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Abstract

Because there are reports on cytotoxic and cytoprotective effects of antipsychotics, the aim of the present study was to evaluate the impacts of different concentrations (1.6–50 μg/mL) of atypical antipsychotics on the survival of human neuronal (neuroblastoma SH-SY5Y) and immune (monocytic U-937) cells and on energy metabolism (ATP level after the incubation with antipsychotics in the concentration of 25 μg/mL). Statistical analysis showed that incubation for 24 h with the antipsychotics quetiapine, risperidone, 9-hydroxyrisperidone and ziprasidone led to a significantly enhanced cell survival in both cell lines in the lower concentrations. Higher concentrations exerted in part cytotoxic effects with the exception of quetiapine, but therapeutically relevant concentrations of the drugs were not cytotoxic in our experiments. Measurement of ATP contents in the neuronal cell line showed significantly increased levels after a 24-h treatment with 25 μg/mL risperidone and 9-hydroxyrisperidone. The other substances produced no effects. Our results show that the antipsychotic substances under investigation exert concentration-dependent effects on cell survival in both cell lines examined.

Key words

antipsychotic; ATP; monocytic cell; neuronal cell; survival

Introduction

Adenosine triphosphate (ATP) is one of the most important molecules in the human organism. It represents the cellular fuel for all energy-requiring reactions, including synthesis processes, membrane transport and muscle contractions, and can serve as a marker for cell toxicity (Fukuzako, 2001; Skinner, *et al*., 1995; Vairetti, *et al*., 1999).

Some studies suggest that a decrease of cellular ATP content may be an early event after the induction of oxidative stress in non-neuronal (Andreoli and Mallett, 1997) and neuronal systems (Aito, *et al*., 2004; Wang, *et al*., 2001). Typical and atypical antipsychotics are known to induce oxidative stress in cell cultures (Behl, *et al*., 1995; Sagara, 1998), in animal models (Agostinho, *et al*., 2007; Polydoro, *et al*., 2004; Reinke, *et al*., 2004) and in patients (Fehsel, *et al*., 2005). However, several studies described cytoprotective effects of atypical antipsychotics such as clozapine, olanzapine or quetiapine (Bai, *et al*., 2002; Qing, *et al*., 2003; Tan, *et al*., 2007; Wang, *et al*., 2005; Wei, *et al*., 2003).

Furthermore, antipsychotics are able to induce side-effects such as tardive dyskinesia (TD), a movement disorder that occurs in 20–40% of patients chronically treated with antipsychotics (Morgenstern and Glazer, 1993). In the development of this disorder, reactive oxygen species (ROS) seem to play a substantial role (Lohr, *et al*., 1990; Peet, *et al*., 1993; Tsai,

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et al., 1998). Especially typical antipsychotics are known to exert adverse effects on the extrapyramidal system.

In the context with schizophrenia, levels of high-energy phosphates have been controversially discussed (for review see Fukuzako, 2001). For example, Pettegrew, *et al*. (1991) reported higher ATP levels in the prefrontal cortex of drug-naive schizophrenic patients compared with controls, whereas reduced ATP contents were seen in the frontal lobes of antipsychotic-free patients (Volz, *et al*., 2000).

Concerning effects of antipsychotics on ATP levels with regard to animal studies, Vairetti, *et al*. (1999) described significantly decreased ATP contents in the cerebellum, the striatum and the cortex of haloperidol-treated rats.

Summarising the known effects of antipsychotics on the metabolism of ROS and the alterations of ATP contents discussed in patients with schizophrenia, it is of interest to closer examine such effects of these substances on cell survival as well as on energy metabolism.

In our first study, we examined the effects of haloperidol, clozapine and olanzapine on cell survival and energy metabolism in human neuronal (SH-SY5Y) and immune (U937) cell lines and found that these antipsychotics exerted differential metabolic effects in the cell lines examined (Heiser, *et al*., 2007). With the present study, we wanted to determine the effects of quetiapine, risperidone, 9-hydroxyrisperidone and ziprasidone on the survival of SH-SY5Y and U-937 cells as well as on energy metabolism in neuronal SH-SY5Y cells.

