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Neurotoxic/neuroprotective activity of haloperidol, risperidone and paliperidone in neuroblastoma cells

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ABSTRACT

The neurotoxicity of antipsychotic (AP) drugs seems to be linked with neurological side effects like extrapyramidal symptoms (EPS). On the other hand, neuroprotective effects can mitigate or slow the progressive degenerative structural changes in the brain leading to improved outcome of schizophrenia. First and second-generation antipsychotics may differ in their neurotoxic and neuroprotective properties. The aim of this study was to compare the neurotoxic/neuroprotective activity of haloperidol, a first-generation antipsychotic, and risperidone, a second-generation one, with paliperidone, a relatively new second-generation antipsychotic, in SK-N-SH cells. Haloperidol, risperidone and paliperidone (10, 50, 100 μM) were administered, either alone or in combination with dopamine (100 μM), to human neuroblastoma SK-N-SH. We examined the effects of the drugs on cell viability (measured by alamarBlue®), caspase-3 activity (measured by fluorimetric assay) and cell death (by measuring the externalization of phosphatidylserine). Haloperidol significantly decreased cell viability and increased caspase-3 activity and cell death. Risperidone and paliperidone did not affect cell viability or cell death. Both second-generation APs decreased caspase-3 activity, especially paliperidone. In cells treated with dopamine in combination with antipsychotics, only paliperidone (10 μM) induced a slight improvement in cell viability. While haloperidol potentiated the dopamine-induced increase in caspase-3 activity, risperidone and paliperidone reduced this effect. The results indicate that haloperidol induces apoptosis, whereas risperidone and paliperidone may afford protection against it. Of the APs tested, paliperidone always showed the strongest neuroprotective effect. The different antipsychotic effects on survival and cell death might be related to differences in their capacity to induce EPS.

1. Introduction

Although schizophrenia is considered to be a neurodevelopmental disorder, there is evidence that it is also progressive and possibly neurodegenerative. Antipsychotic (AP) drugs modulate dysfunction in chemical neurotransmission, which plays an important role in reducing the symptoms of schizophrenia but also in their potential role for neuroprotection and neurotoxicity. Research suggests that some second-generation antipsychotics (SGA) may have a neuroprotective effect which would be involved in their therapeutic properties such as cognitive enhancement or prevention of disease progression and clinical deterioration, although these effects have not been consistently demonstrated (Lieberman et al., 2008). On the other hand, the neurotoxic effect of APs may be related to their capacity to produce side effects, including extrapyramidal symptoms (EPS) and tardive dyskinesia (TD). Even though the exact mechanism underlying AP-induced EPS is not clear, the important role of serotonergic neurotransmission aside, striatal dopamine D2 receptor (DRD2) blockade is believed to be the

Abbreviations: 5-HT, 5-hydroxytryptamine; Ac-DEVD, Acetyl-Asp-Glu-Val-Asp; Ac-DEVD-CHO, Acetyl-Asp-Glu-Val-Asp-al; AMC, 7-amino-4-methyl-coumarin; AP, antipsychotic; BDNF, brain-derived neurotrophic factor; Ca^{2+}/CaM, Ca^{2+}-calmodulin-dependent; Protein kinasa DA, dopamine; DMSO, dimethyl sulfoxide; DRD2, dopamine D2 receptor; EPS, extrapyramidal symptoms; ERK, extracellular signal-regulated kinase; FBS, fetal bovine serum; FGA, first-generation antipsychotic; FITC, fluorescein isothiocyanate; MEM, Minimum essential medium; NGF, nerve growth factor; NMDA, N-methyl D-aspartate; PBS, phosphate buffered saline; PI, propidium iodide; PI3K-Akt, 3-kinase-protein kinase B; PKA, protein kinase A; PS, phosphatidylserine; RGS2, regulator of G-protein signaling 2; SGA, second-generation antipsychotic; TD, tardive dyskinesia.

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main cause (Kapur et al., 2000). It is well known that first-generation antipsychotics (FGA) have higher affinity for DRD2 and higher potential to produce EPS than SGAs (Carpenter and Buchanan, 1994; Farde et al., 1992). Attempts have been made to establish a relationship between the neurotoxic activity of antipsychotics and their capacity to cause EPS (Gil-ad et al., 2001; Ukai et al., 2004). It has been demonstrated that first-generation antipsychotic drugs, including haloperidol, decrease the viability of neuronal cells in vitro (Gil-ad et al., 2001), causing apoptosis, necrosis and oxidative stress (Galili et al., 2000; Noh et al., 2000). On the other hand, studies in vitro have shown that while haloperidol is apparently neurotoxic, second-generation antipsychotics offer protection against serum withdrawal-induced apoptosis in SH-SY5Y cells (Kim et al., 2008), or against cytotoxin-induced apoptosis in the pheochromocytoma (PC12) cell line (Qing et al., 2003; Wei et al., 2003a, 2003b). However, the possible neuroprotective effect of SGAs is controversial (Lieberman et al., 2008; Molteni et al., 2009). Therefore, more research is needed to identify the effects of first and second-generation antipsychotics on neural survival and death, and to establish whether such effects contribute to the differences in extrapyramidal symptoms.

