J Neurology Neuromedicine
www.jneurology.com
Journal of Neurology & Neuromedicine

Mini Review Open Access

Extracellular uridine diphosphate-mediated microglial inflammation in a mouse model of Sandhoff disease

Eri Kawashita[1], Daisuke Tsuji[2], Kohji Itoh[2]

[1]Department of Pathological Biochemistry, Kyoto Pharmaceutical University, Kyoto, Kyoto, Japan
[2]Department of Medicinal Biotechnology, Institute for Medicinal Research, Graduate School of Pharmaceutical Sciences, Tokushima University, Tokushima, Tokushima, Japan

Article Info

Article Notes
Received: September 14, 2016
Accepted: October 19, 2016

***Correspondence:**
Dr. Kohji Itoh PhD,
Department of Medicinal Biotechnology, Institute for Medicinal Research, Graduate School of Pharmaceutical Sciences, Tokushima University, 1-78 Sho-machi, Tokushima, Tokushima 770-8505, Japan, Telephone: +81-88-633-7290;
Email: kitoh@tokushima-u.ac.jp

Keywords:
Sandhoff disease
Lysosomal storage disease
Microglia
UDP
MIP-1α
Inflammation
P2Y6 receptor
Lipid raft
Microdomain
Nucleotides

ABSTRACT

Sandhoff disease (SD) is an inherited lysosomal storage disease caused by a β-hexosaminidase deficiency involving excessive accumulation of undegraded substrates, including GM2 ganglioside, which leads to neurological symptoms, such as mental retardation, spasms and quadriplegia. Macrophage inflammatory protein-1α (MIP-1α) is a crucial factor for microglia-mediated neuroinflammation in the onset or progression of SD. However, there was no therapeutic approach to control the abnormal production of MIP-1α in the brain of SD, and the mechanisms underlying the MIP-1α production by microglia, especially the transmitter-mediated production, remains unclear.

Extracellular nucleotides, including uridine diphosphate (UDP), are leaked by injured or damaged neurons. It has been shown that the nucleotide leakage activates microglia to trigger chemotaxis, phagocytosis, macropinocytosis and cytokine production, suggesting that extracellular nucleotides may be important neurotransmitters for microglia to regulate their functions physiologically and pathologically.

In the present study, we review the essential roles of extracellular nucleotides in the microglial functions and the UDP-enhanced MIP-1α production by microglia in SD model mice, providing a potential therapeutic approach for SD.

Sandhoff disease

Sandhoff disease (SD) is a progressive neurodegenerative disorder caused by deficiencies of β-hexosaminidase (Hex), HexA (αβ) and HexB (ββ), associated with a defect in the Hex β-subunit gene[1,2]. Both HexA and HexB can degrade the terminal β-linked *N*-acetylglucosamine residues of oligosaccharides, but only HexA degrades GM2 ganglioside containing an *N*-acetylgalactosamine residue. In SD patients, an excessive accumulation of undegraded substrates, including GM2 ganglioside, is observed, particularly within lysosomes in the neuronal cells, due to the deficiencies of HexA and HexB, which leads to neurological symptoms in the central nervous system (CNS), such as mental retardation, spasms and quadriplegia. SD model mice (SD mice), established by means of Hex β-subunit gene disruption, exhibit the accumulation of GM2 gangliosides throughout the CNS and the abnormalities in motor functions, which are quite similar to those observed in SD patients[3]. Several therapeutic approaches for SD have been investigated for decades, including substrate reduction therapy[4-6], bone marrow transplantation[7,8], stem cell therapy[9,10], enzyme replacement

therapy[10-14], gene therapy[15-17] and chaperon therapy[18], where the aim is to reduce the accumulated substrates. However, the disease remains incurable.

