Review

Inhibition of extracellular vesicle pathway using neutral sphingomyelinase inhibitors as a neuroprotective treatment for brain injury

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Abstract

Traumatic brain injury is a sudden trauma or blow on the head, and severe traumatic brain injury is a major cause of death and disability worldwide. The acute and chronic consequences following traumatic brain injury can lead to progressive secondary neurodegenerative changes and cognitive dysfunction. To date, there is no effective pharmaceutical products for the treatment to reduce secondary damage after brain injury. The discovery of extracellular vesicles has attracted considerable scientific attention due to their role in cell-to-cell communication. Extracellular vesicles have shown their potential to carry not only biological molecules but also as a drug delivery vehicle. As a carrier of molecular information, extracellular vesicles have been involved in physiological functions as well as in the modulation of immune responses. Here, we aim to provide new insights into the contrasting role of extracellular vesicles in the propagation of inflammatory responses after brain injury. As a carrier of pro-inflammatory molecules, their role as functional mediators in the pathophysiology of brain injury is discussed, addressing the inhibition of the extracellular vesicle pathway as an anti-inflammatory or neuroprotective approach to improve the outcome of both acute and chronic inflammation following brain injury. Here, we summarize therapeutic strategies to diminish the risk the neurodegeneration post brain injury and propose that neutral sphingomyelinase inhibitors could be used as potentially useful therapeutic agents for the treatment of brain injury associated neuroinflammation.

Key Words: brain injury; ceramide; extracellular vesicles; microglia; neuroinflammation; neuroprotection; neutral sphingomyelinase

Introduction

A rapidly developing field of extracellular vesicles (EVs) research is opening a new dimension of complexity for intercellular communication occurring within the brain. EVs mediate cell-cell communication and modulate immune responses by transporting their cargo, including nucleic acids, proteins, lipids, and non-coding RNAs from one cell to another. The EVs have been characterized based on their biogenesis or release pathways. When EVs directly bud from plasma membranes, they are identified as microparticles (MP; also called microvesicles). EVs that are secreted and released into the extracellular environment when multivesicular bodies fuse with the plasma membrane are termed exosomes (Johnstone et al., 1987; Kumar et al., 2020). Similar to the cellular plasma membrane, EV membranes consist of a proteolipid bilayer and are enriched with disaturated phosphatidylethanolamine, disaturated phosphatidylcholine, sphingomyelin, ganglioside GM3, and cholesterol (Choi et al., 2013). These lipids and lipid-raft-associated proteins present in EV membranes provide stability and structural rigidity. In contrast to cellular membranes, preferentially small EVs contain externalized phosphatidylserine, which may facilitate their recognition and uptake by recipient cells. EV biogenesis is dependent on the endosomal sorting complex transport (ESCRT)-dependent pathway (Colombo et al., 2013). In addition to ESCRT, other mechanisms are also involved such as the ESCRT-independent pathway that requires lipid ceramide and neutral sphingomyelinase (nSMase) to produce EVs (Trajkovic et al., 2008) (**Figure 1**). EVs formed during the ESCRT-independent pathway are enriched with ceramide that are formed after the reaction involving the hydrolysis of sphingomyelin catalyzed by SMases (Clarke et al., 2006; Trajkovic et al., 2008). Ceramide is a class of sphingolipid that provides structural rigidity to the plasma membrane as well as to the EV vesicular membrane. In addition, ceramides have been involved in a variety of cellular processes such as cellular stress leading to apoptosis, autophagy, cell growth, differentiation, senescence, as well as signal transduction (Li et al., 2014; Taniguchi and Okazaki, 2020). Apart from the de novo synthesis of ceramide that occurs in the endoplasmic reticulum, they can also be generated in the plasma membrane by the enzymatic reaction catalyzed by SMases (Trajkovic et al., 2008). Accumulating shreds of evidence suggest that the sphingolipids are involved in both innate and adaptive immune responses, cell death, and cell growth (Olivera and Rivera, 2005; Maceyka and Spiegel, 2014).

