European Journal of Pharmacology 782 (2016) 30–34

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Neuropharmacology and analgesia

Protective effects of a dimeric derivative of ferulic acid in animal models of Alzheimer's disease

Jun-Sub Jung [a,1], Ji-Jing Yan [a,1], Hong-Mei Li [a], Md. Tipu Sultan [a], Jaehoon Yu [b], Hee-Sul Lee [c], Kye-Jung Shin [d,*], Dong-Keun Song [a,*]

[a] Department of Pharmacology, Institute of Natural Medicine, College of Medicine, Hallym University, Chuncheon, Gangwon-do 24252, Republic of Korea
[b] Department of Chemistry and Education, Seoul National University, Seoul 08826, Republic of Korea
[c] Scigenic Co., Ltd., Seoul, Republic of Korea
[d] College of Pharmacy, The Catholic University of Korea, Bucheon, Gyeonggi-do 14662, Republic of Korea

ARTICLE INFO

Article history:
Received 17 December 2015
Received in revised form
20 April 2016
Accepted 22 April 2016
Available online 23 April 2016

Keywords:
Ferulic acid-derivative
Animal models of Alzheimer's disease

ABSTRACT

Ferulic acid is a compound with potent anti-oxidant and anti-inflammatory activities. We previously reported the protective effects of ferulic acid administration against two animal models of Alzheimer's disease (AD): intracerebroventricular (i.c.v.) injection of Aß1-42 in mice and APP/PS1 mutant transgenic mice. In this study using the same AD animal models, we examined the effect of KMS4001, one of dimeric derivatives of ferulic acid. Intragastric pretreatment of mice with KMS4001 (30 mg/kg/day) for 5 days significantly attenuated the Aß1-42 (i.c.v.)-induced memory impairment both in passive avoidance test and in Y-maze test. APP/PS1 mutant transgenic mice at KMS4001 doses of 3 and 30 mg/kg/day via drinking water showed the significantly enhanced novel-object recognition memory at both 1.5 and 3 months after the start of KMS4001 treatment. Treatment of APP/PS1 mutant transgenic mice with KMS4001 for 3 months at the doses of 3 and 30 mg/kg/day markedly decreased Aβ1-40 and Aβ1-42 levels in the frontal cortex. The KMS4001 dose-response relationships for Aβ decrease and for improvement in novel-object recognition test corresponded to each other. Taken together, these results suggest that KMS4001 could be an effective drug candidate against AD.

1. Introduction

Ferulic acid, widely present in various plants, is a compound with potent anti-oxidant and anti-inflammatory activities (Mancuso and Santangelo, 2014). We previously reported that long-term administration of ferulic acid protects against Amyloid ß (Aß)-induced learning and memory impairment in mice (Yan et al., 2001), and inhibits Aß-induced activation of astrocytes and microglia (Cho et al., 2005; Kim et al., 2004). Furthermore, we and others recently reported the protective effects of ferulic acid administration in APP/PS1 mutant transgenic mice (Yan et al., 2013; Mori et al., 2013). Ferulic acid has *in vitro* anti-fibrillogenic and fibril-destabilizing effects for Aß (Ono et al., 2005; Ono and Yamada, 2006). Similar *in vivo* and *in vitro* beneficial effects of ferulic acid derivatives, i.e., sodium ferulate (Jin et al., 2005, 2006), ferulic acid ethyl ester (Mohmmad Abdul and Butterfield, 2005; Joshi et al., 2006; Perluigi et al., 2006; Sultana et al., 2005) and a styryl benzene–ferulic acid hybrid molecule (Lee et al., 2005) have also been reported.

In this study, we examined the effect of KMS4001, a new dimeric derivative of ferulic acid (Fig. 1). Since ferulic acid has anti-fibrillogenic and fibril-destabilizing effects against Aß (Ono et al., 2005; Ono and Yamada, 2006), we thought that the dimerization of this type of small molecules could increase its pharmacological efficiency due to divalent or mutivalent bindings against pathogenic Aβ oligomers. We used two animal models of Alzheimer's disease (AD), i.e. an intracerebroventricular (i.c.v.) injection of Aß1-42 to mice, and APP/PS1 mutant transgenic mice, and found that KMS4001 has beneficial effects in both animal models.

