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Antioxidant properties of atypical antipsychotic drugs used in the treatment of schizophrenia

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ABSTRACT

The aim of this study was to compare the antioxidant activities of six atypical antipsychotic drugs: clozapine (CLZ), quetiapine, olanzapine (OLA), risperidone, ziprasidone, aripiprazole (ARI), as well as a typical antipsychotic drug, haloperidol. Several tests of antioxidant activity were used: protection of thiol groups against oxidation by peroxynitrite (PN) and 3-morpholinosydnonimine (SIN-1, generator of PN), oxidation of dihydrorhodamine 123 by PN, SIN-1 and hypochlorite (NaOCl), bleaching of fluorescein fluorescence by PN, 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH, generator of peroxyl radicals) and NaOCl, radical-scavenging activity with respect to 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) radical, 2,2-diphenyl-1-picrylhydrazyl free radical and the Ferric Reducing Antioxidant Potential. In most of the tests, OLA showed the highest antioxidant activity, followed by CLZ and in some cases ARI, other compounds being much less active or not active. OLA and CLZ exerted limited toxicity on mouse neuroblastoma Neuro-2A (N2A) cells and protected the cells against the toxic action of SIN-1, AAPH and NaOCl in the physiologically relevant concentration range of these oxidants. Both drugs reduced the PN-induced nitration of intracellular proteins. Given that schizophrenia is associated with oxidative and nitrosative stress, the direct antioxidant activity OLA and CLZ may contribute to the therapeutic action of these compounds.

1. Introduction

Schizophrenia (SZ) is one of the most severe and chronic forms of mental disorders, but the etiopathogenesis of this illness has not yet been clarified. Accumulating evidence suggests that mitochondrial dysfunction, oxidative and nitrosative stress contribute to the pathogenesis of SZ (Dietrich-Muszalska et al., 2015; Joshi and Praticò, 2014; Koga et al., 2015).

Great progress in the treatment of SZ was achieved with the introduction of atypical antipsychotics (AAPs) in the early 1990′s. AAPs block dopamine as well as serotonin binding to their respective receptors, and antipsychotics are thought to exert their main therapeutic effects through this process (Kapur and Remington, 2001; Meltzer and Massey, 2011).

Unfortunately, it should be noted that some AAPs such as CLZ cause potentially life-threatening side-effects, such as agranulocytosis, which is thought to occur due to oxidation of the drugs to form a reactive nitrenium ion (Gardner et al., 1998). In addition, serious hepatotoxicity has been reported in patients receiving OLA, mediated by overproduction of reactive oxygen species (ROS), glutathione depletion and lipid peroxidation, and dependent on CYP450 metabolism (Eftekhari et al., 2016). It was also reported that in relatively large doses, CLZ induced myocarditis consistently with increased myocardial oxidative stress, DNA damage and inflammatory cytokines in a rat model (Abdel-Wahab et al., 2014). It is not obvious if this effect can be extrapolated to humans because the half-life of antipsychotics is considerably faster in rodents than in humans, so injections may lead to fluctuating plasma drug concentrations in humans (Kapur et al., 2003).

Recent studies suggest that some AAPs, used for the treatment of SZ, may have protective properties against oxidative stress (Magliaro and

Abbreviations: ABTS*, 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) radical; AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; AAPs, atypical antipsychotics; ARI, aripiprazole; BSA, bovine serum albumin; CLZ, clozapine; DHR123, dihydrorhodamine 123; DMEM, Dulbecco's Modified Eagle Medium; DMSO, dimethylsulfoxide; DPPH*, 2,2-diphenyl-1-picrylhydrazyl free radical; FRAP, The Ferric Reducing Antioxidant Potential; HAL, haloperidol; HOCl, hypochlorous acid; HSA, human serum albumin; N2A, mouse neuroblastoma Neuro-2A cells; NaOCl, sodium hypochlorite; $O_2^{\bullet -}$, superoxide radical; OLA, olanzapine; PBS, phosphate-buffered saline; PN, $ONOO^-$, peroxynitrite; QTP, quetiapine; RISP, risperidone; ROS, reactive oxygen species; SIN-1, 3-Morpholinosydnonimine; SOD, superoxide dismutase; SZ, schizophrenia; ZIP, ziprasidone.