Microglia-mediated inflammation in the brains of SD patients and mice

A progressive increase in microglial activation/expansion and subsequent neuronal apoptosis have been observed in the brains of SD patients and mice, suggesting that microglial inflammation is likely involved in the neurodegenerative mechanism in SD[8,19-21]. We demonstrated that macrophage inflammatory protein-1α (MIP-1α) is upregulated in the brains of SD mice from the age of 1 week, and in microglial cells derived from neonatal SD mice[22,23]. Wu and Proia also demonstrated that the deletion of MIP-1α expression results in not only a substantial decrease in macrophage/microglial-associated pathology together with neuronal apoptosis in SD mice, but also an increase in the life span of SD mice[24]. These studies suggest that MIP-1α is a crucial factor for microglia-mediated neuroinflammation during the pathogenesis of SD, and the downregulation of the abnormal production of MIP-1α by microglia may be another approach to delay the onset or progression of SD. However, the mechanisms underlying the abnormal production of MIP-1α by microglia, especially the transmitter-mediated production, is still poorly understood.

Extracellular nucleotides as signals for microglial activation

Microglia exists in their resting ramified form in the CNS under normal conditions; however, they are transformed into the activated ameboid form when they recognize a pathological state in the brain[25]. Extracellular nucleotides, adenosine triphosphate and uridine diphosphate (ATP and UDP, respectively), are leaked from injured or damaged neuronal cells and activate microglia to trigger cytokine production, chemotaxis, phagocytosis and macropinocytosis. Extracellular ATP and UDP induce the expression or release of cytokines and chemokines, including TNF-α, MCP-1 and MIP-1α, in microglia[26,27]. ATP regulates the microglial branch dynamics in the intact brain, and the ATP leakage from the damaged tissue mediates a rapid microglial response towards injury[28]. UDP is an “eat-me” signal from the dying cells: microglia recognize the extracellular UDP leakage from damaged neuronal cells, leading to the removal of the dying cells or their debris[29,30]. The extracellular nucleotides modulate the cellular functions by activating P2 receptors, which are classified into ionotropic P2X receptors and metabotropic P2Y receptors. Microglia have been shown to express functional P2X4, P2X7, P2Y6 and P2Y12 receptors[29]. These studies suggest that extracellular nucleotide signaling may regulate microglia-mediated physiological or pathological events in the brain.

Enhancement of MIP-1α production by UDP in microglia from SD mice, mediated by the activation of P2Y6 receptor, ERK and JNK

We demonstrated that MIP-1α is prominently upregulated in the brain of SD mice[22], and that the basal production of MIP-1α is higher in microglia derived from SD mice (SD-Mg) than in that from wild-type mice (WT-Mg)[23], suggesting that the higher MIP-1α production is due to the abnormal signal transduction caused by the deficiencies of HexA and HexB in SD-Mg as well as the effects of other neuronal cells, including neurons and astrocytes. We furthermore investigated whether or not extracellular nucleotides enhance the production of MIP-1α by SD-Mg. We found that UDP induces the production of MIP-1α in SD-Mg but not WT-Mg, while ATP has no effect on the production of MIP-1α by WT- or SD-Mg[31]. The UDP leakage from the damaged neurons might enhance the MIP-1α production in microglia of SD mice to recruit other microglia to the damaged area, thereby resulting in a rapid microglial inflammation in the progression of SD. We also showed that SD-Mg is more strongly activated than WT-Mg due to the excessive accumulation of undegraded substrates, based on the observed increase in the IL-1β and TNF-α expression in SD-Mg compared with WT-Mg. The different activation states of WT- and SD-Mg may lead to differences in the response to UDP.

