Int.J.Curr.Microbiol.App.Sci (2023) *12*(08): 1-6

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 12 Number 8 (2023)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2023.1208.001

Enzymatic Synthesis of Memantine (Memary) Oligosaccharides (Gluco-oligosaccharides) and their application as anti-dementia drugs that cross the Blood-Brain Barrier (BBB)

Hiroki Hamada[1]*, Kohji Ishihara[2], Kei Shimoda[3], Atsuhito Kuboki[2], Yuya Kiriake[4] and Ryusuke Hosoda[5]

[1]Meisterbio Co. Ltd., 29-13Ekimoto-cho, Kita-ku, Okayama 700-0024, Japan
[2]Department of Biological Science, Faculty of Life Science, Okayama University of Science, 1-1 Ridai-cho, Kita-ku, Okayama 700-0005, Japan
[3]Department of Biomedical Chemistry, Faculty of Medicine, Oita University, 1-1 Hasama-machi, Oita 879-5593, Japan
[4]Faculty of Medicine and Health Sciences, Yamaguchi University, 1-1-1 Minamikogushi, Ube-shi, Yamaguchi 755-8505, Japan
[5]Department of Pharmacology, School of Medicine, Sapporo Medical University, Sapporo 060-8556, Japan
**Corresponding author*

Keywords

Memantine oligosaccharides, Blood-brain barrier, Spatial learning, Brain-drug-delivery

Article Info

Received:
02 July 2023
Accepted:
05 August 2023
Available Online:
10 August 2023

A B S T R A C T

Memantine (memary) oligosaccharides (gluco-oligosaccharides) were synthesized by enzymatic glycosylation, using cyclodextrin glucanotransferase as a biocatalyst. Although memantine hardly crosses the blood–brain barrier (BBB) in the mouse brain, memantine oligosaccharides crossed the BBB of mouse brain and were incorporated into the mouse brain tissue. Our investigations indicated that memantine modified with oligosaccharides might have gained a BBB-crossing ability. Furthermore, during the Y-maze test using senescence-accelerated mouse prone 8, our study revealed that the time spent in the novel Y-maze arm by the memantine-oligosaccharides-treated mouse was longer than that spent in the novel arm by the memantine-treated-mouse. Therefore, this study established that since memantine oligosaccharides could penetrate the BBB of mouse brain and be incorporated into the mouse's brain tissue, they could also enhance spatial learning.

Introduction

Alzheimer's disease is one of the most intractable neurodegenerative disorders. The β-amyloid peptides in the brain's learning and memory regions, such as the cortex and hippocampus, are typical of Alzheimer's (Berardi *et al.,* 2009). Therefore, preventing β-amyloid peptide aggregation is the primary therapeutic strategy for treating Alzheimer's (Berardi *et al.,* 2009). Earlier, memantine, which is

an uncompetitive glutamatergic N-methyl-D-aspartate (NMDA) receptor antagonist, is used for the treatment of Alzheimer's disease. It has been reported that memantine reduces amyloid-β peptide levels in both neuronal cultures and in brains of animal models of Alzheimer's. Memantine reduces the amounts of both CHAPS-soluble and CHAPS-insoluble amyloid-β peptide in the brain. It also reduces the levels of insoluble Aβ42 in the brains. Moreover, memantine increases the amount of amyloid precursor protein (APP) at the cell surface without changing the total amount of APP (Ito *et al.*, 2017).

The blood–brain barrier (BBB) exists in the brain as a selective semipermeable border that prevents solutes in the circulating blood from non-selectively crossing the extracellular fluid of the central nervous system where neurons exist (Daneman *et al.*, 2015). It comprises endothelial cells of the capillary wall, astrocyte end-feet ensheathing the capillary, and pericytes fixed firmly in the capillary basement membrane (Ballabh *et al.*, 2004). While the BBB system allows the passage of some small molecules by passive diffusion, it also permits the selective transport of various nutrients, ions, organic anions, and macromolecules, such as glucose and amino acids, crucial to neuronal functioning (Ballabh *et al.*, 2004).

We have developed a drug delivery system using drugs' chemical and biological glycosylation. As a result, curcumin was identified as one of the most useful neuroprotective agents. However, when curcumin was intraperitoneally injected into a mouse, while only a small amount could be incorporated into the mouse brain tissue because it hardly crossed the BBB in the mouse brain, the glycosylated curcumin, i.e., curcumin oligosaccharides (gluco-oligosaccharides), successfully crossed the BBB and were incorporated into the mouse brain tissue (Hamada *et al.*, 2020).