Search Strategy and Selection Criteria

We performed a literature search on PubMed and Google Scholar until October 2020 published in English. The

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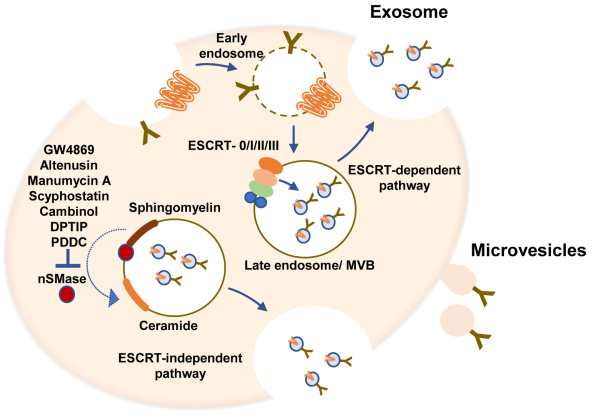

Figure 1 | ESCRT-dependent and ESCRT-independent pathway of extracellular vesicles.
EVs that are secreted and released into the extracellular environment when MVB fuse with the plasma membrane are termed exosomes, whereas EVs directly bud from plasma membranes are identified as microvesicles (or microparticles). In addition to ESCRT, other mechanisms such as the ESCRT independent pathway that requires lipid ceramide and nSMase are also involved in the production of EVs. Inhibiting EV formation and release can be achieved using nSMase inhibitors and could provide a novel therapeutic approach for neurological disorders including brain injury. DPTIP: 2,6-Dimethoxy-4-(5-phenyl-4-thiophen-2-yl-1H-imidazol-2-yl)-phenol; ESCRT: endosomal sorting complex transport; EV: extracellular vesicle; MVB: multivesicular body; nSMase: neutral sphingomyelinase; PDDC: phenyl(R)-(1-(3-(3,4-dimethoxyphenyl)-2,6-dimethylimidazo[1,2-b]pyridazin-8-yl) pyrrolidin-3-yl)-carbamate.

key words/terms were extracellular vesicles, neutral sphingomyelinase inhibitors, neuroinflammation, brain injury and neurodegeneration.

Role of Extracellular Vesicles in Traumatic Brain Injury-Induced Neuroinflammation

Despite many preclinical studies, there are still no novel therapeutic options that alter the microglia-mediated neurotoxicity following brain injury and provide substantial benefit except for routine medical intervention and intensive care of patients with severe brain injury. Following brain injury, microglia activation sustains for years and is believed to contribute to chronic neuronal damage and progressive neurologic deficits (Loane and Byrnes, 2010). Several studies demonstrated that ceramide mediates cell death and tissue damage as a result of physical, biological, or metabolic injury. Prior work indicated that traumatic brain injury (TBI) elicits sphingosine accumulation in mitochondria as a cause of disturbance in mitochondrial-associated sphingolipid-metabolizing enzymes including SphK2 via post-translational mechanisms (Novgorodov et al., 2014). Later they found that Smpd1 gene ablation (acid sphingomyelinase deficiency) preserved mitochondrial function and attenuated sphingosine accumulation in mitochondria in addition to improving brain functional recovery and reducing NLRP3-dependent neuroinflammatory response after TBI (Novgorodov et al., 2019). In a study by Gu et al. (2013), nSMase2/ceramide was found to be involved in ischemia-associated peripheral neuronal damage. In the brain, tissue damage due to trauma can trigger a sequence of events that mainly comprise innate immune response. The primary goal of this initial immune response is to clear dead cells and tissue from the damaged site and to induce tissue-repair mechanisms. However, in the case of substantial brain injury, a well-behaved immune system can turn into a dysregulated system that leads to progressive and secondary damage following injury. This secondary damage resulting from the inflammatory response involves cytokine storm generated by neurotoxic microglia activation and infiltrated leukocyte, generation of reactive oxygen species, and dysregulation of autophagy/phagocytosis (Soares et al., 1995; Lee et al., 2014; Sarkar et al., 2014). In addition, leukocyte- and microglia-derived EVs that carry pro-inflammatory molecules can propagate the inflammatory response. Following brain injury, microglia-derived MP loaded with pro-inflammatory molecules may contribute to progressive neuroinflammatory response in the injured brain, as well as, stimulate systemic immune responses (Kumar et al., 2017). nSMase inhibitors emerged as promising therapeutics not only for inflammatory brain lesions but also for several neurodegenerative diseases (Dinkins et al., 2016; Dickens et al., 2017; Yoo et al., 2020). nSMase inhibitor, scyphostatin, has been shown to inhibit nerve growth factor-induced ceramide generation and prevent neuronal cell death *in vitro*, supporting the role of nSMase in neuronal cell death (Brann et al., 2002). However, physiological importance of nerve growth factor-induced ceramide generation and neuronal cell death is yet to be elucidated *in vivo*.