UDP is a known ligand of the P2Y2 and P2Y6 receptors, in addition to the CysLT1 and CysLT2 receptors, which are receptors for cysteinyl leukotrienes; UTP that is converted from UDP by ecto-nucleoside diphosphokinase binds to P2Y2, P2Y4 and P2Y6[32]. We demonstrated that UDP and potentially UTP converted from UDP induce the production of MIP-1α by SD-Mg via the P2Y6 receptor but not via the P2Y2, P2Y4, CysLT1 or CysLT2 receptors[31]. A recent study reported that P2Y6 receptors are present in neuronal cells as monomeric and dimeric forms[33]. The protein expression of the dimeric P2Y6 receptor as well as the mRNA expression of P2Y6 receptor were found to be increased in SD-Mg in comparison to WT-Mg, suggesting that the increase in the expression of dimeric P2Y6 receptor may cause the enhanced response of SD-Mg to UDP in MIP-1α production compared with that of WT-Mg. We also confirmed that the activation of ERK and JNK was involved in the UDP-induced MIP-1α production in SD-Mg. Our previous study indicated that the activation of PLC, PKC, ERK and JNK mediates the enhanced production of MIP-1α in SD-Mg[23]. P2Y6 receptor couples to G_q protein to activate PLCβ and mobilize intracellular Ca^{2+} [34] and also modulates several cellular functions through the activation of ERK, JNK or PKC[35,36]. These findings suggest that the activation of PLC, PKC, ERK and JNK may be critical signaling events in the transmitter-induced abnormal production of MIP-1α in an autocrine or paracrine manner in SD-Mg.

Enhancement of UDP-induced MIP-1α production by the disruption of the lipid rafts

The dimeric P2Y6 receptors are distributed in a non-raft microdomain and are thought to regulate the uracil nucleotide signaling[33]. Microdomains, such as lipid rafts, are known to be rich in cholesterol and glycosphingolipids including GM1 and GM3 gangliosides, where the gangliosides associate with receptors or signal transducers to modulate their functions. Glycosylation and deglycosylation have been reported to be responsible for the ganglioside composition of the plasma membrane[37]. GD3 synthase and GM2/GD2 synthase double knockout mice have disordered lipid rafts and subsequent inflammation, suggesting that the ganglioside composition is critical in the maintenance of lipid rafts[38]. Our previous studies by immunoblotting and immunocytochemical analyses have demonstrated little difference in the distribution of flotillin-1, a raft marker, between WT- and SD-Mg, but the intensity of flotillin-1 was decreased in SD-Mg compared with WT-Mg, suggesting the disordered maintenance of the lipid rafts in SD-Mg[31]. Previous studies have shown the level of cholesterol in fibroblasts from Tay-Sachs variant GM2 gangliosidosis to be similar to that of control fibroblasts[39], and that the amounts of cholesterol and GM1 ganglioside in the brain did not differ significantly between the *Hexb*$^{-/-}$ and the *Hexb*$^{+/-}$ mice[40]. Thus, the disordered maintenance of the lipid rafts in SD-Mg might be caused by the altered ganglioside composition due to the failure to catalyze GM2 to GM3 ganglioside, with no marked changes in the amounts of cholesterol or GM1 ganglioside. We found that the disruption of the lipid rafts by pretreatment with methyl-β-cyclodextrin enhanced UDP-induced MIP-1α production in both WT- and SD-Mg, suggesting that lipid raft formation plays an important role in regulating the UDP-P2Y6 receptor signaling[31]. The disordered maintenance of the lipid rafts in SD-Mg is likely not involved in the enhanced dimeric formation of P2Y6 receptors[31], and therefore both the increase in the expression of the dimeric P2Y6 receptor and the disruption of the lipid rafts may independently cause the enhanced response of SD-Mg to UDP in MIP-1α production.

Perspectives

A recent study demonstrated that UDP and UTP, as well as uridine, are detectable in the brain extracellular fluid obtained from freely moving rats, and the exposure of striatum to depolarizing concentrations of potassium chloride increases the level of the extracellular uracil nucleotides[41]. The extracellular uracil nucleotides have been shown to play roles in neural precursor cell proliferation and differentiation, and kainic acid-induced neuronal damage[29,42]. Furthermore, the uracil nucleotides are possibly involved in several neurological disorders, including epilepsy, cerebral ischemia, Alzheimer's disease and amyotrophic lateral sclerosis[43-46]; however, the detailed roles of the uracil nucleotides in those diseases remain unclear and thus require further investigation.

