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Therapeutic and pharmacokinetic characterizations of an anti-amyloidogenic bis-styrylbenzene derivative for Alzheimer's disease treatment

Seong Rim Byeon [a], Hyunjin Vincent Kim [a], Mijin Jeon [a], Young Gil Ahn [b], Maeng Sup Kim [b], Jae Yang Kong [c], Hye Yun Kim [a], Young Soo Kim [a], Dong Jin Kim [a,*]

[a] Center for Neuro-Medicine, Brain Science Institute, Korea Institute of Science and Technology, Hwarangno 14-gil 5, Seongbuk-gu, Seoul 136-791, Republic of Korea
[b] Hanmi Research Center, Hanmi Pharmaceutical Co., Ltd, 377-1 Youngchen-ri, Dongtan-myeon, Hwaseong-si, Gyonggi-do, Republic of Korea
[c] College of Pharmacy, Keimyung University, Dalseo-Gu, Daegu 704-701, Republic of Korea

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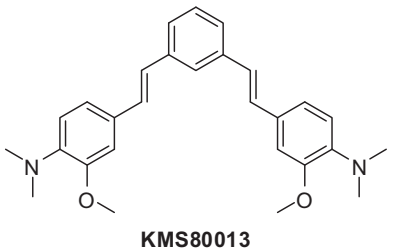
ABSTRACT

Alzheimer's disease drug discovery regarding exploration into the molecules and processes has focused on the intrinsic causes of the brain disorder correlated with the accumulation of amyloid-β. An anti-amyloidogenic bis-styrylbenzene derivative, **KMS80013**, showed excellent oral bioavailability ($F = 46.2\%$), facilitated brain penetration (26%, iv) in mouse and target specific in vivo efficacy in acute AD mouse model attenuating the cognitive deficiency in *Y*-maze test. Acute toxicity (LD_{50} >2000 mg/kg) and hERG channel inhibition (14% at 10 μM) results indicated safety of **KMS80013**.

Senile disease poses one of the greatest threats to the health care systems under the demographic shift to an aging population. Especially Alzheimer's disease (AD) is the most common and fatal neurodegenerative disorder among various senile dementias. AD patients progressively undergo brain injuries linked to cognitive impairment and behavior dysfunction to death. A variety of pharmaceutical approaches targeting AD are currently investigated but none of the approved drugs can solve the fundamental problems such as halting the degeneration of brain cells or reversing the progression of the disease.[1] Although distinct causes of AD genesis are not fully discovered yet, accumulation and aggregation of amyloid β (Aβ) play a causative role in the pathogenesis.[2–4] Therefore AD drug discovery regarding the molecules and processes has been widely advanced and focused on the intrinsic causes of the disorder correlated with the accumulation of Aβ.[5,6] However small molecule tramiprosate (Alzhemed)[19] and monoclonal antibody bapineuzumab (Johnson & Johnson and Pfizer)[20] and solanezumab (Eli Lilly) targeted Aβ were recently failed in phase III clinical trials.[7] None of the molecules based on Aβ hypothesis have been approved yet and there are only several candidates under the clinical trial in spite of challenging and urgency needs to develop better designed compounds and improved trials based on Aβ.

Consequently, we attempted to explore promising small molecules that directly inhibit Aβ aggregation to reduce neurotoxic Aβ species in the brain. And we previously reported bis-styrylbenzene and bis-styrylpyridine derivatives that showed excellent inhibitory activities against in vitro Aβ fibril formation ($IC_{50} = 0.1–2.7$ μM) comparable to a control curcumin ($IC_{50} = 0.8$ μM).[8] Curcumin was reported to prevent neurotoxic Aβ species and disaggregate Aβ effectively in vitro and in vivo.[9–12] Herein, we demonstrated a potency of promising AD drug candidate **KMS80013** (Fig. 1) selected from previous reported chemical library. It displayed such a



KMS80013

Figure 1. Structure of **KMS80013**.

* Corresponding author. Tel.: +82 2 958 5142; fax: +82 2 958 5189.
E-mail address: djk2991@kist.re.kr (D.J. Kim).

