Biomedicine & Pharmacotherapy 81 (2016) 411–415

Available online at
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www.sciencedirect.com

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EM|consulte
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Clozapine and olanzapine are better antioxidants than haloperidol, quetiapine, risperidone and ziprasidone in *in vitro* models

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ARTICLE INFO

Article history:
Received 28 January 2016
Accepted 22 February 2016

Keywords:
Antioxidants
Antipsychotic
Oxidative stress
Schizophrenia

ABSTRACT

Although the etiopathogenic mechanisms of schizophrenia (SCZ) are unknown, evidences suggest that excessive free radical production or oxidative stress may be involved in the pathophysiology of SCZ. Antipsychotics are the drugs used in the treatment of SCZ but it remains controversial the impact that typical vs. atypical antipsychotics has on the oxidative stress status in SCZ patients. *In vitro,* the antioxidant capacity of six antipsychotics was assessed by their ability to: decrease or scavenge reactive oxygen species in the neutrophil respiratory burst; donate hydrogen and stabilize the free radical 2,2-diphenyl-1-picryl-hydrazyl ($DPPH^{\bullet}$); and scavenge 2,2′-azino-di-(3-ethylbenzthiazoline-6-sulphonic acid) ($ABTS^{+}$). This study demonstrated that both clozapine and olanzapine have antioxidant effects, *in vitro,* by scavenging superoxide anion on the respiratory burst, donating electron in the $ABTS^{+}$ assay and stabilizing the radical $DPPH^{\bullet}$. Ziprasidone significantly scavenged $ABTS^{+}$ and stabilized the radical $DPPH^{\bullet}$ whereas risperidone significantly reduced the respiratory burst. Haloperidol and quetiapine lacked antioxidant effects. The chemical structure-related antioxidant capacity suggests a possible neuroprotective mechanism of these drugs on the top of their antipsychotic mechanism of action.

1. Introduction

Schizophrenia (SCZ) is a chronic, severe and disabling psychiatric illness that affects about 1% of the population worldwide [1]. Because of its etiopathogenetic heterogeneity, biological, neurochemical and neuroimaging studies have failed to provide conclusive evidence for any specific etiologic theory for SCZ [2]. Although a clear mechanism underlying the pathogenesis of this disease remains unknown, oxidative stress, that occurs when pro-oxidative processes overwhelm the antioxidant defense system, has become an attractive hypothesis for explaining the pathophysiology of SCZ [1,3].

Antipsychotics have been used to treat SCZ for over 40 years [4]. Presently, pharmacological management of SCZ involves the use of one or more antipsychotics, which are classified as first-generation or typical such as haloperidol (HAL) and second-generation or atypical such as clozapine (CLZ), risperidone (RIS), olanzapine (OLZ), quetiapine (QTP) and ziprasidone (ZPS) [5].

It remains controversial the impact that typical vs. atypical antipsychotics could have on the oxidative stress status in SCZ patients [4]. Typical antipsychotic drugs act blocking the dopamine type 2 receptor [6]. Atypical antipsychotics have lower affinity for the dopaminergic receptors but high affinity for the serotoninergic receptor 5-HT2A [6]. There are also controversies regarding the oxidative stress status in patients treated with typical vs. atypical antipsychotics [4]. Zhang et al. did not find differential effects of typical and atypical antipsychotic drugs on plasmatic antioxidant enzyme activities and lipid peroxidation [7]. In contrast, Padurariu et al. reported that chronic use of typical antipsychotics decreases the activity of plasmaticantioxidant enzymes leading to membrane lipid peroxidation and, consequently, oxidative stress [8]. Free

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http://dx.doi.org/10.1016/j.biopha.2016.02.047
0753-3322/

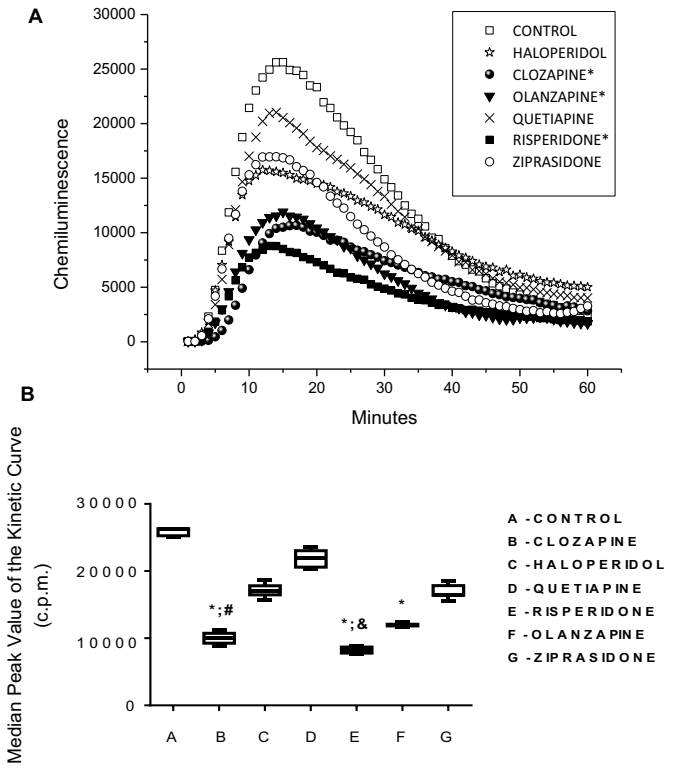

Fig. 1. (A) Effect of antipsychotics drugs (10^{-4} M) on the activation of human neutrophils by PMA. Each group was composed by at least 14 replicates. A kinetic curves was obtained each minute for 60 min. Data were analyzed by Kruskal-Wallis complemented with Dunn's test. * 0.05 compared to control. (B) Effect of antipsychotics drugs (10^{-4} M) on the activation ofhuman neutrophils by PMA. Each group was composed by at least 14 replicates. Data were analyzed by Kruskal-Wallis complemented with Dunn's test. When indicated by symbols, 0.05; * compared to control; # compared to Quetiapine; & compared to haloperidol, quetiapine and ziprasidone. c.p.m. = count per minute.