MIP-1α is a crucial factor for microglia-mediated neuroinflammation in the brain of SD mice, and the downregulation of the abnormal production of MIP-1α by microglia may delay the onset or progression of SD[22-24]. The activation of EP2 and 4/cAMP/PKA signaling has been shown as a potential target to control the abnormal production of MIP-1α in SD-Mg[47]. Our findings additionally provide a new therapeutic approach for SD; that is, the P2Y6 receptor antagonist is thought to be a potential therapeutic target for reducing the UDP-enhanced MIP-1α production in SD. This approach can be used for the other lysosomal storage disease, including Tay-Sachs disease and Gaucher disease, although further investigation of the involvement of the uracil nucleotide signaling is required.

Acknowledgments

This work was supported by MEXT/JSPS KAKENHI (Grant No. 26293120).

References

1. Mahuran DJ. Biochemical consequences of mutations causing the GM2 gangliosidoses. Biochim Biophys Acta. 1999; 1455: 105-138.
2. Gravel RA, Kaback MM, Proia RL, et al. The Metabolic and Molecular Basis of Inherited Disease: Scriver CR, Beaudet AL, Sly WS, Valle D, editors, 8th edit. 2001; 3: 3827-3877.
3. Sango K, Yamanaka S, Hoffmann A, et al. Mouse models of Tay-Sachs and Sandhoff diseases differ in neurologic phenotype and ganglioside metabolism. Nat Genet. 1995; 11: 170-176.
4. Wortmann SB, Lefeber DJ, Dekomien G, et al. Substrate deprivation therapy in juvenile Sandhoff disease. J Inherit Metab Dis. 2009; 32: 307-311.
5. Arthur JR, Wilson MW, Larsen SD, et al. Ethylenedioxy-PIP2 oxalate reduces ganglioside storage in juvenile Sandhoff disease mice. Neurochem Res. 2013; 38: 866-875.
6. Ashe KM, Bangari D, Li L, et al. Iminosugar-based inhibitors of glucosylceramide synthase increase brain glycosphingolipids and survival in a mouse model of Sandhoff disease. PLoS One. 2011; 6: e21758.
7. Norflus F, Tifft CJ, McDonald MP, et al. Bone marrow transplantation prolongs life span and ameliorates neurologic manifestations in Sandhoff disease mice. J Clin Invest. 1998; 101: 1881-1888.
8. Wada R, Tifft CJ, Proia RL. Microglial activation precedes acute neurodegeneration in Sandhoff disease and is suppressed by bone marrow transplantation. Proc Natl Acad Sci U S A. 2000; 97: 10954-10959.
9. Lee JP, Jeyakumar M, Gonzalez R, et al. Stem cells act through multiple mechanisms to benefit mice with neurodegenerative metabolic disease. Nat Med 2007; 13: 439-447.
10. Arthur JR, Lee JP, Snyder EY, et al. Therapeutic effects of stem cells and substrate reduction in juvenile Sandhoff mice. Neurochem Res. 2012; 37:1335-1343.
11. Matsuoka K, Tsuji D, Aikawa, S et al. Introduction of an N-glycan sequon into HEXA enhances human beta-hexosaminidase cellular uptake in a model of Sandhoff disease. Mol Ther. 2010; 18: 1519-1526.

12. Matsuoka K, Tamura T, Tsuji, D et al. Therapeutic potential of intracerebroventricular replacement of modified human β-hexosaminidase B for GM2 gangliosidosis. Mol Ther. 2011; 19: 1017-24.

13. Tsuji D, Akeboshi H, Matsuoka K, et al. Highly phosphomannosylated enzyme replacement therapy for GM2 gangliosidosis. Ann Neurol. 2011; 69: 691-701.