Preventing Extracellular Vesicles Release via Neutral Sphingomyelinase Inhibitors

Therapies that showed positive outcomes in preclinical studies, performed in experimental animal models of TBI, failed to translate to human clinical studies to date. Several studies also indicated animal modeling limitations that might be contributing factors to these failures including secondary insults following brain injury, post traumatic coma, limited intensive care monitoring, and modalities of traumatic events (McAteer et al., 2017). These limitations could arise due to structural differences between the rodent and human brains Importantly rodent lissencephalic brain responds differently to mechanical insults than a human gyrencephalic brain, particularly from a biomechanical and physiological perspective (Morganti-Kossmann et al., 2010; Nyanzu et al., 2017; Vink, 2018). In addition to nSMase, acid sphingomyelinase (aSMase) has also been associated with MP shedding. Inhibition of aSMase using imipramine blocked the release of interleukin-1β by hampering aSMase mediated MP shedding from glial cells (Bianco et al., 2009). Several nSMase inhibitors have been identified so far, however, a lack of information related to specificity, potency, and physicochemical properties, limit their use in the clinic. GW4869 is a selective, noncompetitive nSMase2 inhibitor (Bianco et al., 2009), whereas altenusin is a nonsteroidal fungal metabolite with broader nSMase inhibitor activity (Uchida et al., 1999). Both the compounds lack inhibitory activity towards aSMase. Moreover, manumycin A is a naturally occurring potent but reversible nSMase inhibitor that show an affinity to nSMase comparable to the sphingomyelin, which is a natural substrate for nSMase (Arenz et al., 2001). Also, scyphostatin is known for its inhibitory activity towards nSMase. Although both manumycin A and scyphostatin showed nSMase inhibitory activity, they showed no selectivity towards multiple isoforms of nSMases. In addition, scyphostatin displayed inhibitory activity towards aSMase (Nara et al., 1999). To elucidate the role of ceramide in EV biogenesis, prior studies that mostly used GW4869 to inhibit ceramide synthesis attenuated EV secretion (Trajkovic et al., 2008; Hoshino et al., 2013). Ceramide generation by nSMase2 plays a key role in ESCRT-independent exosome formation (Trajkovic et al., 2008; Elsherbini and Bieberich, 2018). A reduction in the expression of nSMase2 using siRNAs or inhibitors such as GW4869 resulted in the decreased release of proteolipid protein-containing EVs (Menck et al., 2017). This suggested the importance of ceramide in the sorting of proteolipid protein into intraluminal vesicles leading to ESCRT-independent formation of EVs (Trajkovic et al., 2008). Furthermore, the depletion of nSMase2 with GW4869 reduced the secretion of exosomal proteins such as CD63, CD81, and TSG101. Recently, large-scale compounds screening

