Inhibitory effect of chloroquine derivatives on presenilin 1 and ubiquilin 1 expression in Alzheimer’s disease

Fang-Fang Zhang, Jing Li

Department of Neurology, Xinxiang Central Hospital, Xinxiang 453000, China

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Abstract: Alzheimer’s disease is the common cause of dementia characterized by the accumulation of amyloid-β produced by breakage of amyloid-β precursor protein (APP). The present study was designed to synthesize and investigate the effect of chloroquine derivatives on the expression of presenilin. Among the five chloroquine derivatives (D1, D2, D3, D4 and D5) synthesized, D5 with disisopropyl substitution was found to be most effective. The results from western blot analysis showed the inhibition of presenilin 1 protein expression on treatment with chloroquine derivative D5 in Daudi cells. The results were also confirmed by reverse transcriptase-polymerase chain reaction (RT-PCR). It was observed that chloroquine derivative D5 downregulates presenilin expression via the inhibition of ubiquilin 1 expression. Thus our study demonstrates that chloroquine derivative D5 treatment can have preventive against Alzheimer’s disease.

Keywords: Chloroquine, inhibition, presenilin, ubiquilin 1, disisopropyl substitution

Introduction

Chloroquines (CQ) pass through the blood-brain barrier (BBB) and have been reported to exhibit potential antimalarial activity [1]. In addition, chloroquine and its analogs have also shown promising results for the treatment of prion disease [2-5], against hepatitis C virus (HCV) [6, 7] and for cancer treatment [8, 9]. The derivatives of chloroquine like hydroxychloroquine have been used for the treatment of systemic lupus erythematosus [10] and rheumatoid polyarthritis [11] without producing any side effect. Taking into account the accumulation and spread of prion-like proteins in the brain in Alzheimer’s disease (AD) patients [12, 13], we synthesized and evaluated the effect of five chloroquine derivatives (Figure 1) on Alzheimer’s disorder.

Alzheimer’s disease (AD) is the common cause of dementia with no cure reported so far and is characterized by the accumulation of amyloid-β [14]. Amyloid-β is a neurotoxin produced by the breakage of amyloid-β precursor protein (APP). This breakage is induced by the presenilins a catalytic unit of γ-secretase complex when pre-senilin gene suffers mutation [15]. Therefore targeting presenilin can be of therapeutic value for AD disease. The disorders related with amyloid-β are reduced by curcumin in transgenic AD mouse models [16]. In addition, curcumin leads to reversal of structural changes in dystrophic dendrites including abnormal curvature and dystrophy size [17]. Reduction in the expression of presenilin 1 in Jurkat cells was observed on treatment with curcumin [18]. The γ-secretase modulators (GSMs) are the small molecules which modulate the activity of γ-secretase by reducing the long Aβ (1-40 and 1-42) concentration and promoting short Aβ peptides (1-37 and 1-38) concentration.

Materials and methods

Cell culture

The human Daudi cell line was obtained from the American Type of Collection Centre (Manassas, VA, USA) and maintained in DMEM supplemented with 10% fetal bovine serum (FBS) and antibiotics at 37°C in a humidified atmosphere containing 5% CO₂.
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Preparation of reagents

The chloroquine derivatives were synthesized using the procedure developed by Patricia Melnyk et al. [19] and dissolved in DMSO to a stock concentration of 10 mM and stored at -20°C.

Western blot analysis

The cells at a density of 2.5 × 10⁶ were plated for 12 h and treated with chloroquine derivatives derivative or untreated as control for 48 h. The cells were lysed in lysis buffer (50 mMTris-HCl pH 7.4, 137 mM NaCl, 10% glycerol, 100 mM sodium vanadate, 1 mM PMSF, 10 mg/mL aprotinin, 10 mg/mL leupeptin, 1% NP-40, and 5 mM cocktail). The lysate was centrifuged to remove cell debris and concentration of proteins was determined by Protein Assay System (Bio-Rad, Hercules, CA, USA). The protein were resolved by electrophoresis on 10% polyacrylamide gel and transferred to nitrocellulose membranes. The semi-dry method was used to transfer proteins onto a PVDF membrane which was then blocked with 5% non-fat dry milk overnight. Incubation of membranes with anti-ubiquilin 1 (GeneTex Inc., Irvine, CA, USA), anti-presenilin 1 (GenScript USA Inc., Piscataway, NJ, USA), anti-presenilin 2 (GenScript) and anti-Erk2 (Epitomics Inc., Burlingame, CA, USA) antibodies was performed for 2 h. Then the incubation was continued for 1.5 h with horseradish peroxidase-conjugated goat anti-rabbit, goat anti-mouse, or rabbit anti-goat IgG secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) antibodies. ECL chemiluminescence detection system (GE Healthcare) was used for visualizing the signal.

