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Ciproxifan improves cholinergic transmission, attenuates neuroinflammation and oxidative stress but does not reduce amyloid level in transgenic mice

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ABSTRACT

Aim: The present study is aimed to investigate the ability of ciproxifan, a histamine H_3 receptor antagonist to inhibit β-amyloid (Aβ)-induced neurotoxicity in SK-N-SH cells and APP transgenic mouse model.
Materials and methods: In vitro studies was designed to evaluate the neuroprotective effects of ciproxifan in $A\beta_{25-35}$ - induced SK-N-SH cells. For the in vivo study, ciproxifan (1 and 3 mg/kg, i.p.) was administrated to transgenic mice for 15 days and behaviour was assessed using the radial arm maze (RAM). Brain tissues were collected to measure Aβ levels ($A\beta_{1-40}$ and $A\beta_{1-42}$), acetylcholine (ACh), acetylcholinesterase (AChE), nitric oxide (NO), lipid peroxidation (LPO), antioxidant activities, cyclooxygenases (COX) and cytokines (IL-1α, IL-1β and IL-6), while plasma was collected to measure TGF-1β.
Results: The in vitro studies demonstrated neuroprotective effect of ciproxifan by increasing cell viability and inhibiting reactive oxygen species (ROS) in $A\beta_{25-35}$-induced SK-N-SH cells. Ciproxifan significantly improved the behavioural parameters in RAM. Ciproxifan however, did not alter the Aβ levels in APP transgenic mice. Ciproxifan increased ACh and showed anti-oxidant properties by reducing NO and LPO levels as well as enhancing antioxidant levels. The neuroinflammatory analysis showed that ciproxifan reduced both COX-1 and COX-2 activities, decreased the level of pro-inflammatory cytokines IL-1α, IL-1β and IL-6 and increased the level of anti-inflammatory cytokine TGF-1β.
Conclusion: This present study provides scientific evidence of the use of ciproxifan via antioxidant and cholinergic pathways in the management of AD.

1. Introduction

Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder and has been reported as a leading cause of dementia in elderly people affecting approximately 10% of the population older than 65 years of age. This disease is often characterized by progressive memory impairment and cognitive deficits that can affect their daily routine such as thought, learning, memory and speaking. According to World Alzheimer's Report 2013, there are presently 35 million of people with AD globally and this number is expected to double by 2030 and more than triple to 115 million by 2050 [1]. The pathological hallmarks of AD are characterized mainly by the deposition of extracellular neuritic plaques and the formation of intracellular neurofibrillary tangles [2]. The major component of neuritic plaque is the β-amyloid peptide (Aβ), which is produced by proteolytic processing from the sequential cleavage of amyloid precursor protein (APP) carried out by β-secretase and γ-secretase. Although the neurotoxicity of Aβ is still unclear, but various studies have suggested that Aβ has been related to induce neurodegenerative changes including apoptosis, oxidative stress and neuroinflammation. Therefore, prevention of Aβ aggregation is one of promising strategies for the prevention and treatment of AD.

In recent years, histamine H_3 receptor antagonists have received much attention as a potential therapeutic for treating disorders of the central nervous system (CNS) including dementia [3]. Histamine H_3 receptor antagonists have been reported in numerous studies as potential cognitive-enhancing drugs. Histamine H_3 receptors are G-protein

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coupled receptors which act as auto-receptors in regulating the synthesis and release of neurotransmitter histamine as well as a heteroreceptor in regulating the release of other neurotransmitters such as acetylcholine (ACh), norepinephrine (NE) and dopamine that are involved in learning and memory [4]. Upon activation, this receptor can inhibit the release of these neurotransmitters. Various studies have revealed that the blockade of these receptors lead to the release of these neurotransmitters in the hippocampal and prefrontal cortex of the brain [5].

Ciproxifan was first described by Ligneau et al. (1998) as a potent H_3 receptor antagonist [6]. It has been shown to increase the production of histamine, dopamine, norepinephrine as well as acetylcholine in the cortex and hippocampus of the brain [7]. Since the elevation of these neurotransmitters is believed to enhance attention and memory, this compound has been widely studied and explored in behavioural models. Ciproxifan blocks H_3 receptor consequently increasing histamine release that promotes alertness [8]. Ciproxifan has been shown to enhance memory function in several behavioural tasks on normal rats and mice as well as in animal induced with anti-cholinergic and anti-glutamatergic drugs. The role of ciproxifan on pathophysiology of AD however, remains unknown. Therefore, the present study aimed to investigate the ability of ciproxifan to inhibit Aβ-induced neurotoxicity using neuroblastoma SK-N-SH cells in-vitro as well as a neuroprotective agent against brain Aβ peptides level, oxidative stress and neuroinflammation in AD transgenic mouse model.

2. Experimental procedures

2.1. In-vitro BACE-1 assay

The assay was carried out to determine the ability of ciproxifan to inhibit BACE-1 enzyme activity using the BACE-1 assay kit according to the manufacturer's protocol. Final concentrations of ciproxifan were as follow: 0.1, 1, 10, 100 and 1000 μg/ml. A mixture of 10 μl of test compound dissolved in assay buffer, 10 μl of substrate (750 nM Rh-EVNLDAEFK- quencher in 50 mM ammonium bicarbonate) and 10 μl BACE-1 (1.0 Unit/ml) were incubated at room temperature for 60 min in the dark. Fluorescence was read using a fluorescence microplate reader (infinite M200, TECAN) under excitation at 545 nM and emission 585 nM. Percentage inhibition of the enzyme was calculated and $IC_{50(BACE\text{-}1)}$ value was determined. The $IC_{50(BACE\text{-}1)}$ value is defined as the concentration of the BACE1 inhibitor required to reduce 50% of BACE1 activity.