Using our brain–drug-delivery technique and the glycosylation of neuroprotective chemicals, we studied the crossing ability of memantine oligosaccharides (gluco-oligosaccharides) through the BBB in mouse brain. We also investigated whether the gluco-oligosaccharide modification of memantine enhances its crossing ability through the mouse brain's BBB, after which the memantine oligosaccharides' effects on spatial learning of senescence-accelerated mouse prone 8 (SAMP8) were examined.

Materials and Methods

General

Memantine was purchased from FujifilmWako Pure Chemical Co. However, C57BL mice and senescence-accelerated mouse prone 8 (SAMP8) were purchased from Japan SLC Inc.

Enzymatic synthesis of memantine oligosaccharides

Memantine oligosaccharides were prepared using enzymatic procedures. First, the reaction mixture containing memantine glucoside, soluble starch, and cyclodextrin glucanotransferase (CGTase) from *Bacillus macerans*, was incubated in sodium phosphate buffer (pH 7.0) at 40°C for 24 h. Then, the mixture was centrifuged at 3000 ×gfor 10 min. Subsequent investigations revealed that the supernatant contained glycosides, i.e., memantine maltoside, memantine maltotrioside, memantine maltotetraoside, and memantine maltopentaoside.

BBB penetrating ability test of memantine oligosaccharides

The mice were intraperitoneally injected once with memantine oligosaccharides or memantine (control) to test their BBB penetration abilities. One hour later, they were sacrificed by cervical dislocation, after which their brain tissue samples were quickly processed by rinsing with cold sodium phosphate buffer, then frozen and stored at −20°C. Subsequently, memantine was extracted, after which its concentration in the brain sample was determined using HPLC as previously described (Hamada *et al.*,

2020). Tissue samples were first homogenized in sodium acetate buffer (0.1 M, pH 6.0), and tissue homogenates were ultrasonicated in 0.1% Triton X-100 for 10 min. Then, in a flask containing the homogenate mixture with Triton X-100, 10 mg/ml α-glucosidase (Amano enzyme Co. Ltd., Aichi, Japan), 10 mg β-glucosidase (Amano enzyme Co. Ltd., Aichi, Japan), and 10 mg β-glucuronidase (Nacalai Tesque, Inc.) were added and incubated at 36°C for one hour. Organic compounds were finally extracted with ethyl acetate. After three extraction steps, ethyl acetate was evaporated. Samples were dissolved in methanol to analyze them. Finally, the extracted memantine was quantified by HPLC using a reverse-phase column. The mobile phase comprised 31% acetonitrile and 69% water (flow rate: 1.0 ml/min, detector temperature: 40°C).

Y-maze test

A Y-maze with three arms was constructed with gray plastic, then it was equipped with a partition that isolates an arm. The experiment involved a 5-min trial 1, separated by a 40-min interval, followed by a 5-min trial 2. During the familiarization phase (trial 1), one arm (arm C: novel arm) of the Y-maze was closed with a partition. Then, while we placed one SAMP8 in one arm (arm A) of the two remaining arms (arms A and B) and the mouse allowed to explore the maze for five minutes, the partition was removed after a 40-min interval.

Afterward, for five minutes, the mouse had free access to all three arms during the retrieval phase (trial 2). Notably, although the mouse was video recorded during trial 2, the time of the novel arm (arm C) exploration was only recorded when the mouse put his hind feet in that arm. Then, the percentage of time spent in the novel arm C was calculated. Finally, memantine was intraperitoneally injected five times into mouse during five days' investigation period(one injection per day) (the control), whereas memantine oligosaccharides were intraperitoneally injected five times into mouse(one injection per day) (the memantine-oligosaccharides-treated mouse).

Results and Discussion

Enzymatic synthesis of memantine oligosaccharides

Memantine glucoside was subjected to glycosylation, using CGTase as a biocatalyst (Figure 1). Then, it was incubated with soluble starch and CGTase from *Bacillus macerans* for 24 h. After centrifuging the reaction mixture, we observed that the supernatant contained memantine oligosaccharides (Figures 2).