Reverse transcriptase-polymerase chain reaction (RT-PCR)

Ubiquilin 1, presenilin 1 and GAPDH mRNAs were analyzed by semi-quantitative RT-PCR. The cells after chloroquine derivative treatment where lysed and RNA isolation kit (Takara, Japan) was used to extract total RNA. The Phusion RT-PCR kit (NEB) was used to reverse-transcribe two micrograms of total RNA using the manual protocol. The level of ubiquilin 1 and presenilin 1 genes was quantified by employing semi-quantitative RT-PCR using GAPDH as an internal loading control. The reactions for real-time PCR were carried out employing real-time PCR system (Illumina Inc., San Diego, CA, USA).

Results

The Daudi cells were cultured in a medium supplemented with various chloroquine derivatives (D1-D5) or ethanol vehicle as control. We used semi-quantitative RT-PCR analysis for the quantification of ubiquilin 1 and presenilin 1 gene expression. The results revealed that the levels of presenilin 1, presenilin 2 and housekeeping gene GAPDH remained unaltered throughout the treatments in cells cultured in culture medium supplemented with D1, D2, D3, D4 and D5 derivatives compared with the ethanol vehicle (Figure 2). However, the expression level of the...
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ubiquilin 1 gene was markedly decreased upon treatment with chloroquine derivative D5 after 24 h. The inhibition of ubiquilin 1 expression by chloroquine derivative D5 was found to be dose dependent with significant effect at 10 mg/mL.

We also used western blot analysis to investigate the expression status of the ubiquilin 1 and presenilin 1 protein level in the cells treated with the chloroquine derivatives. Treatment of the cells with 10 mg/mL of chloroquine derivative D5 and the tyrosine kinase inhibitor (AG490) significantly inhibited the expression of both ubiquilin 1 and presenilin 1 proteins after 48 h (Figure 3). The cells were treated with different concentrations of chloroquine derivative, D5 to determine its concentration needed to inhibit the protein expression of presenilin 1 and 2. The inhibition of presenilin 1 and 2 in Daudi cells was significant at 10 mg/mL of chloroquine derivative, D5 with no effect on Erk2 (Figure 4). Thus chloroquine derivative, D5 exhibited a concentration dependent effect on the downregulation of presenilin 1 and 2.

Discussion

Estrogen receptor plays a vital role in the regulation of presenilin expression and one of the major presenilin-interacting proteins known to stabilize presenilin is ubiquilin [20]. It is report-ed that AD has a relation with ubiquilin protein expression [21, 22]. Our results demonstrate that chloroquine derivative; D5 inhibits the expression of presenilins in Daudi cells which may be due to the suppression of ubiquilin 1 expression. The mechanism behind the ubiquilin 1 inhibitory effect of chloroquine derivative, D5 is yet to be fully understood but further studies need to be performed to understand the same.

The γ-secretase modulators (GSMs) reduce the long Aβ (1-40 and 1-42) concentration and enhance short Aβ peptides (1-37 and 1-38) concentration thereby modulate the activity of γ-secretase. In the present study, results demonstrate that chloroquine derivative, D5 and another tyrosine kinase inhibitor AG490 inhibited the presenilin protein expression. This inhibition can have a crucial role in γ-secretase activity. Ubiquilin 1 has also been shown to regulate the process of decomposition of proteasomes like presenilin [23]. Since proteasomes are responsible for the removal of oxidatively damaged proteins in the cytosol and nucleus during oxidative stress, it is possible that presenilin 1 may be decomposed by ubiquilin proteosomal pathway. Further studies to investigate the effects of chloroquine derivative, D5 on proteins implicated in Alzheimer’s disease are under process in our laboratory.

Conclusion

Thus chloroquine derivative, D5 induces the inhibition of presenilin 1 which can be of therapeutic value for the treatment of AD.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Fang-Fang Zhang, Department of Neurology, Xinxiang Central Hospital, 56 Jinsui Avenue, Xinxiang 453000, Henan, China. Tel: 0086-373-2022300; Fax: 0086-373-2022300; E-mail: zhangff09@gmail.com
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