was performed in an endeavor to find new nSMase inhibitors with enhanced solubility and improved potency, which led to the identification of cambinol as a novel uncompetitive nSMase2 inhibitor (Figuera-Losada et al., 2015). In addition, cambinol was found to reduce the cytokine-induced secretion of ceramide and cell death in primary neurons. Compared to GW4869, cambinol showed similar potency but improved solubility. Moreover, cambinol showed better inhibition against nSMase2 than altenusin, C11AG, or macquarimicin A. However, the later findings found that cambinol is a metabolically unstable compound with a weak *in vivo* pharmacokinetic profile. The efforts to enhance its potency and stability also failed. Further, in an attempt to find a new nSMase2 inhibitor that exhibits an excellent pharmacokinetic profile and blood-brain barrier (BBB) penetration capacity, a similar group started a high throughput screening campaign and identified a potent inhibitor of nSMase2, termed DPTIP (Rojas et al., 2018). DPTIP not only showed an excellent pharmacokinetic profile and brain penetration capacity, but it was also found to modulate the cytokine-induced release of EVs derived from astrocytes (ADEV). Moreover, DPTIP inhibited ADEV induced peripheral upregulation of cytokine and peripheral leukocyte response to the inflamed brain. These findings indicate that nSMase could regulate leukocyte migration to the inflammatory lesions following brain injury. Further, their genetic or pharmacological inhibition could inhibit the neuroinflammatory responses mediated by the activated glial cells.

Neutral Sphingomyelinase Inhibitors as a Neuroprotective Strategy for Traumatic Brain Injury

A pathological process in TBI is largely associated with progressive neuronal damage that occurs due to neurotoxic microglial activation. However, distinct microglial reactions during inflammation have been challenging to pinpoint, as both neurotoxic and neuroprotective functions of microglia have been proposed in this process (Colonna and Butovsky, 2017). Research conducted in past years witnessed diversified microglial functions that largely depend on pathological and normal physiological states. Microglia under pathological conditions transform into a reactive phenotype and mediate cellular and molecular processes with a primary aim of restoring homeostasis and resolution of inflammation in the brain. However, during this process, due to the continuum of activation, microglia become hyperactivate and initiate both innate and adaptive immune responses and produce excessive pro-inflammatory molecules that may exacerbate brain damage following injury. Pharmacological targeting of microglial activation is one of the methods that counter the negative impact of microglia induced neuroinflammation in TBI. In a prior study, we investigated the therapeutic potential of nSMase inhibitors to regulate neurotoxic microglial activation and neuroinflammation in the mice subjected to moderate level of controlled cortical impact. Controlled cortical impact is a well-characterized experimental model of brain trauma that uses a microprocessor-controlled pneumatic impactor to induce a cortical focal contusion injury in mice (Osier and Dixon, 2016; Kumar et al., 2019). This study was consistent with others indicating that activation of nSMase is positively correlated with neuroinflammatory signaling pathways. Here, we utilized experimental neuroprotection strategies for TBI using the nSMase inhibitors altenusin and GW4869. However, we observed that general inhibition of nSMase activity provides improved effectiveness in neuroinflammation by regulating microglia activation and cytokine production in the brain. We observed that post TBI treatment of altenusin or GW4869 significantly downregulated gene expression of the pro-inflammatory mediators such as proinflammatory cytokines, chemokines, and nitric oxide. Further, it reduced the production of nitric oxide and tumor necrosis factor-α in cultured microglia, indicating that nSMase may play a role in regulating innate immune processes. In addition, nSMase appears to regulate the shedding or budding of EVs, resulting in inhibition of microglial activation markers and associated inflammatory responses (Kumar et al., 2019). Moreover, altenusin treatment reduced the gene expression of pro-inflammatory cytokines and multiple inflammatory markers associated with microglial activation and neuroinflammation after experimental TBI in a mouse model (Kumar et al., 2019).