BBB penetration by memantine oligosaccharides

Brain tissue samples were processed as described in the Materials and Methods. After homogenizing tissue samples in sodium acetate buffer, the homogenates were ultrasonicated and treated by hydrolysis with glycosidases. Afterward, the products were extracted with ethyl acetate to prepare a brain sample. The obtained memantine was subsequently quantified by HPLC analysis of the brain sample using a reverse-phase column. Thus, memantine was detected at 330 ng per 1 g of mouse brain tissue. Subsequently, memantine oligosaccharides were intraperitoneally injected. The HPLC analysis results of the brain sample indicated that memantine oligosaccharides were incorporated into the mouse brain tissue. Investigations also revealed that the brain sample of the mouse treated with memantine (control) contained little amount of memantine, indicating that it hardly migrated to the mouse brain tissue. These results suggest that memantine oligosaccharides, which were intraperitoneally injected into mouse, could smoothly penetrate the BBB in the mouse brain.

Y-maze test findings for memantine oligosaccharides-injected-SAMP8

In the Y-maze test using SAMP8, the time spent in the novel arm of the Y-maze by the mouse intraperitoneally injected with memantine-oligosaccharides (memantine-oligosaccharides-treated mice), was longer than that spent by the

control mouse, into which memantine alone was intraperitoneally injected (Table 1). Investigations also revealed that the percentage of time spent in the novel arm by memantine-oligosaccharides-treated mouse was higher than that of the time spent by the control mouse. These results suggest that memantine oligosaccharides penetrated the BBB and were incorporated into the brain tissue of SAMP8, enhancing spatial learning of the mouse by 49% (approximately 1.5-fold) compared to the control.

Memantine oligosaccharides were synthesized following a biocatalytic synthetic procedure, using CGTase as a biocatalyst. Enzymatic hydrolysis using three glycosidases of the brain tissue homogenates of mouse, to which memantine oligosaccharides were intraperitoneally injected, yielded memantine, suggesting that memantine oligosaccharides migrated into the brain tissue through the BBB in mouse brain. Previous studies have reported that the glucosides of ketoprofen and indomethacin could significantly inhibit the glucose transporter (GluT1)-mediated uptake of glucose, indicating its affinity to the transporter (Gynther *et al.*, 2009; Berardi *et al.*, 2009).

In addition, these glucoconjugates could temperature-dependently penetrate the BBB, indicating that the glucosylation of drugs enhances their BBB-crossing ability and that the brain uptake of the conjugates is carrier-mediated (Gynther *et al.*, 2009). Consistent with these studies, we also observed that memantine oligosaccharides can cross the BBB in the mouse brain and be incorporated into brain tissue.

Table.1 Spatial-learning of SAMP8 in the Y-maze test.

SAMP8 group	Time spent in the novel arm[1] (s)	Percentage total time (%)
Memantine-treated mouse (control)	74	24.7
Memantine-oligosaccharides-treated mouse	110	36.7

[1]The exploration periods of the novel arm (arm C) recorded only when the mouse puts his hind feet in that arm.

Fig.1 Enzymatic synthesis of memantine oligosaccharides (gluco-oligosaccharides) by biocatalytic glycosylation of memantine glucoside with CGTase.