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Saldanha, 2009; Kato et al., 2011; Kracmarova and Pohanka, 2014; Yan et al., 2014). OLA, aripiprazole (ARI) and ziprasidone (ZIP), but not HAL, were found to modulate ROS levels, superoxide dismutase (SOD) activity and BCL2-associated X protein (Bax) expression to provide protective effects against the N-methyl-4-phenylpyridinium (MPP^+) ion-induced oxidative stress in the rat pheochromocytoma (PC12) cells (Park et al., 2011).

Nevertheless, Zhang et al. (2012) suggested that typical antipsychotics may also at least partially normalize abnormal free radical metabolism in SZ. OLA increased mRNA for the copper/zinc isoform of the superoxide dismutase enzyme (SOD-1). Wei et al. (2003) suggested that the neuroprotective action of OLA includes the upregulation of SOD.

More recently, Kato et al. (2011) demonstrated antioxidative effects of ARI via modulating microglial $O_2^{\bullet -}$ generation. These authors reported that ARI inhibited the $O_2^{\bullet -}$ generation by phorbol-myristate-acetate (PMA)-stimulated microglia through the cascade of protein kinase C (PKC) activation, intracellular Ca^{2+} signaling and NADPH oxidase activation via cytosolic $p47^{phox}$ translocation to the plasma/phagosomal membranes (Kato et al., 2011).

The aim of this study was to compare antioxidant activities of six AAPs: CLZ, quetiapine (QTP), OLA, risperidone (RISP), ZIP, ARI, as well as HAL using several tests in cell-free systems. The selected antipsychotics were then tested in a cellular system [mouse neuroblastoma Neuro-2A (N2A) cells]. N2A cells have been used to study neurotoxicity (LePage et al., 2005), Alzheimer's disease (Provost, 2010) and biochemical effects of antipsychotics on neurons (Andres et al., 1999; Budziszewska et al., 2002; Basta-Kaim et al., 2006). This cell line displays a neuron-like phenotype both morphologically and neurochemically (Klebe and Ruddle, 1969; Olmsted et al., 1970). Such a comparison is important to determine where certain compounds exhibit antioxidant activity against physiologically relevant ROS; their dual action may be beneficial as SZ is associated with oxidative and nitrosative stress.

2. Materials and methods

2.1. Materials

All basic reagents were from Sigma-Aldrich (Poznań, Poland), unless indicated otherwise. 3-Morpholinosydnonimine (SIN-1) was obtained from Tocris Bioscience (Bristol, United Kingdom). SIN-1 stock solutions (1 mM) were prepared in phosphate-buffered saline (PBS), and aliquots were frozen immediately at −80 °C until use. Under these conditions, SIN-1 was stable for several months, as assessed by HPLC analysis. Dulbecco's Modified Eagle Medium (DMEM), dihydrorhodamine 123 (DHR123), Hank's Balanced Salt Solution (HBSS), Microplate BCA Protein Assay Kit, Reducing Agent Compatible, trypsin and 4-amino-5-methylamino-2′,7′-difluorofluorescein diacetate (DAF-FM diacetate) were purchased from Thermo Fisher Scientific (Warsaw, Poland). Magnesium chloride solution was obtained from Fluka (Warsaw, Poland). 3-Nitrotyrosine enzyme-linked immunosorbent assay kit was supplied by Merck Millipore (Darmstadt, Germany).

