Journal of Psychiatric Research 43 (2009) 818–823

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/jpsychires

Impact of haloperidol and quetiapine on the expression of genes encoding antioxidant enzymes in human neuroblastoma SH-SY5Y cells

Andreas Johannes Schmidt [a], Ulrich Michael Hemmeter [a], Jürgen-Christian Krieg [a], Helmut Vedder [a], Philip Heiser [a,b,*]

[a] Department of Psychiatry and Psychotherapy, Philipps-University, Marburg, Germany
[b] Department of Child and Adolescent Psychiatry, Albert-Ludwigs-University, Freiburg, Germany

ARTICLE INFO

Article history:
Received 16 January 2008
Received in revised form 7 November 2008
Accepted 7 November 2008

Keywords:
Antipsychotics
Antioxidant enzymes
Glutathione metabolism
Neuroblastoma cells

ABSTRACT

Antipsychotics are known to alter antioxidant activities in vivo. Therefore, the aim of the present study was to examine in the human neuroblastoma SH-SY5Y cell line the impact of a typical (haloperidol) and an atypical (quetiapine) antipsychotic on the expression of genes encoding the key enzymes of the antioxidant metabolism (Cu, Zn superoxide dismutase; Mn superoxide dismutase; glutathione peroxidase; catalase) and enzymes of the glutathione metabolism (γ-glutamyl cysteine synthetase, glutathione-S-transferase, γ-glutamyltranspeptidase, glutathione reductase). The cells were incubated for 24 h with 0.3, 3, 30 and 300 μM haloperidol and quetiapine, respectively; mRNA levels were measured by polymerase chain reaction. In the present study, we observed mostly significant decreases of mRNA contents. With respect to the key pathways, we detected mainly effects on the mRNA levels of the hydrogen peroxide detoxifying enzymes. Among the enzymes of the glutathione metabolism, glutathione-S-transferase- and γ-glutamyltranspeptidase-mRNA levels showed the most prominent effects. Taken together, our results demonstrate a significantly reduced expression of genes encoding for antioxidant enzymes after treatment with the antipsychotics, haloperidol and quetiapine.

1. Introduction

There is a growing body of evidence that alterations of the metabolism of reactive oxygen species (ROS) and their detoxifying enzymes play an important role in the pathophysiology and treatment of psychiatric disorders. In schizophrenia, for example, indirect signs of ROS actions like altered levels of the products of lipid peroxidation (e.g. Dakhale et al., 2004; Evans et al., 2003; Herken et al., 2001; Sarandol et al., 2007; Skinner et al., 2005), of protein (Young et al., 2007) and DNA (Nishioka and Arnold, 2004; Young et al., 2007) damages as well as changed activities of antioxidative enzymes are widely and especially in the case of lipid peroxidation controversially discussed.

Alterations of antioxidant enzyme activities have been described in untreated schizophrenic patients (Dakhale et al., 2004; Evans et al., 2003; Li et al., 2006; Mukhrjee et al., 1996; Sarandol et al., 2007; Srivastava et al., 2001; Yao et al., 1998; Zhang et al., 2003a) as well as in patients treated with antipsychotics (Akyol et al., 2002; Alevizos and Stefanis, 1980; Altuntas et al., 2000; Ben Othmen et al., 2008; Dietrich-Muszalka et al., 2005; Evans et al., 2003; Gama et al., 2006; Herken et al., 2001; Ranjekar et al., 2003; Reddy et al., 1991; Zhang et al., 2006). These studies have focused on the main antioxidant enzymes [superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase] and have shown contrasting results. Interestingly, the study of Ben Othmen et al. (2008) found in part similar effects in treated schizophrenic patients and their unaffected siblings. Post-mortem studies also revealed significant alterations of the key antioxidant enzyme activities in different brain regions of treated patients (Loven et al., 1996; Michel et al., 2004; Yao et al., 2006).