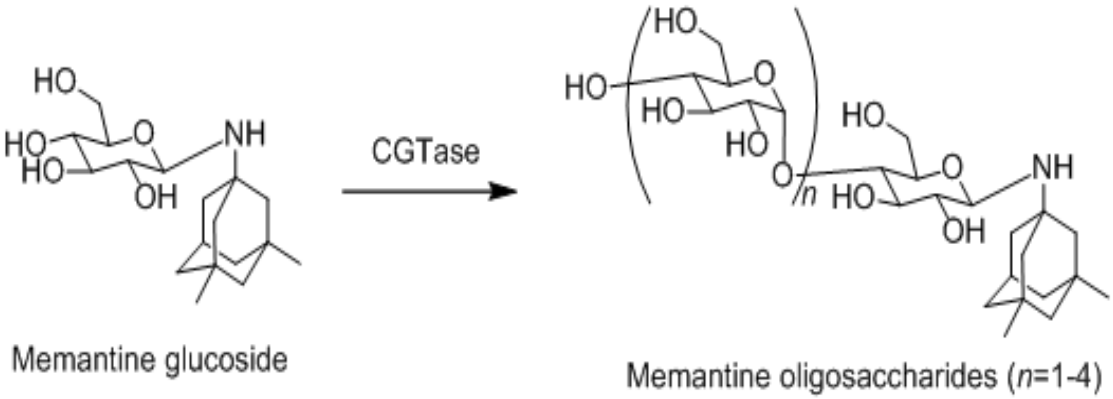
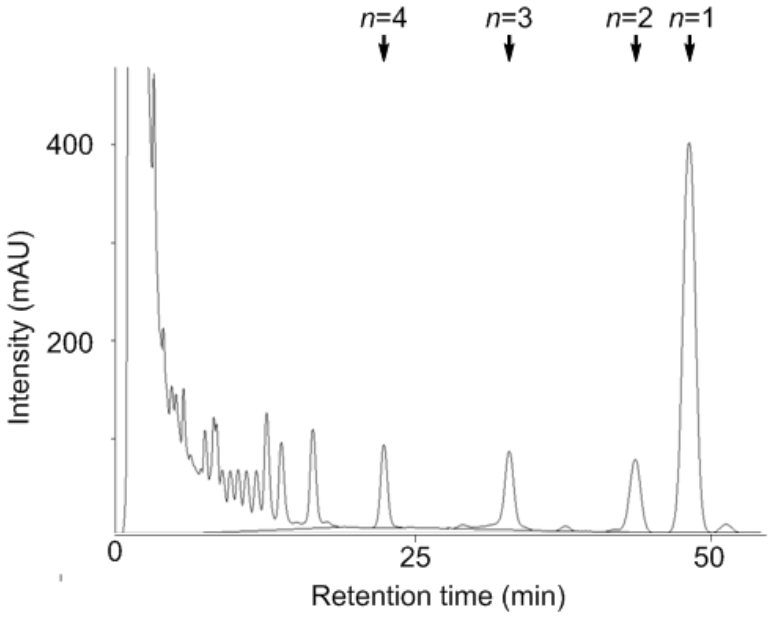


Fig.2 HPLC analysis results of memantine oligosaccharides (gluco-oligosaccharides).



Alzheimer's disease is an irreversible and progressive neurodegenerative disease. According to clinical evidence, amyloid plaques were observed in central nervous system of Alzheimer's patients. So, inhibiting β-amyloid aggregation may be useful to control Alzheimer disease. It has been shown memantine decreases β-amyloid levels via increase in secretion of amyloid precursor protein and activation of α-secretase (Niles *et al.,* 2006; Shan *et al.,* 2014; Hashemi *et al.,* 2022). On the other hand, the hippocampus is a critical brain area for cognitive and memory functions, making it a sensitive area in Alzheimer's (Berardi *et al.,* 2009). Memantine has been shown to improve learning and memory in several pharmacological models of Alzheimer's disease. For example, the study of the effects of memantine on locomotor activity, social behavior, and spatial learning assessed in a transgenic mouse model of Alzheimer's disease indicated that memantine improves hippocampus-based spatial learning in a transgenic mouse model of Alzheimer's disease without producing nonspecific effects on locomotion/exploratory activity (Evers *et al.,* 2004; Minkeviciene *et al.,* 2004; Rimando *et al.,* 2004).

These previous findings are consistent with our study, which suggests that memantine oligosaccharides are chemopreventive agents that can protect neurons against the β-amyloid-induced disruption of spatial learning and memory in the hippocampus of SAMP8 and enhance spatial learning. Additionally, we recently reported that the glycosylated curcumin, i.e., curcumin oligosaccharides (gluco-oligosaccharides), successfully penetrated the BBB in the mouse brain and can be incorporated into their brain tissue (Hamada *et al.,* 2020). Therefore, based on these results, our findings suggest that the gluco-oligosaccharide modification of neuroprotective chemicals, such as memantine and curcumin, enhances their crossing ability through the BBB in the brain, thus, proposing that the brain–drug-delivery technique of neuroprotective chemicals by glycoside (gluco-oligosaccharide) modification is useful for preparing new anti-dementia drugs.

Further studies on the transporter that recognizes oligosaccharide conjugates as substrates and the neuroprotective property of oligosaccharide conjugates are now in progress in our laboratory.