Methods and materials

Preparation and incubation of cells

Human SH-SY5Y neuroblastoma and U-937 monocytic immune cells were cultured in a 5% CO_2 atmosphere in heat-inactivated Roswell Park Memorial Institute medium (RPMI) (Gibco/BRL, Eggenstein, Germany) supplemented with 15% foetal calf serum (FCS) (Biochrom, Berlin, Germany), 1% penicillin–streptomycin, and 1% glutamine (SH-SY5Y) or with 10% FCS, 1% gentamycin, and 0.5% glutamine (U-937), respectively. Quetiapine was obtained from AstraZeneca Pharmaceuticals LP (Wilmington, USA), risperidone and 9-hydroxyrisperidone from Janssen-Cilag (Neuss, Germany) and ziprasidone from Pfizer (Karlsruhe, Germany). Risperidone and 9-hydroxyrisperidone were dissolved in ethanol, quetiapine and ziprasidone in dimethylsulphoxide (DMSO). The antipsychotics were used in concentrations of 1.6, 3.13, 6.25, 12.5, 25 and 50 μg/mL. For the control conditions, the respective dissolvents were applied.

Measurement of metabolic activity and survival

In 96-well dishes, 36,000 cells per well were exposed to the antipsychotics under investigation for 24 h at 37 °C in the concentrations mentioned above. Cell survival was determined by a biochemical assay using a modified tetrazolium method (EZ4U, Biozol, Eching, Germany). The test is based on the ability of living cells to transform colourless tetrazolium salts into deeply coloured formazans by mitochondrial dehydrogenases (Klöcking, *et al*., 1995). Cell viability was quantified by photometric determination of formazan-like products with an ELISA-reader (Dynex, Stuttgart, Germany).

Measurement of ATP content

A total of 36,000 cells per well were plated into 96-well dishes and exposed to the antipsychotics in a concentration of 25 μg/mL for 24 h at 37 °C. The ATP content of the cells was determined by the ATP Bioluminescence Assay (Boehringer, Ingelheim, Germany)

The amounts of ATP were measured with a microplate scintillation counter 'TopCount' (Perkin Elmer, Ueberlingen, Germany) enabling quantitative measurement via luminescence detected by single photon counting.

Statistical analysis

Data are expressed in percentage of the respective control and displayed as mean values ± standard error of the mean (SEM).

To evaluate the treatment effects, Kruskal–Wallis tests were used to determine differences among the treatment groups. In case of significance, Dunn's method was performed. The respective degrees of freedom (DF), H statistics (H) and *P* values are presented. The level of significance was set to $P < 0.05$. Statistical evaluation was carried out using SigmaStat software (Jandel Scientific, Erkrath, Germany).

Results

Kruskal–Wallis analysis showed the following significant effects of the atypical antipsychotics on the survival of immune (U-937) and neuronal (SH-SY5Y) cells (Table 1): in both cell lines, treatment with quetiapine showed significant increases of cell survival in all concentrations used with the exception of 50 μg/mL (Figure 1). Risperidone showed a dose-dependent

Table 1 Statistical results of Dunn's test of the metabolic activity after different treatments with four antipsychotics

Cellular system	Treatment	DF	H	*P*
U-937 cells	Quetiapine	6	109	<0.05
SH-SY5Y cells	Quetiapine	6	185	<0.05
U-937 cells	Risperidone	6	156	<0.05
SH-SY5Y cells	Risperidone	6	121	<0.05
U-937 cells	9-hydroxyrisperidone	6	111	<0.05
SH-SY5Y cells	9-hydroxyrisperidone	6	132	<0.05
U-937 cells	Ziprasidone	6	97	<0.05
SH-SY5Y cells	Ziprasidone	6	118	<0.05