radical-mediated abnormalities may contribute to the development of clinical symptoms such as negative symptoms, tardive dyskinesia, parkinsonism and neurological "soft" symptom [9]. It has also been suggested that some typical antipsychotics may have pro-oxidant effects leading to oxidative cell injury [4]. On the other hand, Miljevic at al. reported that aripiprazole and QTP increase and protect Cu, Zn-superoxide dismutase activity during incubation *in vitro*, in comparison with controls and may preserve the anti-oxidative role of erythrocytes and have positive systemic effects [10].

The present study aimed to comparatively evaluate, *in vitro*, the antioxidant potential of typical (HAL) and atypical antipsychotics (CLZ, OLZ, QTP, RIS, ZPS). To our knowledge, this is the first study aiming to investigate the relationship between a possible antioxidant effect and the chemical structure of the selected antipsychotics.

2. Material and methods

2.1. Chemicals

The drugs used were: CLZ (Leponex™, Novartis, Brazil), HAL (Halo™, Cristalia, Brazil), OLZ (Zap™, Eurofarma, Brazil), QTP (Queopine™, GlaxoSmithKline, Brazil), RIS (Zargus™, Biosintética, Brazil) and ZPS (Geodon™, Pfizer, Brazil). The drugs were macerated and dissolved in dimethylsulfoxide (DMSO: Synth, Brazil). The reagents used for the assays were: luminol (Acros, USA), phorbolmyristate acetate (PMA), histopaque, 3-ethylbenzothiazoline-6-sulfonic acid (2,2′-azinobis) ($ABTS^+$), 2,2-diphenyl-1-picrylhydrazyl ($DPPH^•$), all from Sigma-Aldrich, USA. All other reagents were of the highest grade available commercially.

2.2. Reactive oxygen species production by neutrophils (respiratory burst)

Reactive oxygen species production by neutrophils was evaluted by chemiluminescence according to an adaptation of the method described by Freitas et al. and Huber, Krötz-fahning, Hock in a multilabel plate reader (Victor X-3, PerkinElmer™, USA) [11,12].

Human neutrophils were isolated from whole blood through gradient density centrifugation. Neutrophil burst was induced by PMA in the presence of 10^{-4} M of CLZ, HAL, OLZ, QTP, RIS, ZPS or phosphate buffer saline (PBS: control group). The DMSO used to dissolve the drugs does not influence the respiratory burst as demonstrated in pilot experiments conducted in our laboratory (data not show). The reaction medium in each well was composed by 200 μL neutrophils (2.5×10^6 cells/mL), 50 μL of luminol 20 mM, 10 μL of the solutions of the test drugs and 50 μL of PMA 5 mM. After fast homogenization, reading (response range between 300 and 620 nm) was conducted for 60 min (one read/min) under 30 ± 1 °C. Results are expressed as count per minute (c.p.m.). Each experimental group was composed by at least 14 replicates. For statistical analysis, the peak value of each curve was used, independently of the time it occurred [13].

2.3. Reduction of free radical $DPPH^•$

The measurement of free radical scavenging was conducted according to Blois, with some modifications [14]. Antipsychotics were added to the reaction mixture containing 1 mL 0.1 M acetate buffer (pH 5.5), 1 mL of ethanol and 0.5 mL of ethanolic solution of $DPPH^•$ 50 M. The drugs concentrations in the reaction were 0.0005–0.008 mg/mL of CLZ, 0.0005–1.0 mg/mL of HAL, 0.05–0.08 mg/mL of OLZ, 0.5–25 mg/mL of QTP, 0.5–2 mg/mL of RIS and 0.0005–1.0 mg/mL of ZPS. The reduction of $DPPH^•$ radical was determined by the change in absorbance measured at 517 nm. The suppression of the colored radical results in absorbance decrease. The positive control was prepared in the absence of the test drugs in order to determine the maximum odd electrons of $DPPH^•$, which was considered 100% of free radicals in the solution and used to calculate the hydrogen donating ability (%) of the drugs evaluated. The blank was prepared from the reaction mixture without $DPPH^•$ solution. Samples were analyzed in triplicate. The results were expressed as percentage of activity by the following equation:

$$\% \text{ of activity} = [1 - (\text{sample absorbance}/\text{control absorbance})] \times 100.$$

2.4. $ABTS^+$ free radical scavenging assay

The ability to scavenge the $ABTS^+$ free radical was carried out according to Sánchez-Gonzáles, Jiménez-Escrig, Saura-Calixto with some modifications [15]. $ABTS^+$ solution was obtained after the reaction of 7 mM $ABTS^+$ with 2.45 mM potassium persulphate. This solution was diluted in phosphate buffer (pH 7.4, 0.1 M) until it reached an absorbance of 0.7–0.8 at 730 nm. Different concentrations of CLZ 0.0005–0.005 mg/mL, HAL 0.05-1.0 mg/mL, OLZ 0.0005–0.04 mg/mL, QTP 0.5–25 mg/mL, RIS 0.5–2 mg/mL and ZPS 0.0005–0.15 mg/mL were evaluated. The suppression of the colored radical on the medium was monitored through the decrease in absorbance. The positive control was prepared in the absence of the test drugs and was considered 100% of free