A prior study identified the cellular mechanism and function of microglia-derived MP, a member of the EV family, in neuroinflammation after experimental TBI (Kumar et al., 2017). They showed that tissue damage leads to the unresolved or dysregulated immune response soon after TBI, resulting in chronic activation of microglia. Further, they indicated that acute brain tissue damage or injury is attributable to secondary processes related to neurodegeneration, due to excessive and neurotoxic microglial activation. In response to secondary injury after TBI, microglia release MP that are not only involved in intercellular communication, but also propagate neuroinflammatory response by transporting a cargo of pro-inflammatory cytokines in the brain following trauma. Because of the involvement of MP, which in turn induce pro-inflammatory effects upon chronic TBI, the development of novel inhibitors that regulate the production of EVs may be needed. Based on prior studies, a broader inhibition of nSMase activity may provide protection against progressive neuronal damage following brain injury. However, the off-target activity of these inhibitors cannot be denied, and pose a limitation in the pharmaceutical intervention. For instance, altenusin has been shown to have inhibitory potential against a number of protein kinases including Aurora-A/B, ARK5, CDK4/CycD1, and SRC (Aly et al., 2008). In addition, altenusin is found to be an agonist of the Farnesoid X receptor, a member of the nuclear receptor subfamily (Zheng et al., 2017). Some nSMase inhibitors such as GW4869 have low aqueous solubility that makes them poor drug delivery candidate for clinical studies. Therefore, an intense research is required to develop inhibitors with improved solubility and drug-like physicochemical properties with BBB penetration ability. A recent study identified phenyl(R)-(1-(3-(3,4-dimethoxyphenyl)-2,6-dimethylimidazo[1,2-b]pyridazin-8-yl) pyrrolidin-3-yl)-carbamate (PDDC), a novel nSMase2 inhibitor that not only displayed favorable pharmacodynamics and pharmacokinetic parameters, but also possessed substantial oral bioavailability and BBB penetration (Rojas et al., 2019). These studies demonstrated that PDDC inhibited the release of ADEV and leukocyte transmigration in response to a focal inflammatory brain lesion. In addition, PDDC significantly reversed cognitive impairment in the 5XFAD mouse model of Alzheimer's disease (Sala et al., 2020).

Conclusion

In conclusion, based on studies conducted in recent years EVs appear to be promising for therapeutic interventions. However, studies need to be performed to validate the use of EVs as drug delivery vehicles, especially to target TBI. Moreover, targeting the nSMase pathway may have significant therapeutic value. However, in the context of translational effectiveness of pharmacological inhibition or gene ablation of nSMase, a further preclinical investigation is warranted to elucidate the role of nSMase inhibitors on brain functional recovery following brain injury. In the future, we may witness a remarkable increase in our understanding of the roles and regulation of EVs, which may facilitate the development of novel therapeutic strategies against various diseases, including TBI.

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References

Arenz C, Thutewohl M, Block O, Waldmann H, Altenbach HJ, Giannis A (2001) Manumycin A and its analogues are irreversible inhibitors of neutral sphingomyelinase. Chembiochem 2:141-143.

Bianco F, Perrotta C, Novellino L, Francolini M, Riganti L, Menna E, Saglietti L, Schuchman EH, Furlan R, Clementi E, Matteoli M, Verderio C (2009) Acid sphingomyelinase activity triggers microparticle release from glial cells. EMBO J 28:1043-1054.

Brann AB, Tcherpakov M, Williams IM, Futerman AH, Fainzilber M (2002) Nerve growth factor-induced p75-mediated death of cultured hippocampal neurons is age-dependent and transduced through ceramide generated by neutral sphingomyelinase. J Biol Chem 277:9812-9818.

Choi DS, Kim DK, Kim YK, Gho YS (2013) Proteomics, transcriptomics and lipidomics of exosomes and ectosomes. Proteomics 13:1554-1571.

Clarke CJ, Snook CF, Tani M, Matmati N, Marchesini N, Hannun YA (2006) The extended family of neutral sphingomyelinases. Biochemistry 45:11247-11256.

Colombo M, Moita C, van Niel G, Kowal J, Vigneron J, Benaroch P, Manel N, Moita LF, Thery C, Raposo G (2013) Analysis of ESCRT functions in exosome biogenesis, composition and secretion highlights the heterogeneity of extracellular vesicles. J Cell Sci 126:5553-5565.