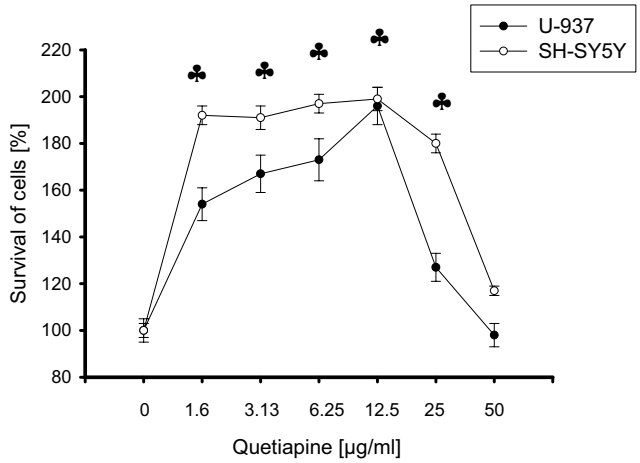

Figure 1 Survival rate of SH-SY5Y and U-937 cells after treatment with quetiapine (♣, for both cell lines significant compared with control condition).

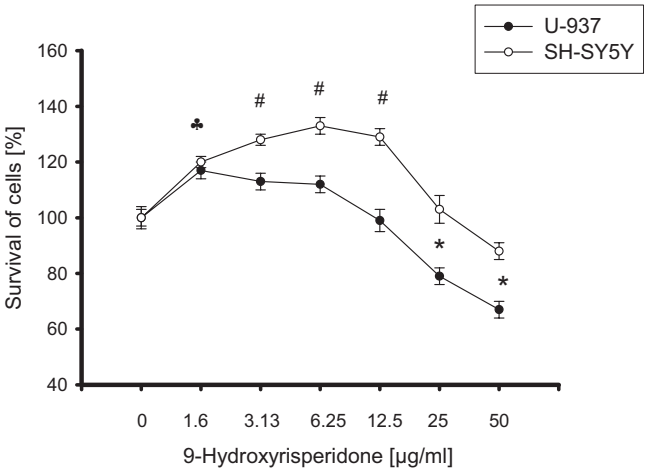

Figure 3 Survival rate of SH-SY5Y and U-937 cells after treatment with 9-hydroxyrisperidone (*, for U-937 cells significant compared with control condition; #, for SH-S5Y5 cells significant compared with control condition; ♣, for both cell lines significant compared with control condition).

reaction in both cell lines, too (Figure 2). Low concentrations resulted in an increased cell survival. The significance level was reached in U-937 cells after treatment with 1.6, 3.13, 6.25 µg/mL and in SH-SY5Y cells after treatment with 3.13 and 6.25 µg/mL, respectively. Incubation with 25 µg/mL risperidone resulted in a significant decrease of cell survival in U-937 cells and with 50 µg/mL in both cell lines. Similar results were found for 9-hydroxyrisperidone (Figure 3). A significant increase of survival was shown after 1.6 µg/mL for U-937 cells and after 1.6, 3.13, 6.25, 12.5 µg/mL for SH-SY5Y cells, whereas a treatment with 25 and 50 µg/mL led to a significantly decreased survival of U-937 cells. Incubation with ziprasidone resulted in a significantly increased cell survival in both cell lines in concentrations ranging from 1.6 to 12.5 µg/mL and 25 µg/mL in the U-937 cell line only; 50 µg/mL significantly decreased the survival of the SH-SY5Y cells (Figure 4).