Peroxynitrite (PN; $ONOO^-$; anion of peroxynitrous acid HONOO) was synthesized according to Pryor et al. (1995). This method provides PN of low ionic strength that does not contain hydrogen peroxide as an impurity. The final concentration of PN was about 75–85 mM. Stock solutions of PN were stored at −80 °C and used within 4–6 weeks after synthesis. Before each experiment the concentration of PN was estimated spectrophotometrically at 302 nm in 0.1 M NaOH (ε_M = 1670 M^{-1} cm^{-1}). Also, before each experiment the concentration of hypochlorite ions (OCl^-) was estimated spectrophotometrically at 290 nm in 0.1 M NaOH (ε_M = 350 M^{-1} cm^{-1}). Fluorimetric and absorptiometric measurements were done in a Tecan Infinite 200 PRO multimode reader (Tecan Group Ltd.; Männedorf, Switzerland or in an EnVision Multilabel Plate Reader (Perkin-Elmer; Überlingen, Germany).

The levels of ROS in N2A cell line were estimated with flow cytometry using an LSRII apparatus (BD Biosciences; San Jose California, USA).

All measurements were performed in triplicate and repeated a minimum of three times. Selected AAPs as well as HAL were dissolved in dimethylsulfoxide (DMSO) or ethanol (in studies of the effects of sodium hypochlorite, NaOCl). Minimal amounts of the solvents present in the samples had a small effect on the protection (up to several %). The effect of DMSO or ethanol was subtracted from the effects of substances introduced in this solvent. In cell-free systems, ascorbate and Trolox were used as reference antioxidants.

2.2. Principles of antioxidant essays employed

2.2.1. Protection against protein thiol oxidation

Various oxidants, among them PN and SIN-1 (a compound generating PN), oxidize thiol groups of proteins, which may inactivate proteins. Antioxidants prevent this reaction in a concentration-dependent manner. Human serum albumin (HSA) is used as a substrate protein in this assay. Per cent inhibition of HSA thiol oxidation is a measure of the efficacy of the antioxidant.

2.2.2. Protection against oxidation of DHR123

DHR123 is a model substance that is easily oxidized by various oxidants, among them SIN-1, 2, 2′-azobis(2-amidinopropane) dihydrochloride (AAPH) and NaOCl, to the fluorescent rhodamine 123. AAPH is a source of peroxyl radicals; radicals of this type are produced in the body, e.g. during lipid peroxidation; NaOCl is a product of myeloperoxidase reaction. Prevention of DHR123 oxidation by a compound is a measure of its property to react with a given oxidant. The lower is the concentration of a compound inhibiting DHR oxidation by 50%, the higher is the antioxidant potency of this compound with respect to a given oxidant.

2.2.3. Protection of fluorescein against bleaching induced by PN or NaOCl

The fluorescence of fluorescein can be bleached by various oxidants, including NaOCl, due to oxidation. PN also destroys fluorescein fluorescence, but in this case the loss of fluorescence is due to nitration of this compound (Sadowska-Bartosz et al., 2015). Inhibition of fluorescein bleaching is thus a measure of a given compound to prevent reactions of oxidation by NaOCl or nitration by PN.

2.2.4. Antiradical activity

Antiradical activity is a measure of the ability of a given compound to react with free radicals. One stable free radical employed in such reactions is the 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) radical (ABTS*). Standard antioxidants react rapidly with ABTS* (within seconds; "fast antioxidants") while some react at a lower rate ("slow antioxidants"; Bartosz, 2003). 2,2-Diphenyl-1-picrylhydrazyl free radical (DPPH*) is another stable free radical reduced by antioxidants. Both radicals have different structures and reactivities for various compounds (Janaszewska and Bartosz, 2002).

2.2.5. FRAP assay

The Ferric Reducing Antioxidant Potential assay measures the ability of antioxidants to reduce ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions (Benzie and Strain, 1996). A narrower class of compounds shows antioxidant activity in this test as compared with antiradical activity assays. The reactivity of drugs with iron ions is important as an increase in redox-active iron levels in the brain may cause cognitive impairment (Bókkon and Antal, 2011).

2.2.6. Comparison with standard antioxidants

In order to evaluate the antioxidant power of the compounds studied, standard antioxidants [ascorbic acid (Vitamin C) and Trolox, a water-soluble analog of Vitamin E] were assayed in parallel. When it was possible, activities of the drugs studied were related to the activity of Trolox, dividing the activity of the drug by the activity of Trolox. The results were expressed as moles of Trolox equivalents per mole of the

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