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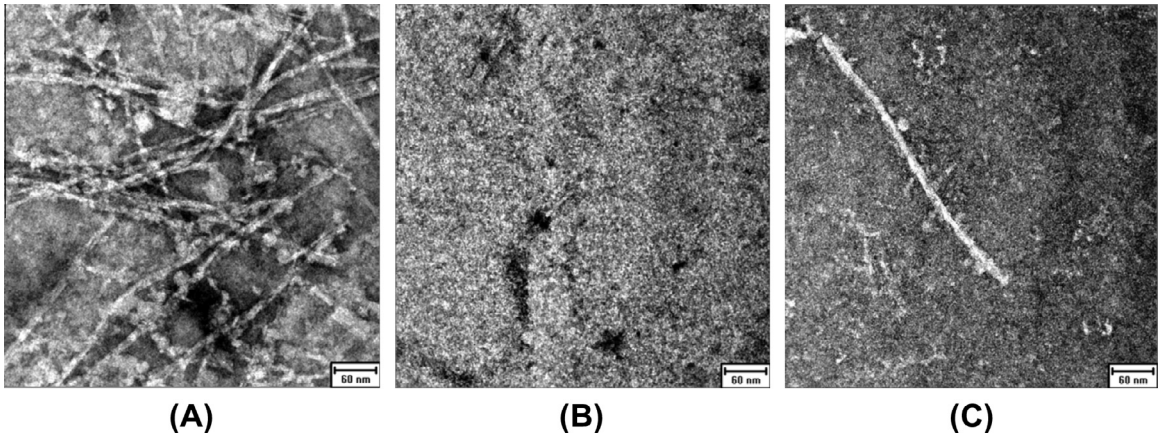

Figure 2. (A) TEM images of Aβ42 (50 μM) without an inhibitor; (B) with **KMS80013** (100 μM); (C) with curcumin (100 μM). All of the concentrations in A–C (Aβ42 and compounds) are final concentrations. The scale bars represent 60 nm. Each mixture of freshly prepared Aβ42 solution (10 μL, 100 μM in 10 mM sodium phosphate, pH 7.4) and inhibitors (10 μL, 100 μM or 200 μM in 10 mM sodium phosphate, 2% DMSO, pH 7.4) was prepared. After all test solutions were incubated at 37 °C and 5% CO_2 for 20 h, each of them (5 μL) was immediately dropped on carbon coated grid. The grid was negatively stained with 1% uranyl acetate before drying for 2 h. TEM image was obtained using a electron microscope (Phillips, CM-30).

Table 1
PK parameters of **KMS80013** in plasma and brain of mice

Parameters	KMS80013			
	po		iv	
Region (n = 3)	Plasma	Brain	Plasma	Brain
Dose (mg)	20	20	10	10
AUC_{0-24}[a]	29210 ± 4422	4526 ± 255	31593 ± 3780	8101 ± 816
C_{max}[b]	3489 ± 636	395 ± 110	13073 ± 585	1413 ± 158
T_{max}[c]	1.0 ± 0.9	4.7 ± 4.6	0.08 ± 0	0.4 ± 0.3
$t_{1/2}$[c]	9.4 ± 3.1	14.5 ± 0.0	7.3 ± 0.5	6.04 ± 0.72
F[d] (%)	46.2			
Brain distribution[e] (%)	15		26	

[a] 0–24 (ng h/mL).
[b] (ng/mL).
[c] (h).
[d] BA. F = $(AUC_{po}/AUC_{iv}) \times 100$
[e] Brain distribution = $(AUC_{brain}/AUC_{plasma}) \times 100$. All values are represented as mean ± SEM.

dramatic pharmacokinetic (PK) profiles among several compounds that was selected for further biological and pharmacological study including animal behavior test (Supplementary data, SI Fig. 2). Inhibition activity against Aβ fibril formation in transmission electron microscopy (TEM) image and blood brain barrier (BBB) permeability in a tight junction co-culture system were evaluated in vitro. PK property involving oral bioavailability (BA) and brain distribution were measured in vivo. The safety validation of animal acute toxicity and in vitro human ether-a-go-go related gene (hERG) channel activity was performed. Finally, in vivo efficacy was assessed in an acute mouse model.

Corresponding with the previous thioflavin T assay result of **KMS80013** (IC_{50} = 1.0 μM), the reduction of Aβ fibrils in structural morphology was confirmed in the TEM analysis (Fig. 2).[13] The image of Aβ (50 μM) without the compound (Fig. 2a) showed a large amount of long and thick fibril aggregates. In contrast, the image of Aβ with **KMS80013** (100 μM, Fig. 2b) and curcumin (100 μM, Fig. 2c) showed significantly reduced fibril aggregates. Surprisingly, the image of Aβ with **KMS80013** (100 μM, Fig. 2b) did not display any long and thick fibril species at all indicating more prominent inhibition effect than curcumin.