Furthermore, we examined differential effects of the antipsychotics on energy metabolism in the human neuroblastoma SH-SY5Y cells. Data of the Kruskal–Wallis analyses are shown in Table 2. Treatment with risperidone and 9-hydroxyrisperidone in a concentration of 25 µg/mL resulted in

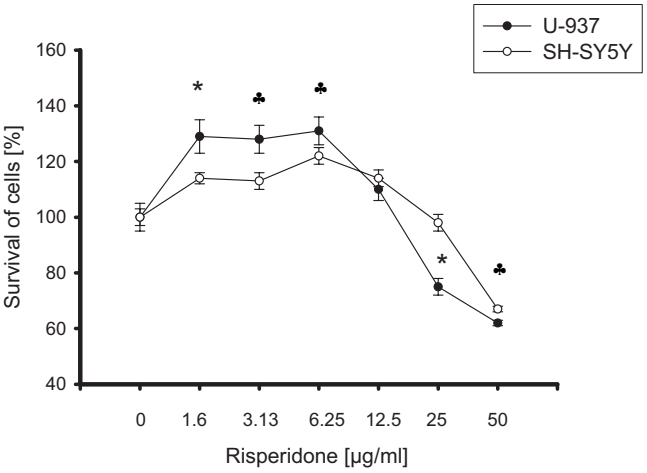

Figure 2 Survival rate of SH-SY5Y and U-937 cells after treatment with risperidone (*, for U-937 cells significant compared with control condition; ♣, for both cell lines significant compared with control condition).

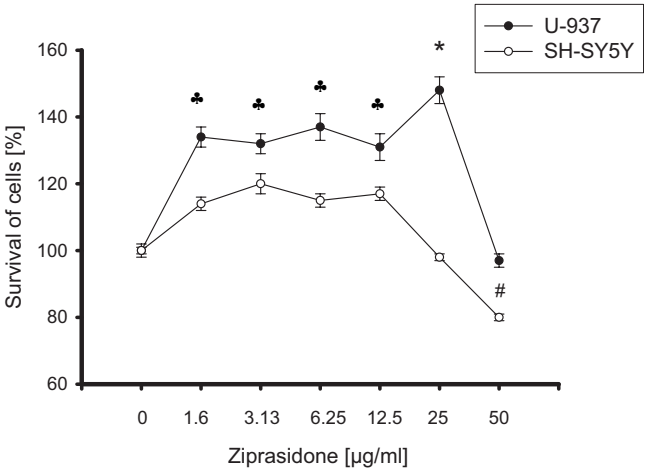

Figure 4 Survival rate of SH-SY5Y and U-937 cells after treatment with ziprasidone (*, for U-937 significant compared with control condition; #, for SH-S5Y5 cells significant compared with control condition; ♣, for both cell lines significant compared with control condition).

Table 2 Statistical results of Dunn's test of the ATP content after different treatments with four antipsychotics

Cellular system	Treatment	DF	H	*P*
SH-SY5Y cells	Quetiapine	2	14	NS
SH-SY5Y cells	Risperidone	2	8	<0.05
SH-SY5Y cells	9-hydroxyrisperidone	2	8	<0.05
SH-SY5Y cells	Ziprasidone	2	14	NS

significant increases of ATP-levels in this cell type, whereas the other two substances failed to induce significant effects (Figure 5).

Discussion

In the present study, we examined alterations of cell survival in human neuroblastoma SH-SY5Y as well as in peripheral human monocytic U-937 cells after treatment with the atypical antipsychotics quetiapine, risperidone, 9-hydroxyrisperidone and ziprasidone in a concentration range from 1.6 to 50 μg/mL. Furthermore, we measured the ATP content in SH-SY5Y cells after treatment with the four antipsychotics. Because antipsychotics may induce neuro-psychiatric as well as immunological side effects, we investigated both, a neurological and immune cell line, under similar experimental conditions.