2. Materials and methods

2.1. Preparation of KMS4001

Synthesis of 1,2-di[2-(4-methoxybenzyloxy)-5-formyl]phenoxyethane: 10 g (38.7 mmol) of 4-(4-methoxybenzyloxy)-3-

Abbreviations: AD, Alzheimer's disease; Aß, Amyloid ß; i.c.v., intracerebroventricular

* Corresponding authors.
E-mail addresses: kyejung@catholic.ac.kr (K.-J. Shin), dksong@hallym.ac.kr (D.-K. Song).

[1] These authors contribute equally to this study.

http://dx.doi.org/10.1016/j.ejphar.2016.04.047
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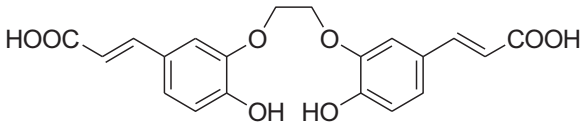

Fig. 1. Structure of KMS4001.

hydroxybenzaldehyde was dissolved in 200 ml of anhydrous dimethylformamide, and then 1.63 g (40.6 mmol) of 60% NaH was slowly added at room temperature. The reaction mixture was stirred for 30 min, wherein 7.17 g (19.4 mmol) of ethyleneglycol ditosylate was added. The reaction mixture was stirred at 80 °C for 5 h, and then cooled to room temperature after the completion of reaction was confirmed by thin layer chromatography. The reaction mixture was added to 1000 ml of water and stirred vigorously. The solid product was filtered, washed with 1000 ml of water and 500 ml of hexane, and then dried in a vacuum dryer to yield 9.25 g (87.9%) of 1,2-di[2-(4-methoxybenzyloxy)-5-formyl]phenoxyethane as light yellow solids. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.79 (s, 2H), 7.49 (d, J=1.9 Hz, 2H), 7.42 (dd, J=1.9, 8.2 Hz, 2H), 7.29 (d, J=8.7 Hz, 4H), 7.00 (d, J=8.2 Hz, 2H), 7.27 (d, J=8.7 Hz, 4H), 5.10 (s, 4H), 4.47 (s, 4H), 3.77 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 190.82, 159.50, 154.30, 149.28, 130.28, 128.97, 128.12, 126.78, 114.01, 113.50, 112.51, 70.82, 67.78, 55.26; Anal. Calcd for $C_{32}H_{30}O_8$: C, 70.84; H. 5.57. Found: C, 70.9; H 5.5.

Synthesis of 1,2-di(2-hydroxy-5-formyl)phenoxyethane: To a stirred solution of 6.87 g (12.8 mmol) of 1,2-di[2-(4-methoxybenzyloxy)-5-formyl]phenoxyethane in 250 ml of EtOH was added 100 ml of 1N HCl solution. The reaction mixture was refluxed for 2 h, and then cooled to room temperature after the completion of reaction was confirmed by thin layer chromatography. The excess EtOH was evaporated in vacuo. The resulting solid was filtered, washed with 500 ml of water and 500 ml of hexane, and then dried in a vacuum dryer to yield 3.70 g (97%) of 1,2-di(2-hydroxy-5-formyl)phenoxyethane as white solids. ^{1}H NMR (500 MHz, DMSO) δ 9.79 (s, 2 h), 7.50 (d, J=1.8 Hz, 2H), 7.46 (dd, J=1.8, 8.1 Hz, 2H), 7.01 (d, J=8.1 Hz, 2H), 4.43 (s, 4H); ^{13}C NMR (125 MHz, DMSO) δ 191.47, 153.69, 147.60, 129.18, 126.50, 116.09, 113.12, 67.65; Anal. Calcd for $C_{16}H_{14}O_6$: C, 63.57; H, 4.67. Found: C, 63.4; H, 4.7.