Dickens AM, Tovar YRLB, Yoo SW, Trout AL, Bae M, Kanmogne M, Megra B, Williams DW, Witwer KW, Gacias M, Tabatadze N, Cole RN, Casaccia P, Berman JW, Anthony DC, Haughey NJ (2017) Astrocyte-shed extracellular vesicles regulate the peripheral leukocyte response to inflammatory brain lesions. Sci Signal 10:eaai7696.

Dinkins MB, Enasko J, Hernandez C, Wang G, Kong J, Helwa I, Liu Y, Terry AV, Jr., Bieberich E (2016) Neutral sphingomyelinase-2 deficiency ameliorates Alzheimer's disease pathology and improves cognition in the 5XFAD mouse. J Neurosci 36:8653-8667.

Elsherbini A, Bieberich E (2018) Ceramide and exosomes: a novel target in cancer biology and therapy. Adv Cancer Res 140:121-154.

Figuera-Losada M, Stathis M, Dorskind JM, Thomas AG, Bandaru VV, Yoo SW, Westwood NJ, Rogers GW, McArthur JC, Haughey NJ, Slusher BS, Rojas C (2015) Cambinol, a novel inhibitor of neutral sphingomyelinase 2 shows neuroprotective properties. PLoS One 10:e0124481.

Hoshino D, Kirkbride KC, Costello K, Clark ES, Sinha S, Grega-Larson N, Tyska MJ, Weaver AM (2013) Exosome secretion is enhanced by invadopodia and drives invasive behavior. Cell Rep 5:1159-1168.

Johnstone RM, Adam M, Hammond JR, Orr L, Turbide C (1987) Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). J Biol Chem 262:9412-9420.

Kumar A, Henry RJ, Stoica BA, Loane DJ, Abulwerdi G, Bhat SA, Faden AI (2019) Neutral sphingomyelinase inhibition alleviates LPS-induced microglia activation and neuroinflammation after experimental traumatic brain injury. J Pharmacol Exp Ther 368:338-352.

Kumar A, Kodidela S, Tadrous E, Cory TJ, Walker CM, Smith AM, Mukherjee A, Kumar S (2020) Extracellular vesicles in viral replication and pathogenesis and their potential role in therapeutic intervention. Viruses 12:887.

Kumar A, Stoica BA, Loane DJ, Yang M, Abulwerdi G, Khan N, Kumar A, Thom SR, Faden AI (2017) Microglial-derived microparticles mediate neuroinflammation after traumatic brain injury. J Neuroinflammation 14:47.

Lee J, Costantini TW, D'Mello R, Eliceiri BP, Coimbra R, Bansal V (2014) Altering leukocyte recruitment following traumatic brain injury with ghrelin therapy. J Trauma Acute Care Surg 77:709-715.

Li Y, Li S, Qin X, Hou W, Dong H, Yao L, Xiong L (2014) The pleiotropic roles of sphingolipid signaling in autophagy. Cell Death Dis 5:e1245.

Loane DJ, Byrnes KR (2010) Role of microglia in neurotrauma. Neurotherapeutics 7:366-377.

Maceyka M, Spiegel S (2014) Sphingolipid metabolites in inflammatory disease. Nature 510:58-67.

McAteer KM, Turner RJ, Corrigan F (2017) Animal models of chronic traumatic encephalopathy. Concussion 2:CNC32.

Menck K, Sonmezer C, Worst TS, Schulz M, Dihazi GH, Streit F, Erdmann G, Kling S, Boutros M, Binder C, Gross JC (2017) Neutral sphingomyelinases control extracellular vesicles budding from the plasma membrane. J Extracell Vesicles 6:1378056.

Morganti-Kossmann MC, Yan E, Bye N (2010) Animal models of traumatic brain injury: is there an optimal model to reproduce human brain injury in the laboratory? Injury 41 Suppl 1:S10-13.

Nara F, Tanaka M, Masuda-Inoue S, Yamasato Y, Doi-Yoshioka H, Suzuki-Konagai K, Kumakura S, Ogita T (1999) Biological activities of scyphostatin, a neutral sphingomyelinase inhibitor from a discomycete, Trichopeziza mollissima. J Antibiot (Tokyo) 52:531-535.