A previous examination of our group showed toxic effects of the typical antipsychotic haloperidol and the atypical one clozapine in both cell lines, whereas olanzapine induced an increase of metabolic activity in the neuronal cell line (Heiser, *et al*., 2007). In the current study, the atypical antipsychotics under investigation led to a significantly increased cell survival

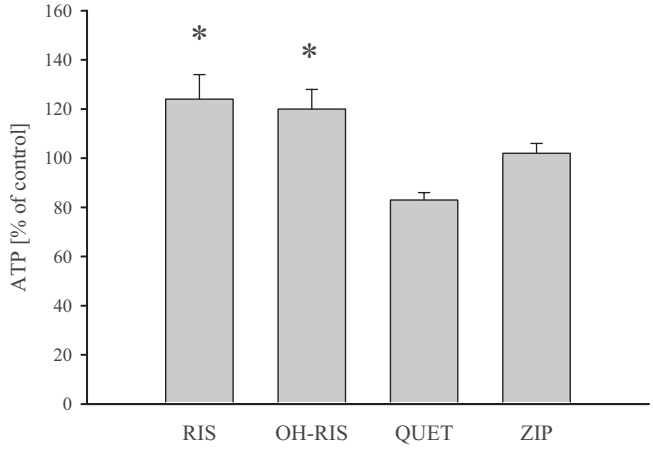

Figure 5 ATP content in SH-SY5Y cells after different treatments with four antipsychotics (Ris, risperidone; OH-RIS, 9-hydroxyrisperidone; QUET, quetiapine; ZIP, ziprasidone) (*, significant compared with control condition).

in both cell lines in the lower concentrations. Higher concentrations exerted in part cytotoxic effects with the exception of quetiapine, but therapeutically relevant concentrations of the drugs were not cytotoxic in our experiments. The concentrations of drugs used in the course of our experiments were about 100–1000 times higher than the serum levels in patients treated with those antipsychotics. For haloperidol it is known that the brain-to-serum ratio for haloperidol is about 22 in rats (Sunderland and Cohen, 1987, Tsuneizumi, *et al*., 1992); therefore, this is probably also true for the other antipsychotics tested in our study. We also applied the higher concentrations to evoke detectable effects. Therefore, the concentrations used represent to some extent a compromise between therapeutical and toxic ones. It has to be pointed out that in our former study therapeutically relevant concentrations of haloperidol and clozapine were not cytotoxic (Heiser, *et al*., 2007). The incubation time of 24 h was chosen to compare our results of the cell survival with our previous data where we incubated both cell lines with haloperidol, clozapine and olanzapine for 4 (data not shown) as well as for 24 h and did not find any significant differences.

In the literature, various cytoprotective effects of atypical antipsychotics have been described. For example, quetiapine and risperidone have been shown to exert protective effects against serum withdrawal (Bai, *et al*., 2002) and rotenone- (Tan, *et al*., 2007) and MPP^+-induced cell death (Qing, *et al*., 2003) in PC 12 cells, whereas such effects were not detectable after haloperidol treatment. Quetiapine has also been reported to protect cells under conditions of incubation with β-amyloid (25–35) (Wang, *et al*., 2005). Gil-ad, *et al*. (2001) showed that the antipsychotics clozapine and haloperidol, but not risperidone, decreased the viability of human SK-N-SH neuroblastoma cells and that haloperidol and clozapine, but not risperidone, induced neurotoxic effects in mouse primary neurons. On the contrary to risperidone, haloperidol induced apoptotic injury in cultured rat cortical neurons in another study (Ukai, *et al*., 2004).

These results of in-vitro studies are in line with our findings that quetiapine treatment results in an enhanced cell survival in both cell lines examined and in nearly all concentrations used. Risperidone induced an increased cell survival after treatment at low concentrations. Treatment with the metabolite of risperidone, 9-hydroxyrisperidone, resulted in a significant enhanced survival of SH-SY5Y cells (1.6–12.5 μg/mL), whereas U-937 cells showed a significant decrease of cell survival after 25 and 50 μg/mL and a significant increase after 1.6 μg/mL. An incubation of the cells with ziprasidone showed a significant increased cell survival (1.6–12.5 μg/mL) in both cell lines and in U-937 cells after 25 μg/mL, whereas 50 μg/mL led to a significant decrease in SH-SY5Y cell numbers. To our current knowledge, similar studies with 9-hydroxyrisperidone and ziprasidone have not yet been reported in other cell types or experimental models.