Several studies have tried to identify potential risk factors for EPS, such as younger age (Lewis, 1998), male gender (Dayalu and Chou, 2008), psychiatric diagnosis (McIntyre and Konarski, 2005), AP dosage and AP potency of the DRD2 blockade (Farde et al., 1992; Remington and Kapur, 1999). Even when all these factors are taken into account, there is still considerable patient-to-patient variability in the propensity for extrapyramidalism, indicating that genetic factors could play an important role. Our group is seeking to identify genetic polymorphisms involved in the metabolism and transport of antipsychotics and dopamine (Crescenti et al., 2008; Gassó et al., 2010; Lafuente et al., 2007; Mas et al., 2010), as well as those affecting AP targets (Gassó et al., 2009; 2011; Lafuente et al., 2008). However, genetic factors involved in the neurotoxic mechanisms might be also involved.

The aim of this study was to compare, for the first time in the literature, the neurotoxic/neuroprotective activity of haloperidol, a reference FGA, and risperidone, a commonly used SGA, with paliperidone (9-hydroxyrisperidone), a relatively new second-generation antipsychotic. By using a dopaminergic cell model (neuroblastoma cells SK-N-SH), we measured changes in viability, caspase-3 activity and cell death produced by antipsychotics alone and in combination with a high dopamine (DA) concentration. The results may help us to identify differences in the cytotoxic mechanisms of antipsychotics in order to understand the etiopathology of EPS and thus explore new pharmacogenetic targets.

2. Methods

2.1. Reagents

Minimum essential medium (MEM), fetal bovine serum (FBS), L-glutamine, sodium pyruvate, penicillin, streptomicin, phosphate buffered saline (PBS) and trypsin were purchased from Invitrogen (Carlsbad, CA, USA). Dopamine (DA), haloperidol, risperidone, paliperidone and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Cell culture

SK-N-SH human neuroblastoma cells were purchased from the American Type Cell Culture (ATCC, Manassas, VA, USA) and were cultured in MEM supplemented with 10% FBS, 2 mM L-glutamine, 500 μM sodium pyruvate, 50 units/ml penicillin and 50 μg/ml streptomycin. Cells were grown in a humidified incubator with 5% CO_2 at 37 °C. The culture medium was changed every 2–3 days.

2.3. Selection of antipsychotic and dopamine concentrations

Antipsychotic and dopamine concentrations were selected after preliminary experiments and after an intensive search in the literature to establish the range of dosage that would produce an effect on the SK-N-SH cell line. Taking into account the different potencies and dopamine receptor D2 affinities of the antipsychotics tested (haloperidol>risperidone and paliperidone), we carried out the study with 10, 50 and 100 μM of each antipsychotic to evaluate a wide range of concentrations. 100 μM of dopamine was the concentration selected to evaluate the capacity of antipsychotics to protect against, or to enhance, damage to the cell culture.

2.4. Measurement of cell viability

SK-N-SH cells were seeded on 24-well plates at a density of 2×10^5 cells/well and treated with haloperidol, risperidone and paliperidone at concentrations of 10, 50 and 100 μM, either alone or in combination with dopamine 100 μM. Controls were treated with vehicle (0.4% DMSO, v/v) either alone or in combination with DA. Each condition was assessed at least in triplicate. Cell viability was determined by alamarBlue® (Sigma, St. Louis, Missouri, USA). Resazurin, a non-fluorescent indicator dye, is converted to bright red-fluorescent resorufin via the reduction reactions of metabolically active cells. The amount of fluorescence produced is proportional to the number of living cells. After 24 h of incubation, 50 μl of alamarBlue® was added to each well and incubated for 2 h. Fluorescence was measured at the excitation wavelength of 540 nm and the emission wavelength of 610 nm using a microplate reader (Wallac Victor 2 I420 Multilabel Counter, PerkinElmer, Waltham, MA). Each measurement was done at least in duplicate. Cell viability is expressed as a percentage of control (vehicle-treated) or DA-treated cells.

2.5. Measurement of caspase-3 activity as apoptotic marker

Cells were cultured at a density of 1×10^5 cells/well on 24-well plates until 70–80% confluence. Then, normal culture medium was replaced by culture medium containing haloperidol, risperidone and paliperidone at concentrations of 10, 50 and 100 μM, either alone or in combination with dopamine 100 μM. Culture medium for controls contained vehicle (0.4% DMSO, v/v) either alone or in combination with DA. Each condition was assessed at least in triplicate. After 12 h or 24 h, caspase-3 activity was measured by the cleavage of Acetyl-Asp-Glu-Val-Asp (Ac-DEVD) peptide-conjugated 7-amino-4-methyl-coumarin (AMC) using the Caspase-3 Fluorimetric Assay Kit (Sigma, St. Louis, Missouri, USA). The cells were incubated with 100 μl of ice-cold cell lysis buffer on ice for 20 min. The lysate was centrifuged at 4 °C for 5 min at 10,000×g. 20 μl of the supernatant was transferred to a 96-well plate, then 200 μl of reaction buffer containing the caspase-3 substrate (DEVD-AMC) was added to each well. To verify that the signal detected by the reaction was due to protease activity, an induced sample was incubated with caspase-3 inhibitor Acetyl-Asp-Glu-Val-Asp-al (Ac-DEVD-CHO) before adding the substrate. After incubation at 37 °C for 1 h, the fluorescence counts of AMC in the wells with a 355 nm excitation filter and 460 nm emission filter were measured, at least, in duplicate using a microplate reader (Wallac Victor 2 I420 Multilabel Counter, PerkinElmer, Waltham, MA). Fluorescence of blanks was subtracted from each value. Fluorescence values were converted to caspase-3 activity using a standard curve for AMC. Caspase-3 activity was normalized to the total protein content of the cell extracts, as measured by a DC protein assay kit (BioRad, Hemel Hempstead, UK). Results are expressed as percentage of control (vehicle-treated) or DA-treated cells.