Synthesis of 1,2-di[2-hydroxy-5-(2-carboxyvinyl)]phenoxyethane (KMS4001): 1 g (3.31 mmol) of 1,2-di(2-hydroxy-5-formyl)phenoxyethane and 1.38 g (13.2 mmol) of malonic acid were fully dissolved in 27 ml of anhydrous pyridine, and then 0.5 ml of piperidine was added. The reaction mixture was stirred at 80 °C for 4 h, and then cooled to room temperature after the completion of reaction had been confirmed. After filtering, the solid was washed with 200 ml of ethanol, and then dried in a vacuum dryer to obtain 0.67 g (52.4%) of 1,2-di[2-hydroxy-5-(2-carboxylvinyl)] phenoxyethane as white crystals. ^{1}H NMR (500 MHz, DMSO) δ 12.18 (bs, 2H), 9.61 (s, 2H), 7.50 (d, J=15.9 Hz, 2H), 7.39 (d, J=1.8 Hz, 2H), 7.13 (dd, J=1.8, 8.2 Hz, 2H), 6.84 (d, J=8.2 Hz, 2H), 6.39 (d, J=15.9 Hz, 2H), 4.40 (s, 4H); ^{13}C NMR (125 MHz, DMSO) δ 168.49, 149.77, 147.37, 144.92, 126.30, 123.62, 116.23, 116.17, 113.24, 67.77; Anal. Calcd for $C_{20}H_{18}O_8$: C, 62.17; H, 4.70. Found: C, 62.1; H, 4.8.

2.2. I.c.v. injection of Aß1-42 in mice

Male ICR mice (4–5 weeks old) were used. Procedures for animal experiments were approved by the Animal Experimentation Committee at Hallym University. The i.c.v. administration of Aβ1-42 (410 pmol) was performed according to the procedure established by Laursen and Belknap (1986). Briefly, each mouse was injected at bregma with a 50 μl Hamilton microsyringe fitted with a 26-gauge needle that was inserted to a depth of 2.4 mm. The injection volume was 5 μl.

2.3. Passive avoidance performance

One day after Aβ1-42 injection, mice were trained on a one-trial step-through passive avoidance task. The passive avoidance box was divided into 2 compartments (one illuminated and one dark), equipped with a grid floor. During the training trial, each mouse was placed in the lighted compartment; as soon as it entered the dark compartment, the door was closed and the mouse received an inescapable shock (0.25 mA, 1 s). In the testing trial, given 1 day after the training trial, the mouse was again placed in the lighted compartment and the time until it re-entered the dark compartment was measured (the step-through latency maximum testing limit was 300 s).

2.4. Y-maze task

Immediate working memory performance was assessed by recording spontaneous alternation behavior in a Y-maze (Sarter et al., 1988; Kim et al., 2014). The Y-maze task was carried out on days 3 and 4 after Aβ1-42 administration. The maze was made of black-painted wood and each arm was 25 cm long, 14 cm high, 5 cm wide and positioned at equal angles. Mice were placed at the end of one arm and allowed to move freely through the maze during an 8-min session. The series of arm entries was recorded visually. Arm entry was considered to be complete when the hind paws of the mouse were completely placed in the arm. Alternation was defined as successive entries into the three arms on overlapping triplet sets. The percentage alternation was calculated as the ratio of actual to possible alternations (defined as the total number of arm entries minus two), multiplied by 100.

2.5. APP/PS1 Tg mice

APP/PS1 Tg (B6C3-Tg(APPswe, PSEN1dE9)85Dbo/J) mice were allowed to drink water ad libitum, which contains various concentrations of KMS4001. The concentration of KMS4001 (mol. wt. 430.32) was adjusted to allow each mouse to take KMS4001 approximately at the doses of 3 and 30 mg/kg/day for 3 months (KMS4001, 3 mg/315 ml, and 30 mg/315 ml; solution was made fresh every 2 days) from the age of 9 months to 12 months. Mice ingested about 7 ml/day on average. At the start of experiment, 7 mice were allocated per group. At 1.5 and 3 months after the start of KMS4001 treatment, the numbers of mice survived per group were 6, 6 and 7 (for control, 3 and 30 mg/kg/day KMS4001, respectively) at 1.5 month, 5, 5 and 7 (for control, 3 and 30 mg/kg/day KMS4001, respectively) at 3 month after KMS4001 administration. At 1.5 (45 days) and 3 (90 days) months after the start of KMS4001 treatment, mice were put to novel-object recognition test, and at 3 months after the start of KMS4001 treatment, mice were killed to obtain frontal cortex to assay Aβ (Aβ1-42 and Aβ1-40), TNF-α and IL-1β levels.

2.6. Novel-object recognition test

Novel-object recognition test was done as previously described (Tang et al., 1999). Briefly, mice were exposed to an empty cage for 5 min for 2 consecutive days to accommodate to the experiment cage. On the third and fourth day, mice were exposed to cage with 2 objects. On the fifth day, one of the objects was replaced with a new one. The percentage of time spent exploring one of the two objects (training session) or the new object (retention session) over the total time spent exploring both objects (exploratory preference) was calculated.