The ATP levels were also measured in this study because alterations of ATP levels have been shown in animals and cell cultures after treatment with antipsychotics. Vairetti, *et al*. (1999) reported significantly decreased ATP contents in the cer-

ebellum, the striatum and the cortex of haloperidol-treated rats. In SH-SY5Y and in U-973 cells, Heiser, *et al.* (2007) reported significantly lower ATP levels after an incubation with haloperidol. The atypical antipsychotics investigated at that time – that is, clozapine and olanzapine – exerted no statistically significant effects, although increased ATP contents in SH-SY5Y cells after clozapine – and in U-937 cells after olanzapine treatment were observed. In our present study, treatment of SH-SY5Y cells with risperidone and 9-hydroxyrisperidone led to small, albeit significant, increases of ATP contents. The decrease after quetiapine treatment failed to reach a statistically significant level, whereas ziprasidone did not show any effect. Maurer and Möller (1997) examined effects of antipsychotics on the activity of mitochondrial respiratory chain enzyme complexes, which produce ATP via oxidative phosphorylation. Complex I (NADH-CoQ reductase) was progressively inhibited by all antipsychotics used including risperidone, clozapine and haloperidol; we, however, found significant increases of ATP contents in SH-SY5Y cells after treatment with risperidone. Several studies examined ATP levels in drug-naive as well as in medicated schizophrenic patients. With respect to antipsychotic-free patients, Pettegrew, *et al.* (1991) reported higher β-ATP levels in the prefrontal cortex, whereas Volz, *et al.* (2000) observed decreased ATP contents in the frontal lobe of patients compared with controls. In medicated schizophrenic patients, alterations of ATP levels were shown in frontal lobes, but the directions of these changes were not consistent across several studies. Volz, *et al.* (1997) found that ATP levels may be inversely correlated with the degree of negative symptoms in patients with schizophrenia. Furthermore, two studies described lower ATP concentrations in the basal ganglia of medicated schizophrenic patients (Deicken, *et al.*, 1995; Fujimoto, *et al.*, 1992). Summarising effects of antipsychotics on ATP, it had to be mentioned that changes of ATP levels induced by antipsychotic treatment led also to no consistent direction in the frontal lobes of schizophrenic people (for review see Fukuzako, 2001). An animal study showed significant decreased ATP contents in several brain areas and the liver of rats after haloperidol administration (Vairetti, *et al.*, 1999), whereas Skinner, *et al.* (1995) found increased ATP levels in brain of rats after treatment with haloperidol and clozapine. Our first study regarding this topic (Heiser, *et al.*, 2007) showed significant decreases after ATP measurement following incubation of U-937 and SH-SY5Y cells with haloperidol. Atypical antipsychotics showed like in the present study not a clear direction of effects. Further studies are necessary to evaluate the exact mechanism of action of antipsychotics on energy metabolism. One reason for ATP depletion could be oxidative stress induced by antipsychotics, which could be due to the inhibiton of the complex 1 of the mitochondrial respiratory chain enzyme complexes (Maurer and Möller, 1997). Impacts of typical and atypical antipsychotics on indirect signs of free oxygen species have been described as well as oxidative stress induced decreases of ATP levels in non-neuronal (Andreoli and Mallet, 1997) and neuronal cells (Aito, *et al.*, 2004; Teepker, *et al.*, 2007; Wang, *et al.*, 2001).

In conclusion, we were able to find a wide range of effects of the applied atypical antipsychotics on cell survival of neuronal and immune cells as well as on energy metabolism in neuronal cells. Although it is necessary to be cautious when constructing a link between in-vitro findings obtained on immortalised cells and in-vivo mechanisms, the use of such cell systems may be helpful for the further examination and differentiation of cellular and toxic effects of antipsychotics in the nervous as well as in the immune system.

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