Pharmacokinetic study is essential for drug development because it reflects absorption, distribution, metabolism and excretion of an administered drug and gives information of potential pharmacodynamic properties. The plasma and brain concentrations, bioavailability (BA) and brain distribution (%) were measured in ICR male mice (10 weeks old, n = 3) with oral (20 mg/kg, po) and intravenous administration (10 mg/kg, iv). Plasma and brain PK parameters of **KMS80013** in mice are summarized in Table 1. Surprisingly, area under the concentration-versus-time curve (AUC_{0-24}) of **KMS80013** was 29,210 ng h/mL demonstrating very strong absorption. Also a maximum plasma drug concentration (C_{max}) of **KMS80013** was dramatically high as 3489 ng h/mL. **KMS80013** exhibited good bioavailability (F = 46.2%) and long plasma half-life ($t_{1/2}$ = 9.4 h, po) as well as rapid plasma absorption (T_{max} = 1 h, po). As being expected through high apparent permeability coefficients (Papp) in vitro BBB permeability assay (Supplementary data), brain distribution of **KMS80013** (15%, po; 26%, iv) was moderate demonstrating its acceptable penetration across the BBB.

KMS80013 was then subjected to in vivo single dose toxicity test to establish the appropriate drug dose. **KMS80013** was orally administrated in SD male rats (2 weeks old, n = 3) at dose levels of 500, 1000 and 2000 mg/kg. Toxicity was assessed based on mortality and body weight change during 14-day observation period (Supplementary data). No SD rats fed with **KMS80013** showed abnormal change of body weight (Supplementary data) compared to vehicle control group. **KMS80013** was safe with no toxic effects at the dose level of 2000 mg/kg. The medium lethal dose (LD_{50}) value would be estimated to be over 2000 mg/kg.

Since the blockade of hERG potassium channels causes serious cardiovascular problems leading to electrocardiogram prolongation risk and potentially to ventricular arrhythmias, it is recognized

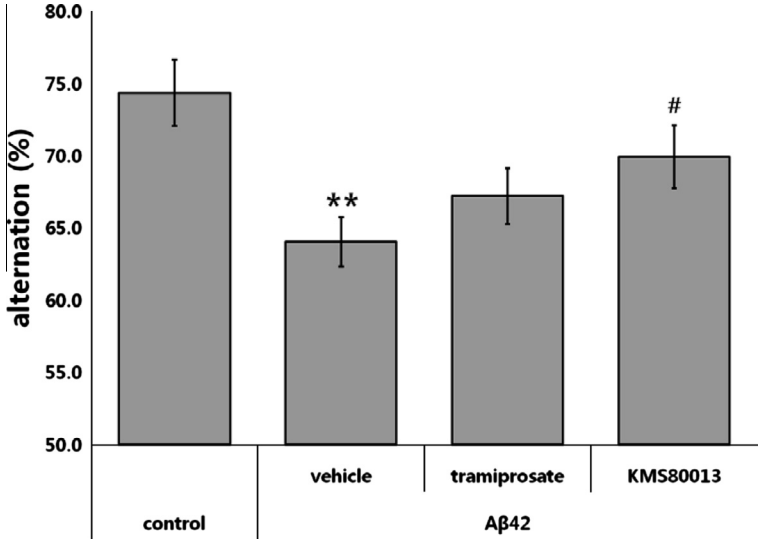

Figure 3. Therapeutic effect of *Y*-maze test in Aβ42-injected ICR mice (6 weeks old, $n = 10$). Aβ42 was injected to icv and each compound to ip. The same amount of solvent (DMSO/PBS, 1/9) was administered into the control mouse with vehicle or compound treated mouse. $^{\#}p < 0.05$ compared to Aβ42; $^{**}p < 0.01$ compared to control. The data are represented as mean ± SEM. Statistical significance was calculated using the Student's two-tailed *t*-test.

as a major hurdle in a new drug development. Therefore, the inhibition activity of hERG channels was assessed in the early development stage. **KMS80013** barely inhibited the hERG potassium channel (14%) at 10 μM. Drug candidates with IC_{50} values greater than 10 μM are considered not to interact with the hERG channel (Supplementary data).[14]

