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Haloperidol and Risperidone Induce Apoptosis Neuronal Cell: In vivo Study

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A B S T R A C T

Introduction: Antipsychotics are drugs that are widely prescribed for mental disorders, such as schizophrenia and psychosis. Recent in vitro studies show antipsychotics play a role in the initiation of neuronal cell apoptosis. This study aims to determine the effect of haloperidol and risperidone on neuronal cell apoptosis in Wistar white rats. **Methods:** Male Wistar rats aged 8 weeks (n = 30) were used in this study. Wistar rats were randomized into 6 groups. Group A: 5 Wistar rats as the control without induced schizophrenia, aquadest, and drugs. Group B: 5 Wistar-induced psychotic mice (using 30 mg / kgBB ketamine, intraperitoneal injection for 5 days) and aquadest. Group C: 5 rats were induced psychotic and were given haloperidol or 0.05 mg / kgBB orally, for 28 days. Group D: 5 mice were induced psychotic and were given haloperidol 0.1 mg/kg orally, for 28 days. Group E: 5 mice were induced psychotic and were given risperidone 0.05 mg / kgBB orally, for 28 days. Group F: 5 mice were induced psychotic and given risperidone 0.1 mg / kgBB orally, for 28 days. Apoptosis of neuronal cells in the ventral tegmental area was assessed by caspase-3 immunohistochemistry. The colored area will be calculated as a total percentage using the ImageJ program. **Results:** Risperidone and haloperidol increase caspase-3 activity, but haloperidol increases caspase- 3 activity more than risperidone. **Conclusion:** Risperidone and haloperidol induce apoptosis of neuronal cells and tardive dyskinesia in Wistar rats with psychotic models.

1. Introduction

Schizophrenia is a psychiatric disorder that occurs in about 1% of the world's population. Genetic influence is believed to have a role in psychiatric disorders, especially if the disorder has occurred in young adults or adolescents. Clinical symptoms are characterized by positive symptoms (delusions, hallucinations, disorganization of the mind, speech, and behavior), negative symptoms (loss of motivation, withdrawal from social life, having poor social relations), and cognitive deficits .[1,2]

Antipsychotics are agents used in the treatment of schizophrenia. There are two generations of antipsychotics, namely first-generation antipsychotic (FGA), one example is haloperidol and second-generation antipsychotics (SGA), one example is risperidone. FGA is an antipsychotic generation that works by blocking dopamine 2 receptor activity, especially in the striatal and mesolimbic areas. SGA is an antipsychotic generation that does not directly block dopamine 2 receptors but through the 5HT2A receptor blockade. Extrapyramidal symptoms are a side effect of dopamine 2 receptor blockade in the nigrostriatal area, in the form of symptoms that mimic Parkinson's disorders. If this blockade goes on chronically it will cause hyperkinetic movements known as tardive dyskinesia. This disorder is

manifested as facial and tongue movements that spontaneously resemble people who chew.[3-5]

Activation of the dopamine 2 receptor will also activate β-arrestin-2 which inhibits Akt activity and increase GSK-3 protein. GSK-3 is a signaling protein that plays a role in neuronal cell survival. If there is an inhibition of dopamine 2 receptor activity by antipsychotics, it will cause activation of Akt and a decrease in GSK-3 activity so there will be a decrease in neuronal cell survival. Decreased dopaminergic cells are believed to have an impact on the onset of a continuous hyperkinetic movement known as dyskinesia. Tardive dyskinesia is an unexpected effect resulting from a blockade of dopamine activity. [6-8]

This research would examine the effect of giving haloperidol and risperidone on neuronal cell apoptosis and the incidence of dyskinesia in Wistar white mice with psychotic models. It was expected that risperidone which is an SGA antipsychotic can minimize apoptosis and dyskinesia tardive events when compared to haloperidol which is an FGA antipsychotic group. SGA plays a role in blocking dopamine activity indirectly by blocking 5HT2A activity, while FGA plays a role in blocking dopamine activity directly on dopamine receptors. Other studies have shown that the administration of risperidone can minimize the apoptosis of neuroblastoma cells.[9]

2. Methods

Wistar white rats aged 8 weeks old (bodyweight 150-200 grams) were obtained from the Preclinical testing laboratory Faculty of Medicine Universitas Sriwijaya, Palembang, Indonesia. This study was approved by the Ethics Unit of the Bioethics and Humanities Faculty of Medicine Universitas Sriwijaya (FK Unsri) (No. 213 / kptfkunsri-rsmh / 2019). Rats were placed in separate cages, with food and drink administered ad libitum, at room temperature 220 C. The study was conducted at the FK Unsri Biomedical Laboratory.