Declaration of Conflicting Interests

The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

References

Ballabh, P., Braun, A. and Nedergaard, M. 2004. The blood-brain barrier: an overview: structure, regulation, and clinical implications. Neur. Dis. 16:1-13. https://doi.org/10.1016/j.nbd.2003.12.016

Berardi, V., Ricci, F., Castelli, M., Galati, G. and Risuleo, G. 2009. Resveratrol exhibits a strong cytotoxic activity in cultured cells and has an antiviral action against polyomavirus: potential clinical use. J. Exper. Clin. Cancer Res. 28:96. https://doi.org/10.1186/1756-9966-28-96

Daneman, R. and Prat, A. 2015. The blood-brain barrier. Cold Spr. Harb Persp. Biol. 7:1-23. https://doi.org/10.1101/cshperspect.a020412

Evers, D., Wang, X., Huong, S.-M., Huang, D. Y. and Huang, E.-S. 2004. 3,4',5-Trihydroxy-trans-stilbene (resveratrol) inhibits human cytomegalovirus replication and virus-induced cellular signaling. Antiv. Res. 63:85-95. https://doi.org/10.1016/j.antiviral.2004.03.002

Gynther, M., Ropponen, J., Laine, K., Leppanen, J., Haapakoski, P., Peura, L., Jarvinen, T. and Rautio, J. 2009. Glucose promoiety enables glucose transporter mediated brain uptake of ketoprofen and indomethacin prodrugs in rats. J. Med. Chem. 52:3348-3353. https://doi.org/10.1021/jm8015409

Hamada, H., Nakayama, T., Shimoda, K., Matsuura, N., Hamada, H., Iwaki, T. and Kiriake, Y. 2020. Curcumin oligosaccharides (gluco-oligosaccharides) penetrate the blood-brain barrier in mouse brain: glycoside (polysaccharide) modification approach for brain drug delivery across the blood-brain barrier and tumor drug delivery. Nat. Prod. Commun. 15:1-4. https://doi.org/10.1177/1934578X20953653

Hashemi, M., Roohi-Azizi, M., Hashemi, F. 2022. Anti-apoptotic effect of memantine in a rat hippocampal cell model of Alzheimer's disease: morphology and cellular mechanism. Res. Square 3703:1-21. https://doi.org/10.21203/rs.3.rs-1693554/v1

Ito, K., Tatebe, T., Suzuki, K., Hirayama, T. 2017. Memantine reduces the production of amyloid-β peptides through modulation of amyloid precursor protein trafficking. Eur. J. Pharmacol. 798:16-25. https://doi.org/10.1016/j.ejphar.2017.02.001

Minkeviciene, R., Banerjee, P., Tanila, H. 2004. Memantine improves spatial learning in a transgenic mouse model of Alzheimer's disease. J. Pharm. Exp. Therap. 311:677-682. https://doi.org/10.1124/jpet.104.071027

Niles, R. M., Cook, C. P., Meadows, G. G., Fu, Y. M., McLaughlin, J. L. and Rankin, G. O. 2006. Resveratrol is rapidly metabolized in athymic (nu/nu) mice and does not inhibit human melanoma xenograft tumor growth. J. Nutr.136:2542-2548. https://doi.org/10.1093/jn/136.10.2542

Rimando, A. M., Kalt, W., Magee, J. B., Dewey, J. and Ballington, J. R. 2004. Resveratrol, pterostilbene, and piceatannol in vaccinium berries. J. Agric. Food Chem..28:4713-4719. https://doi.org/10.1021/jf040095e

Shan, Z., Yang, G., Xiang, W., Pei-jun, W. and Bin, Z. 2014. Effects of resveratrol on oral squamous cell carcinoma (OSCC) cells in vitro. J. Cancer Res. Clin. Oncol.140:371-374. https://doi.org/10.1007/s00432-013-1575-1

How to cite this article:

Hiroki Hamada, Kohji Ishihara, Kei Shimoda, Atsuhito Kuboki, Yuya Kiriake and Ryusuke Hosoda. 2023. Enzymatic Synthesis of Memantine (Memary) Oligosaccharides (Gluco-oligosaccharides) and their Application as Anti-dementia Drugs that Cross the Blood-brain Barrier (BBB). *Int.J.Curr.Microbiol.App.Sci.* 12(08): 1-6. **doi:** https://doi.org/10.20546/ijcmas.2023.1208.001