2.2. In vitro SK-N-SH cells

2.2.1. Cell culture and treatment

Human neuroblastoma SK-N-SH cells (ATCC HTB-11) were cultured routinely in Eagle's minimum essential medium (MEM) supplemented with 10% (v/v) fetal bovine serum, 1% penicillin/streptomycin at 37 °C under 5% CO_2. Medium was changed every 3 days and subcultured once the cells reached 75% confluence. Cell cultures were maintained in a humidified incubator (NuAire Inc., Plymouth, MN, USA), at 37 °C in an atmosphere with 5% CO_2. To induce cell toxicity, SK-N-SH cells were incubated with 75 μM $A\beta_{25-35}$ for 24 h. Aβ (400 μM) was prepared by dissolving in deionized distilled water and incubated in a 37 °C water bath for 3 days to induce aggregation. The stock solution was diluted to the desired concentrations immediately before use and added to the culture medium to induce cell toxicity in SK-N-SH cells.

2.2.2. Neuroprotective effect of ciproxifan

Neuroprotective effects of ciproxifan against β-amyloid$_{25-35}$–induced neurotoxicity in SK-N-SH cells were measured using MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. In brief, cells were seeded onto a 96-flat bottom well plate at a density of 2×10^4 cells per well. The cells were treated with or without ciproxifan (0.01 to 100 μg/ml) and incubated for 24 h. The α-tocopherol (10 μg/ml) served as positive control. The cells were then washed with phosphate buffer saline (PBS) prior exposure to 20 μM $A\beta_{25-35}$ for 24 h to induce toxicity. MTT solutions was added at a final concentration of 0.5 mg/ml and incubated at 37 °C for 4 h. The formazan crystals were dissolved with dimethyl sulfoxide (DMSO) and absorbance was measured at 570 nm using the TECAN Infinite M200 microplate reader (Tecan, Durham, USA).

2.2.3. Measurement of intracellular ROS level

The SK-N-SH cells were cultured in 96 well plates at a density 2×10^4 cells/well and allowed to grow for 24 h. The cells were then treated with $A\beta_{25-35}$ with or without prior incubation with ciproxifan (0.001–1000 μg/ml) for 24 h. The cells were then incubated with 10 μM H_2-DCF-DA for 30 min at 37 °C in the dark. Cells were washed twice with Hanks buffer before quantification by a fluorescence microplate reader (Tecan) at excitation and emission wavelengths of 485 nm and 535 nm respectively.

2.3. Using transgenic mouse model.

2.3.1. Animal

All experimental protocols described in this study were approved by the Research Committee on the Ethical Use in Research (UiTM Care) [600-FF (PT.5/20)] Universiti Teknologi MARA, Malaysia. The care of the laboratory animals was taken as per the guidelines of the Guide for the Care and Use of Laboratory Animals (National Institute of Health Publication, 2011). Male B6.129-Tg(APPSw)40Btla/J mice expressing all mRNA and protein isoforms of the human amyloid beta (A4) precursor protein APP containing the Familial Alzheimer's Disease (FAD) Swedish mutation K670N/M671L with age of three month were purchased from the Jackson Laboratory, Bar Harbor, USA. A group of age matching male C57BL/6J was used as a wild type control. The animals were housed with three per cage in a laboratory animal room under reverse 12 h dark:light cycle and maintained up to the age of twelve months. The temperature and relative humidity were maintained at 21 ± 2 °C and 45–55% respectively. During the above period, animals were given free access to standard laboratory food pellet and water ad libitum. All the animals were monitored daily for signs of distress, and any health concerns were brought to the attention of the institutional veterinarian.

2.3.2. Drug treatment

Animals were divided into four groups; transgenic control, wild-type control that received saline (0.9% NaCl, i.p.), and transgenic groups that received 1 and 3 mg/kg of ciproxifan. The doses of ciproxifan were chosen based on previous efficacy studies that improved memory, attention and spatial learning in rodents [6,7,9]. Ciproxifan hydrochloride was dissolved in sterile isotonic saline solution. Ciproxifan (1 or 3 mg/kg) or saline (10 ml/kg) were administered intraperitoneally in the mice for fifteen consecutive days and the treatment was 45 min prior to training or testing using the radial arm maze. At the end of the maze test, the animals were sacrificed and brains were collected for various biochemical evaluations (Fig. 1).

2.3.3. Behavioural assessment of memory by radial arm maze

Radial arm maze test was performed to measure spatial learning and memory of mouse. This maze consists of 8 spaced arms numbered from 1 to 8 and elevated 50 cm above the floor. Each arm is 48 cm × 12 cm and has a central platform approximately 32 cm in diameter to where the animal is placed at the start of the experiment. Five arms (arm no. 1, 3, 4, 6 and 7) were selected to serve as the baited arms while another three arms (arm no. 2, 5 and 8) were not-baited. Each arm was distinguished by a geometric-shaped paper located at the end of each arm. These cues are present in different geometric shape for each arm and remained in the same location throughout the task. The task consists of 3 phases which include diet restriction, training and memory