This study used 30 experimental animals which were randomized into six groups. Group A: 5 mice as normal controls; group B: experimental animals induced by psychotic for 10 days, then given aquadest 1 mL for 28 days. Group C: experimental animals were induced by psychotic for 10 days, then given a haloperidol dose of 0.05 mg / kg for 28 days. Group D: experimental animals were induced by psychotic for 10 days, then given a haloperidol dose 0.1 mg / kg for 28 days. Group E: experimental animals induced by psychotic for 10 days, then given risperidone dose of 0.05 mg/kg for 28 days. Group F: experimental animals induced by psychotic for 10 days, then given risperidone dose 0.1 mg / kgBB for 28 days.
Experimental animals were injected with saline solution or 30 mg / kgBB of ketamine (Sigma) intraperitoneal (i.p.) for 5 days. Meanwhile, the normal control group was not given an injection of saline or ketamine for 5 days.

The success of psychotic induction in experimental animals was assessed by the locomotor activity test (LAT) and social interactivity test (SIT). LAT is a test used to assess impulsivity that reflects positive symptoms from animal models of psychotic labor. LAT is assessed by measuring the distance traveled by experimental animals. Measurements were assessed for 30 minutes. LAT is stated positively if there is a significant difference between the groups that are induced and those that are not induced.

SIT is a test used to assess the negative symptoms of experimental animals using psychotic models. SIT is assessed by measuring the length of time spent by experimental animals interacting with fellow experimental animals. Measurements were assessed for 15 minutes. SIT is stated as positive if there is a significant difference between the groups that are induced and those that are not induced.

Detection of caspase 3 activity is performed by immunohistochemical techniques. Experimental animals were treated with deep anesthesia using ketamine 60 mg / kgBB, i.p., and fused through the left ventricle.

In short, under the influence of anesthesia, animals try to undergo surgery in the chest and perfusion must begin less than 1 minute after surgery. Perfusion fluid is used for 2 minutes and followed by

fixative fluid. Perfusion liquids contain 0.1% (w / v) Tris, 0.59% (w / v) calcium chloride and 0.09% (w / v) sodium chloride. The fixative fluid composition consists of 2% (v / v) paraformaldehyde, 2.5% (v / v) glutaraldehyde, 0.1% (w / v) Tris, 0.59% (w / v) calcium chloride, and 0.36% (w / v) sodium chloride, with a final pH of 7.5. Evacuation of the animal brain from the skull was not done after perfusion, but 24 hours later to avoid the formation of artifacts. This procedure is very important and effective to avoid damage to neuron tissue after perfusion.

At the end of the perfusion process, the animal's brain tries to be evacuated from the skull. The brain will be fixed in the same solution for 2 hours, placed in Tris phosphate-buffered saline (PBS) / 30% sucrose (pH 7.4, 4 ° C) for 24 hours, and serially divided into 30 mm thick coronal sections using cryotomes. The preparations were then blocked with Tris PBS containing 3% normal serum goat (NGS) at a concentration of 1: 1000 in 3% NGS and 0.3% Triton X-100 for 1 hour, incubated in serum anti-caspase-3 rabbit (Sigma) at concentrations of 1: 1000 in 3% NGS and 0.3% Triton X-10 overnight at room temperature, then washed with Tris PBS three times, each for 10 minutes and then incubated in avidin-biotin-peroxidase complex (Sigma) for 1 hour.

To identify immunoreaction against the product, horseradish peroxidase was visualized with diaminobenzidine (DAB) and glucose oxidase, with nickel intensification. Coronal section estimated at -3.5 mm to bregma is used for Caspase- 3 expressions in the Ventral Tegmental Area. Both sides of several sections from each level are evaluated to ensure the results of each representative region for the results of this study.