Novgorodov SA, Voltin JR, Wang W, Tomlinson S, Riley CL, Gudz TI (2019) Acid sphingomyelinase deficiency protects mitochondria and improves function recovery after brain injury. J Lipid Res 60:609-623.

Novgorodov SA, Riley CL, Yu J, Borg KT, Hannun YA, Proia RL, Kindy MS, Gudz TI (2014) Essential roles of neutral ceramidase and sphingosine in mitochondrial dysfunction due to traumatic brain injury. J Biol Chem 289:13142-13154.

Nyanzu M, Siaw-Debrah F, Ni H, Xu Z, Wang H, Lin X, Zhuge Q, Huang L (2017) Improving on laboratory traumatic brain injury models to achieve better results. Int J Med Sci 14:494-505.

Olivera A, Rivera J (2005) Sphingolipids and the balancing of immune cell function: lessons from the mast cell. J Immunol 174:1153-1158.

Osier N, Dixon CE (2016) The controlled cortical impact model of experimental brain trauma: overview, research applications, and protocol. Methods Mol Biol 1462:177-192.

Rojas C, Sala M, Thomas AG, Datta Chaudhuri A, Yoo SW, Li Z, Dash RP, Rais R, Haughey NJ, Nencka R, Slusher B (2019) A novel and potent brain penetrant inhibitor of extracellular vesicle release. Br J Pharmacol 176:3857-3870.

Rojas C, Barnaeva E, Thomas AG, Hu X, Southall N, Marugan J, Chaudhuri AD, Yoo SW, Hin N, Stepanek O, Wu Y, Zimmermann SC, Gadiano AG, Tsukamoto T, Rais R, Haughey N, Ferrer M, Slusher BS (2018) DPTIP, a newly identified potent brain penetrant neutral sphingomyelinase 2 inhibitor, regulates astrocyte-peripheral immune communication following brain inflammation. Sci Rep 8:17715.

Sala M, Hollinger KR, Thomas AG, Dash RP, Tallon C, Veeravalli V, Lovell L, Kogler M, Hrebabecky H, Prochazkova E, Nesuta O, Donoghue A, Lam J, Rais R, Rojas C, Slusher BS, Nencka R (2020) Novel human neutral sphingomyelinase 2 inhibitors as potential therapeutics for Alzheimer's disease. J Med Chem 63:6028-6056.

Sarkar C, Zhao Z, Aungst S, Sabirzhanov B, Faden AI, Lipinski MM (2014) Impaired autophagy flux is associated with neuronal cell death after traumatic brain injury. Autophagy 10:2208-2222.

Soares HD, Hicks RR, Smith D, McIntosh TK (1995) Inflammatory leukocytic recruitment and diffuse neuronal degeneration are separate pathological processes resulting from traumatic brain injury. J Neurosci 15:8223-8233.

Taniguchi M, Okazaki T (2020) Ceramide/sphingomyelin rheostat regulated by sphingomyelin synthases and chronic diseases in murine models. J Lipid Atheroscler 9:380-405.

Trajkovic K, Hsu C, Chiantia S, Rajendran L, Wenzel D, Wieland F, Schwille P, Brugger B, Simons M (2008) Ceramide triggers budding of exosome vesicles into multivesicular endosomes. Science 319:1244-1247.

Uchida R, Tomoda H, Dong Y, Omura S (1999) Alutenusin, a specific neutral sphingomyelinase inhibitor, produced by Penicillium sp. FO-7436. J Antibiot (Tokyo) 52:572-574.

Vink R (2018) Large animal models of traumatic brain injury. J Neurosci Res 96:527-535.

Yoo SW, Agarwal A, Smith MD, Khuder SS, Baxi EG, Thomas AG, Rojas C, Moniruzzaman M, Slusher BS, Bergles DE, Calabresi PA, Haughey NJ (2020) Inhibition of neutral sphingomyelinase 2 promotes remyelination. Sci Adv 6:eaba5210.

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