14. Kitakaze K, Mizutani Y, Sugiyama E, et al. Protease-resistant modified human β-hexosaminidase B ameliorates symptoms in GM2 gangliosidosis model. J Clin Invest. 2016; 126: 1691-1703.

15. Sargeant TJ, Wang S, Bradley J, et al. Adeno-associated virus-mediated expression of β-hexosaminidase prevents neuronal loss in the Sandhoff mouse brain. Hum Mol Genet. 2011; 20: 4371-4380.

16. Osmon KJ, Woodley E, Thompson P, et al. Systemic Gene Transfer of a Hexosaminidase Variant Using an scAAV9.47 Vector Corrects GM2 Gangliosidosis in Sandhoff Mice. Hum Gene Ther. 2016; 27 :497-508.

17. Walia JS, Altaleb N, Bello A, et al. Long-term correction of Sandhoff disease following intravenous delivery of rAAV9 to mouse neonates. Mol Ther. 2015; 23: 414-422.

18. Chiricozzi E, Niemir N, Aureli M, et al. Chaperone therapy for GM2 gangliosidosis: effects of pyrimethamine on β-hexosaminidase activity in Sandhoff fibroblasts. Mol Neurobiol. 2014; 50: 159-167.

19. Huang JQ, Trasler JM, Igdoura S, et al. Apoptotic cell death in mouse models of GM2 gangliosidosis and observations on human Tay-Sachs and Sandhoff diseases. Hum Mol Genet. 1997; 6: 1879-1885.

20. Myerowitz R, Lawson D, Mizukami H, et al. Molecular pathophysiology in Tay-Sachs and Sandhoff diseases as revealed by gene expression profiling. Hum Mol Genet. 2002; 11: 1343-1350.

21. Jeyakumar M, Thomas R, Elliot-Smith E, et al. Central nervous system inflammation is a hallmark of pathogenesis in mouse models of GM1 and GM2 gangliosidosis. Brain. 2003; 126: 974-987.

22. Tsuji D, Kuroki A, Ishibashi Y, et al. Specific induction of macrophage inflammatory protein 1-alpha in glial cells of Sandhoff disease model mice associated with accumulation of N-acetylhexosaminyl glycoconjugates. J Neurochem. 2005; 92: 1497-1507.

23. Kawashita E, Tsuji D, Kawashima N, Itoh K. Abnormal production of macrophage inflammatory protein-1alpha by microglial cell lines derived from neonatal brains of Sandhoff disease model mice. J Neurochem. 2009; 109: 1215-1224.

24. Wu YP, Proia RL. Deletion of macrophage-inflammatory protein 1 alpha retards neurodegeneration in Sandhoff disease mice. Proc Natl Acad Sci U S A. 2004; 101: 8425-8430.

25. Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 2005; 308: 1314-1318.

26. Davalos D, Grutzendler J, Yang G, et al. ATP mediates rapid microglial response to local brain injury in vivo. Nat Neurosci. 2005; 8: 752-758.

27. Kim B, Jeong HK, Kim JH, Lee SY, Jou I, Joe EH. Uridine 5'-diphosphate induces chemokine expression in microglia and astrocytes through activation of the P2Y6 receptor. J Immunol. 2001; 186: 3701-3709.

28. Ikeda M, Tsuno S, Sugiyama T, et al. Ca(2+) spiking activity caused by the activation of store-operated Ca(2+) channels mediates TNF-alpha release from microglial cells under chronic purinergic stimulation. Biochim Biophys Acta. 2013; 1833: 2573-2585.

29. Koizumi S, Shigemoto-Mogami Y, Nasu-Tada K, et al. UDP acting at P2Y6 receptors is a mediator of microglial phagocytosis. Nature. 2007; 446: 1091-1095.

30. Uesugi A, Kataoka A, Tozaki-Saitoh H, et al. Involvement of protein kinase D in uridine diphosphate-induced microglial macropinocytosis and phagocytosis. Glia. 2012; 60: 1094-1105.