Caspase-3 activity will be assessed in the nigrostriatal area by immunohistochemistry. Digital image analysis is used to analyze caspase 3 activity with the imageJ program.

3. Results

Induction of experimental animals was done by giving ketamine 50 mg / kgBW intraperitoneally for 5 days, then for the next 5 days, the experimental groups were not given ketamine. The success of animal induction was assessed by the locomotor activity test (LAT) and social interactivity test (SIT).

Table 1 shows that there are differences in the average mileage in the LAT test between the control group and the induced group. The ketamine-induced group showed more distance than the control group.

Table 1. Mean distance traveled by experimental animals on LAT

Mean Distance traveled by The control group on LAT (cm)*	**Mean Distance traveled by experimental animals (cm)***
82,23 ± 4,98	105,78 ± 6,12

* p<0,05

Table 2 shows the difference in mean interaction times of experimental animals in the SIT test. The interaction time of experimental animals in the induced group was shorter compared to the control group.

Table 2. Mean interaction time of experimental animals on SIT

Mean Interaction Time of Control Group (minutes)*	**Mean Interaction Time of Induction Group (minutes)***
12,13 ± 2,12	5,21 ± 1,87

* p<0,05

The percentage of caspase-3 area in the treatment group with haloperidol and risperidone is wider than the area of the treatment group with distilled water (Figure 1). Caspase-3 activity was more active in the treatment group with haloperidol and risperidone.

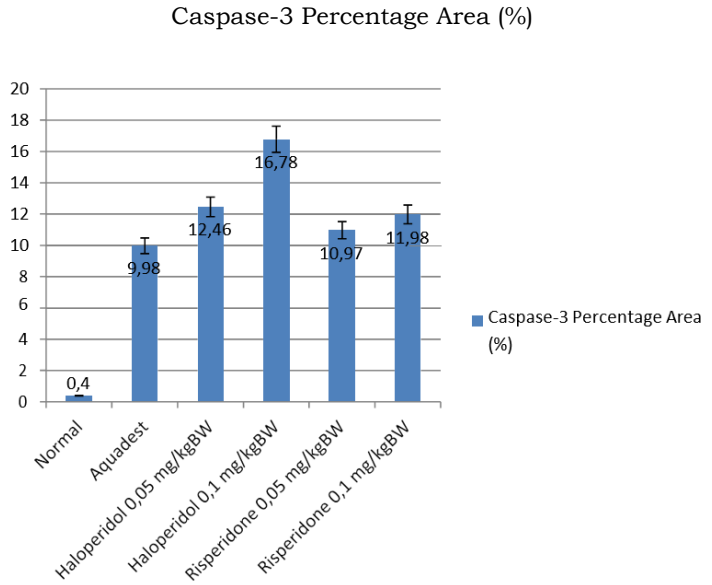

Figure 1. Caspase-3 Area Percentage (%)

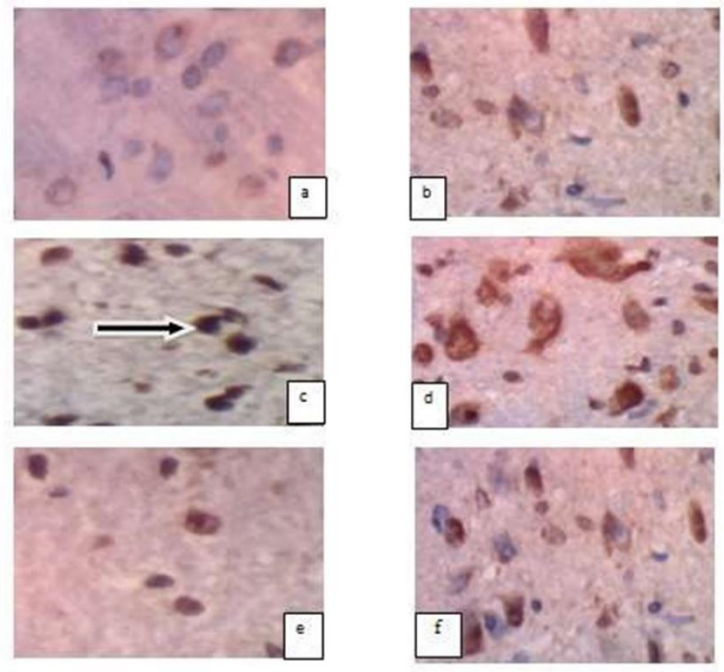

Figure 2. Immunohistochemistry of caspase-3 in VTA activity (magnification 40x)