Impacts of typical and atypical antipsychotics on oxidative stress parameters like altered activities and expression of antioxidant enzymes as well as on glutathione levels have been described in experimental (Agostinho et al., 2007; Li et al., 1999; Parikh et al., 2003; Pillai et al., 2007; Roy et al., 1984; Vairetti et al., 1999) and in clinical studies (Dakhale et al., 2004; Evans et al., 2003; Yao et al., 1998; Zhang et al., 2003b).

Furthermore, studies have revealed toxic effects of antipsychotics. Thus, cell culture (Behl et al., 1995; Heiser et al., 2007; Sagara, 1998) and animal studies (Polydoro et al., 2004) suggest that typical and atypical antipsychotics are able to induce oxidative damage and cell death. Moreover, similar effects have been detected

* Corresponding author. Address: Department of Child and Adolescent Psychiatry, Albert-Ludwigs-University, Freiburg, Germany. Tel.: +49 7612706606; fax: +49 7612706649.
E-mail address: philip.heiser@uniklinik-freiburg.de (P. Heiser).

0022-3956/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jpsychires.2008.11.005

in clinical studies: Fehsel et al. (2005) demonstrated that clozapine induced oxidative stress as well as pro-apoptotic gene expression in neutrophils of schizophrenic patients and Kropp et al. (2005) showed a significantly higher lipid peroxidation in the plasma of patients treated with typical antipsychotics like haloperidol and flupentixol compared to atypical antipsychotics like amisulpride, risperidone, clozapine, olanzapine and quetiapine.

On the other hand, several studies have described cytoprotective effects of typical as well as atypical antipsychotics including impacts on key enzymes of the antioxidant metabolism. Already, phenothiazines like chlorpromazine as the first typical antipsychotics are known to exert cytoprotective effects under certain circumstances in vitro (Babson et al., 1994; Ueda et al., 1997) and atypical antipsychotics have been demonstrated to be protective against the treatment with several toxic agents (Bai et al., 2002; Quing et al., 2003; Wang et al., 2005; Wei et al., 2003). In the course of an in vitro study, Dalla Libera et al. (1998) found that chlorpromazine, trifluoperazine and clozapine are effective antioxidants and Jeding et al. (1995) described antipsychotics as inhibitors of lipid peroxidation and radical scavengers in vitro. Further on Tan et al. (2007) found that in contrast to haloperidol, risperidone, olanzapine and quetiapine at low doses counteracted the rotenone-induced cytotoxicity in PC12 cells.

The studies citied above focused mostly on basal mechanisms with regard to antioxidant metabolism. The aim of the present investigation was to determine the effects of a typical (haloperidol) and an atypical (quetiapine) antipsychotic on the expression of genes encoding for antioxidant enzymes in human SH-SY5Y neuroblastoma cells.

2. Materials and methods

2.1. Cell culture

Neuroblastoma SH-SY5Y cells were cultured in heat-inactivated Roswell Park Memorial Institute medium (RPMI) (Gibco/BRL, Eggenstein, Germany) supplemented with 15% fetal calf serum (FCS) (Biochrom, Berlin, Germany), 1% penicillin-streptomycin and 1% glutamine in a 5% CO_2 atmosphere. For further studies, cells were plated at a number of 225.000 cells/dish in 10 mm culture dishes. Antipsychotics and dimethylsulfoxide-control treatments were performed at a density of 450.000 cells/dish. The antipsychotics haloperidol (Sigma, Deisenhofen, Germany) and quetiapine (AstraZeneca, London, United Kingdom) were dissolved in dimethylsulfoxide and were further diluted in culture medium. The cells were exposed to haloperidol and quetiapine in concentrations of 0.3, 3, 30 and 300 μM for 24 h at 37 °C.

2.2. RNA extraction

As described in the manufacturer's instructions, RNA was extracted from the SH-S5Y5 cells using Trizol® reagent. Optical methods at A_{260}/A_{280} (Bio-Photometer, Eppendorf, Hamburg, Germany) were used to quantify the amount of extracted total RNA. Furthermore, structural integrity was checked by agarose gel electrophoresis (GIBCO, Eggenstein, Germany).