To study in vivo target specific efficacy, *Y*-maze test was performed in acute AD mice model.[15,16] Spontaneous alternation behavior was measured (Fig. 3) to determine the effect of **KMS80013** treatment for short-term spatial working memory. To mimic aspects of AD (deleterious effects on cognition and behaviors), Aβ42 monomer (100 μM, 5 μL) dissolved in DMSO/PBS (1/9) was injected to ICR male mice (6 weeks old, $n = 10$) by icv for 5 days.[17,18] Animal models based on Aβ infusion support the Aβ cascade hypothesis which could provide insight in mechanisms as well as secondary effects of Aβ toxicity and allow preclinical evaluation of drugs specifically targeting Aβ. The vehicle (DMSO/PBS) and Aβ42-injected mice were administered with compounds (30 mg/kg/day, ip). Four groups of mice were employed for this behavioral test: control group (Aβ(−) vehicle icv, compound(−) vehicle ip), Aβ/vehicle group (Aβ(+) vehicle icv, compound(−) vehicle ip), Aβ/tramiprosate group (Aβ(+) vehicle icv, tramiprosate(+) vehicle ip), and Aβ/KMS80013 group (Aβ(+) vehicle icv, KMS80013(+) vehicle ip). **KMS80013** (30 mg/kg) treated mice showed improved alternation performance (70.0%, $p < 0.049$) compared to vehicle treated group (Aβ42 only, 64.1%, $p < 0.002$). This result was comparable to that of wild type (control, 74.4%). Tramiprosate, an ionic small molecule to inhibit Aβ deposition for the oral treatment of mild to moderate AD patients but recently discontinued in the late stages of a phase III clinical trial, was used as a positive control.[19] Tramiprosate treated mice revealed lower level of alternation (67.3%) than **KMS80013** treated group. The result of *Y*-maze test indicated that **KMS80013** significantly enhanced spatial learning and memory by inhibition of Aβ fibril formation.

In conclusion, we found a potent AD drug candidate, **KMS80013**, having synthetic feasibility. All results taken together in this study demonstrated that **KMS80013** was predominantly absorbed with high BA, crossed BBB, bound with soluble Aβ and efficiently prevented larger aggregation. Consequently, **KMS80013** attenuated the cognitive deficiency blocking the AD phenotypes induced by aggregated Aβ toxicity in animal model.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.02.104.

References and notes

1. Cutler, N. R.; Sramek, J. J. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2001**, *25*, 27.
2. Hardy, J. A.; Higgins, G. A. *Science* **1992**, *256*, 184.
3. Nitsch, R. M.; Hock, C. *Neurotherapeutics* **2008**, *5*, 415.
4. Hardy, J.; Selkoe, D. J. *Science* **2002**, *297*, 353.
5. Meyer-luemen, M.; Stalder, M.; Herzig, M. C.; Kaeser, S. A.; Kohler, E.; Pfeifer, M. *Nat. Neurosci.* **2003**, *6*, 370.
6. Roberson, E. D.; Mucke, L. *Science* **2006**, *314*, 781.
7. Callaway, E. *Nature* **2012**, *489*, 13.
8. Byeon, S. R.; Lee, J. H.; Sohn, J.-H.; Kim, D. C.; Shin, K. J.; Yoo, K. H., et al *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1466.
9. Chainani-Wu, N. *J. Altern. Complement. Med.* **2003**, *9*, 161.
10. Raja, K. S.; Balambika, R.; Dolai, S.; Shi, W. *Mini-Rev. Org. Chem.* **2009**, *6*, 152.
11. Shi, W.; Dolai, S.; Rizk, S.; Hussain, A.; Tariq, H.; Averick, S., et al *Org. Lett.* **2007**, *9*, 5461.
12. Ma, Q. L.; Yang, F.; Calon, F.; Ubeda, O. J.; Hansen, J. E.; Weisbart, R. H., et al *J. Biol. Chem.* **2008**, *283*, 14132.
13. Harris, J. R. *Micron* **2002**, *33*, 609.
14. Dumotier, B. M.; Deurinck, M.; Yang, Y.; Traebert, M.; Suter, W. *Pharmacol. Ther.* **2008**, *119*, 152.
15. Lee, Y. S.; Kim, H. Y.; Kim, Y.; Seo, J. H.; Roh, E. J.; Han, H., et al *Bioorg. Med. Chem.* **2012**, *20*, 4921.
16. Maurice, T.; Lockhart, B. P.; Privat, A. *Brain Res.* **1996**, *706*, 181.
17. Choi, J. W.; Kim, H. Y.; Jeon, M.; Kim, D. J.; Kim, Y. *Amyloid* **2012**, *19*, 133.
18. Kim, Y. S.; Moss, J. A.; Janda, K. D. *J. Org. chem.* **2004**, *69*, 7776.
19. Wright, T. M. *Drugs Today Barc* **2006**, *42*, 291.
20. Cutler, N. R.; Sramek, J. J. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2001**, *25*, 27.