Notes: Group A: 5 rats as normal controls; group B: experimental animals induced by psychotic for 10 days, then given aquadest 1 mL for 28 days. Group C: experimental animals were induced by psychotic for 10 days, then given haloperidol dose of 0.05 mg / kg for 28 days. Group D: experimental animals were induced by psychotic for 10 days, then given haloperidol dose 0.1 mg / kg for 28 days. Group E: experimental animals induced by psychotic for 10 days, then given risperidone dose of 0.05 mg / kg for 28 days. Group F: experimental animals induced by psychotic for 10 days, then given risperidone dose 0.1 mg / kgBB for 28 days.

4. Discussion

This research showed that ketamine acts as a psychotic induction agent in experimental animals. This is consistent with research that states that the administration of ketamine at a dose of 25 mg / kgBB for 1 week will increase the impulsivity of experimental animals assessed by LAT and increase the number of stereotype movements in experimental animals. Meanwhile, other studies have shown that administration of ketamine at a dose of 30 mg / kgBB for 1 week will reduce the ability of social interaction of experimental animals as assessed by SIT. Antipsychotics are divided into two generations, namely first-generation antipsychotics (FGA), an example is a haloperidol, and second-generation antipsychotics (SGA), one example is risperidone. FGA is an antipsychotic generation that works by blocking dopamine 2 receptor activity, especially in the striatal and mesolimbic areas. SGA is an antipsychotic generation that does not directly block dopamine 2 receptors but through the 5HT2A receptor blockade. Extrapyramidal symptoms are a side effect of dopamine 2 receptor blockade in the nigrostriatal area, in the form of symptoms that mimic Parkinson's disorders. If this blockade goes on chronically it will cause hyperkinetic movements known as tardive dyskinesia. This disorder is manifested as facial and tongue movements that spontaneously resemble those who chewing.[10,11]

The dopamine ligand will bind to the dopamine 2 receptor and will be activated by the Gai Protein which will inhibit adenylate cyclase and inhibit cAMP activity. Activation of the dopamine 2 receptor will also activate β-arrestin-2 which inhibits Akt activity and an increase in GSK-3 protein. GSK-3 is a signaling protein that plays a role in neuronal cell survival. If there is an inhibition of dopamine 2 receptor activity by antipsychotics, it will cause activation of Akt and a decrease in GSK-3 activity so that there will be a decrease in neuronal cell survival. Decreased dopaminergic cells will certainly have an impact on the onset of hyperkinetic movements known as dyskinesia dyskinesia.[12-15]

In this study, the results of caspase-3 activity were more active in the treatment group with haloperidol and risperidone. Caspase 3 activation acts as a regulator of neuronal cell apoptosis. Apoptosis is programmed cell death. Various conditions initiate the activation of the apoptotic process. Akt protein and GSK-3 are signaling proteins that play a role in apoptotic activation. Defosforilation of GSK-3 Akt and phosphorylation will cause dephosphorylation of the anti-apoptotic protein, Bcl-2. Furthermore, mitochondrial depolarization occurs resulting in an increase in cell membrane permeability, cytochrome-c release into the cytosol, and caspase-3 activation which acts as an apoptotic regulator. The results of this study are in line with studies that prove that atypical and typical antipsychotic administration induces apoptosis in the frontal cortex of experimental animals by activating protein caspase 3.[16,17]

5. Conclusion

Haloperidol and risperidone affect the incidence of neuronal nigrostriatal cell apoptosis that is characterized by caspase-3 activity in white-psychotic Wistar rats.

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