31. Kawashita E, Tsuji D, Kanno Y, et al. Enhancement by uridine diphosphate of macrophage inflammatory protein-1 alpha production in microglia derived from Sandhoff disease model mice. JIMD Rep. 2015 [Epub ahead of print].

32. von Kugelgen I. Pharmacological profiles of cloned mammalian P2Y-receptor subtypes. Pharmacol Ther. 2006; 110: 415-432.

33. D'Ambrosi N, Iafrate M, Saba E, et al. Comparative analysis of P2Y4 and P2Y6 receptor architecture in native and transfected neuronal systems. Biochim Biophys Acta 2007; 1768: 1592-1599.

34. Erb L, Weisman GA. Coupling of P2Y receptors to G proteins and other signaling pathways. Wiley Interdiscip Rev Membr Transp Signal. 2012; 1: 789-803.

35. Kim SG, Gao ZG, Soltysiak KA, et al. P2Y6 nucleotide receptor activates PKC to protect 1321N1 astrocytoma cells against tumor necrosis factor-induced apoptosis. Cell Mol Neurobiol 2003; 23: 401-418.

36. Li R, Tan B, Yan Y, et al. Extracellular UDP and P2Y6 function as a danger signal to protect mice from vesicular stomatitis virus infection through an increase in IFN-beta production. J Immunol 2014; 193: 4515-4526.

37. Prinetti A, Loberto N, Chigorno V, et al. Glycosphingolipid behaviour in complex membranes. Biochim Biophys Acta. 2009; 1788: 184-193.

38. Ohmi Y, Tajima O, Ohkawa Y, et al. Gangliosides are essential in the protection of inflammation and neurodegeneration via maintenance of lipid rafts: elucidation by a series of ganglioside-deficient mutant mice. J Neurochem. 2011; 116: 926-935.

39. Puri V, Watanabe R, Dominguez M, et al. Cholesterol modulates membrane traffic along the endocytic pathway in sphingolipid-storage diseases. Nat Cell Biol. 1999; 1: 386-388.

40. Denny CA, Kasperzyk JL, Gorham KN. et al. Influence of caloric restriction on motor behavior, longevity, and brain lipid composition in Sandhoff disease mice. J Neurosci Res. 2006; 83: 1028-1038.

41. Cansev M, Orhan F, Yaylagul EO, et al. Evidence for the existence of pyrimidinergic transmission in rat brain. Neuropharmacology 2015; 91: 77-86.

42. Milosevic J, Brandt A, Roemuss U, et al. Uracil nucleotides stimulate human neural precursor cell proliferation and dopaminergic differentiation: involvement of MEK/ERK signalling. J Neurochem. 2006; 99: 913-923.

43. Kovács Z, Slézia A, Bali ZK, et al. Uridine modulates neuronal activity and inhibits spike-wave discharges of absence epileptic Long Evans and Wistar Albino Glaxo/Rijswijk rats. Brain Res Bull. 2013; 97:16-23.

44. Woods LT, Ajit D, Camden JM, et al. Purinergic receptors as potential therapeutic targets in Alzheimer's disease. Neuropharmacology. 2016; 104: 169-179.

45. Tian ML, Zou Z, Yuan HB, et al. Uridine 5'-triphosphate (UTP) protects against cerebral ischemia reperfusion injury in rats. Neurosci Lett. 2009; 465: 55-60.

46. Volonté C, Apolloni S, Parisi C, et al. Purinergic contribution to amyotrophic lateral sclerosis. Neuropharmacology. 2016; 104: 180-193.

47. Kawashita E, Tsuji D, Toyoshima M, Kanno Y, Matsuno H, Itoh K. Prostaglandin E2 reverses aberrant production of an inflammatory chemokine by microglia from Sandhoff disease model mice through the cAMP-PKA pathway. PLoS One 2011; 6: e16269.