2.3. Reverse transcriptase–polymerase chain reaction

The transcription of the antioxidant enzymes- and the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase-mRNA was analysed by reverse transcriptase–polymerase chain reaction as described previously (Heiser et al., 2006).

The primer pairs used are listed in Table 1. With a polymerase chain reaction cycler (Biometra Trio, Göttingen, Germany), aliquots of 1 μl cDNA for antioxidant enzymes and glyceralaldehyde-3-phosphate dehydrogenase were amplified using the corresponding primers. Polymerase chain reaction products for the enzymes were analysed after amplification and gel electrophoresis in 1% agarose gels. Semiquantitative determination was made by digitization with a Polaroid video system (Rothaar and Schröder, Heidelberg, Germany) and further densitometric evaluation with the Gelscan 4.0 Professional Program (LTF/BioSciTec, Landau/Frankfurt, Germany).

2.4. Statistical evaluation of results

Data are presented in percent of the mean of the respective control samples and are shown as mean ± S.E.M. values. The results from at least three independent different experiments were pooled and analysed by Mann-Whitney Rank Sum tests. A $p < 0.05$ (*) was accepted as a statistically significant difference. For statistical evaluations, Sigmastat (Jandel Scientific, Kerpenich, Germany) was applied.

3. Results

The effects of haloperidol and quetiapine in concentrations of 0.3, 3, 30 and 300 μM on the expression of genes encoding antioxidant enzymes in the human neuroblastoma SH-SY5Y cell line

Table 1
List of applied primers (Abbreviations: SOD1 = Cu, Zn superoxide dismutase; SOD 2 = Mn superoxide dismutase, GPx = glutathione peroxidase, CAT = catalase, GCS = γ-glutamylcysteine synthetase, GST = glutathione-S-transferase, γ-GT = γ-glutamyltranspeptidase, GR = glutathione reductase).

Gene	Primer	Reference
SOD 1	Sense: 5'-AAG GCC GTG TGC GTG CTG AA-3' Antisense: 5'-CAG GTC TCC AAC ATG CCT CT-3'	El Mouatassim et al. (1999)
SOD 2	Sense: 5'-GCA CAT TAA CGC GCA GAT CA-3' Antisense: 5'-AGC CTC CAG CAA CTC TCC TT-3'	El Mouatassim et al. (1999)
GPx	Sense: 5'-GTG TAT GCC TTC TCG GCG CG-3' Antisense: 5'-CGT TGC GAC ACA CCG GAG AC-3'	Matsuo et al. (2004)
CAT	Sense: 5'- AAG GTT TGG CCT CAC AAG G-3' Antisense: 5'- CGG CAA TGT TCT CAC ACA G-3'	Sinha (2005)
GCS	Sense: 5'- GGG GAA CCT GCT GAA CTG-3' Antisense: 5'-GCT CCA AGG AAA GAT TAA CTC C-3'	Johnson (2008)
GST	Sense: 5'-CGG AGA CCT CAC CCT GTA CCA GTC-3' Antisense: 5'-GCA GCA AGT CCA GCA GGT TGT AGT CA-3'	Kenney et al. (2005)
γ-GT	Sense: 5'-GAT CCT GTC AGC CCT GGG TTG TAA GA-3' Antisense: 5'-CGT CTA CGA TGA TAT GAC CCT TGT CAT T-3'	Kenney et al. (2005)
GR	Sense: 5'-CAG TGG GAC TCA CGG AAG AT-3' Antisense: 5'-TTC ACT GCA ACA GCA AAA CC-3'	Sonoda et al. (2004)
β-Actin	Sense: 5'-GAG GCC CAG AGC AAG AGA GG-3' Antisense: 5'-TCA CCG GAG TCC ATC ACG GAT-3'	Heiser et al. (2006)