., Knasmuller, S., 2000. Single cell gel electrophoresis assay: a new technique for human biomonitoring studies. *Mutation Research* 463, 13–31.
- King, T.D., Jope, R.S., 2005. Inhibition of glycogen synthase kinase-3 protects cells from intrinsic but not extrinsic oxidative stress. *Neuroreport* 16, 597–601.
- Kupfer, D.J., 2005. The increasing medical burden in bipolar disorder. *JAMA: Journal of the American Medical Association* 293, 2528–2530.
- Maluf, S.W., Erdtmann, B., 2000. Follow-up study of the genetic damage in lymphocytes of pharmacists and nurses handling antineoplastic drugs evaluated by cytokinesis-block micronuclei analysis and single cell gel electrophoresis assay. *Mutation Research* 471, 21–27.
- Migliore, L., Fontana, I., Colognato, R., Coppede, F., Siciliano, G., Murri, L., 2005. Searching for the role and the most suitable biomarkers of oxidative stress in Alzheimer's disease and in other neurodegenerative diseases. *Neurobiology of Aging* 26, 587–595.
- Mora, A., Rosa, A., Fuentes, J.M., Soler, G., Centeno, F., 1999. Different mechanisms of protection against by valproate and of lithium. *European Journal of Biochemistry* 266, 886–891.
- Mora, A., Sabio, G., Soler, G., Centeno, F., 2002. Different dependence of lithium and valproate on PI3K/PKB pathway. *Bipolar Disorders* 4, 195–200.
- Murray, C.J., Lopez, A.D., 1997. The utility of DALYs for public health policy and research: a reply. *Bulletin of the World Health Organization* 75 (4), 377–381.
- Psimadas, D., Messini-Nikolaki, N., Zafropoulou, M., Fortos, A., Tsilimigaki, S., Piperakis, S.M., 2004. DNA damage and repair efficiency in lymphocytes from schizophrenic patients. *Cancer Letters* 204 (1), 33–40.
- Ranjekar, P.K., Hinge, A., Hegde, M.V., Ghate, M., Kale, A., Sitasawad, S., Wagh, U.V., Debsikdar, V.B., Mahadik, S.P., 2003. Decreased antioxidant enzymes and membrane essential polyunsaturated fatty acids in schizophrenic and bipolar mood disorder patients. *Psychiatry Research* 121, 109–122.
- Saxena, R., Ahuja, Y.R., 1988. Genotoxicity evaluation of the tricyclic antidepressants amitriptyline and imipramine using human lymphocyte cultures. *Environmental and Molecular Mutagenesis* 12, 421–430.
- Shao, L., Young, L.T., Wang, J.F., 2005. Chronic treatment with mood stabilizers lithium and valproate prevents excitotoxicity by inhibiting oxidative stress in rat cerebral cortical cells. *Biological Psychiatry* 58, 879–884.
- Singh, N.P., McCoy, M.T., Tice, R.R., Schneider, E.L., 1988. A simple technique for quantization of low levels of DNA damage in individual cells. *Experimental Cell Research* 175, 184–191.
- Singh, G., Driever, P.H., Sander, J.W., 2005. Cancer risk in people with epilepsy: the role of antiepileptic drugs. *Brain* 128, 7–17.
- Spanova, A., Kovaru, H., Lisa, V., Lukasova, E., Rittich, B., 1997. Estimation of apoptosis in C6 glioma cells treated with antidepressants. *Physiological Research* 46, 161–164.
- Tice, R.R., Agurell, E., Anderson, D., Burlinson, B., Hartmann, A., Kobayashi, H., Miyamae, Y., Rojas, E., Ryu, J.C., Sasaki, Y.F., 2000. Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. *Environmental Molecular Mutagenesis* 35, 206–221.
- Wang, J.F., Shao, L., Sun, X., Young, L.T., 2004. Glutathione S-transferase is a novel target for mood stabilizing drugs in primary cultured neurons. *Journal of Neurochemistry* 88, 1477–1484.
- Young, R.C., Biggs, J.T., Ziegler, V.E., Meyer, D.A., 1978. A rating scale for mania: reliability, validity, and sensitivity. *British Journal of Psychiatry* 133, 429–435.