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CURRENT STATUS OF DRUG THERAPY DEVELOPMENT FOR NIEMANN-PICK TYPE C DISEASE

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ABSTRACT

Niemann-Pick type C (NPC) disease is a very rare, fatal disorder due to mutations in either of two different genes, NPC1 or NPC2, which are involved in intracellular lipid trafficking but for which the specific functions have yet to be fully elaborated. Mutations in either gene lead to the same disease phenotype, characterized by abnormal accumulation of free cholesterol, sphingolipids and other lipids in late endosomes or lysosomes in tissues throughout the body. This accumulation is associated with severe neurodegeneration and dysfunction of visceral organs such as the liver and spleen. This review summarizes the many approaches that have been investigated for developing small-molecule drug therapies for NPC. Although some compounds, such as miglustat, allopregnanolone, oxysterols and cyclodextrins, are able to slow the progress of the disease, no drug is yet known that provides an effective long-term treatment and rescue of the phenotype. Possible new directions for NPC drug discovery are discussed.

BACKGROUND

Niemann-Pick type C disease (NPC) is one of a very large number of lysosomal storage disorders (1-13), including the related Niemann-Pick types A and B (NPA and NPB), Gaucher's disease, Tay-Sachs disease, Fabry's disease, Schindler's disease, Wolman's disease, Krabbe's disease, and Hunter's syndrome. Knowledge of the Niemann-Pick group of diseases can be traced back to the pioneering descriptions of NPA by the German physicians Albert Niemann (14) and Ludwig Pick (15) in the 1910s and 1920s, followed by a more definitive description by Crocker and Farber (16) in the 1950s.

NPC is a very rare, inherited, autosomal recessive disease that by one estimate has an occurrence of 1 in 150,000, with only several hundred diagnosed cases (3). It has been diagnosed in prenatal, infantile, juvenile and adult stages of development. The phenotype is characterized by abnormally high accumulation of several types of lipids (Fig. 1), including free cholesterol (unesterified), sphingosine, sphingomyelin, other phospholipids and glycosphingolipids (e.g., glucosylceramide and gangliosides G_{M2} and G_{M3}). The accumulation occurs in lysosomes and late endosomes of cells throughout the body, with pronounced concentrations in the liver and the spleen. In the brain, glycosphingolipids rather than cholesterol are seen at especially high levels. Normally, lipids such as cholesterol are transported for further processing from lysosomes to the endoplasmic reticulum (ER), the trans-Golgi network and the plasma membrane. In NPC, this transport is disrupted, with a consequent increase in lysosomal lipid loading. Another abnormality in NPC is the biosynthesis and extent of esterification and phosphorylation of dolichol (17). The combined accumulation of several lipid classes distinguishes NPC from NPA (neurological) and NPB (spleen and liver), which are primarily sphingomyelin lipidoses due to sphingomyelinase deficiency. A previously described disorder, Niemann-Pick type D (NPD), is now recognized as a genetic isolate with a founder mutation in the *NPC1* gene (3).

Symptoms of NPC are most commonly associated with severe neurodegenerative disease progressing over several years and with 100% lethality due most commonly to brainstem failure and subsequent ventilatory failure, uncontrolled seizures or aspiration pneumonia. Death from hepatic or pulmonary failure is seen in some neonatal and infantile cases. Neurodegenerative symptoms include abnormal eye movements (vertical gaze palsy), poor coordination of voluntary muscle action (ataxia), improper muscle tone (dystonia), difficulty in swallowing (dysphagia), seizures, cataplexy, dementia and psychiatric illness (18). Examination of the brains of patients shows abnormal lipid storage in neurons, ectopic dendritogenesis, meganeurite formation, loss of Purkinje cells and the development

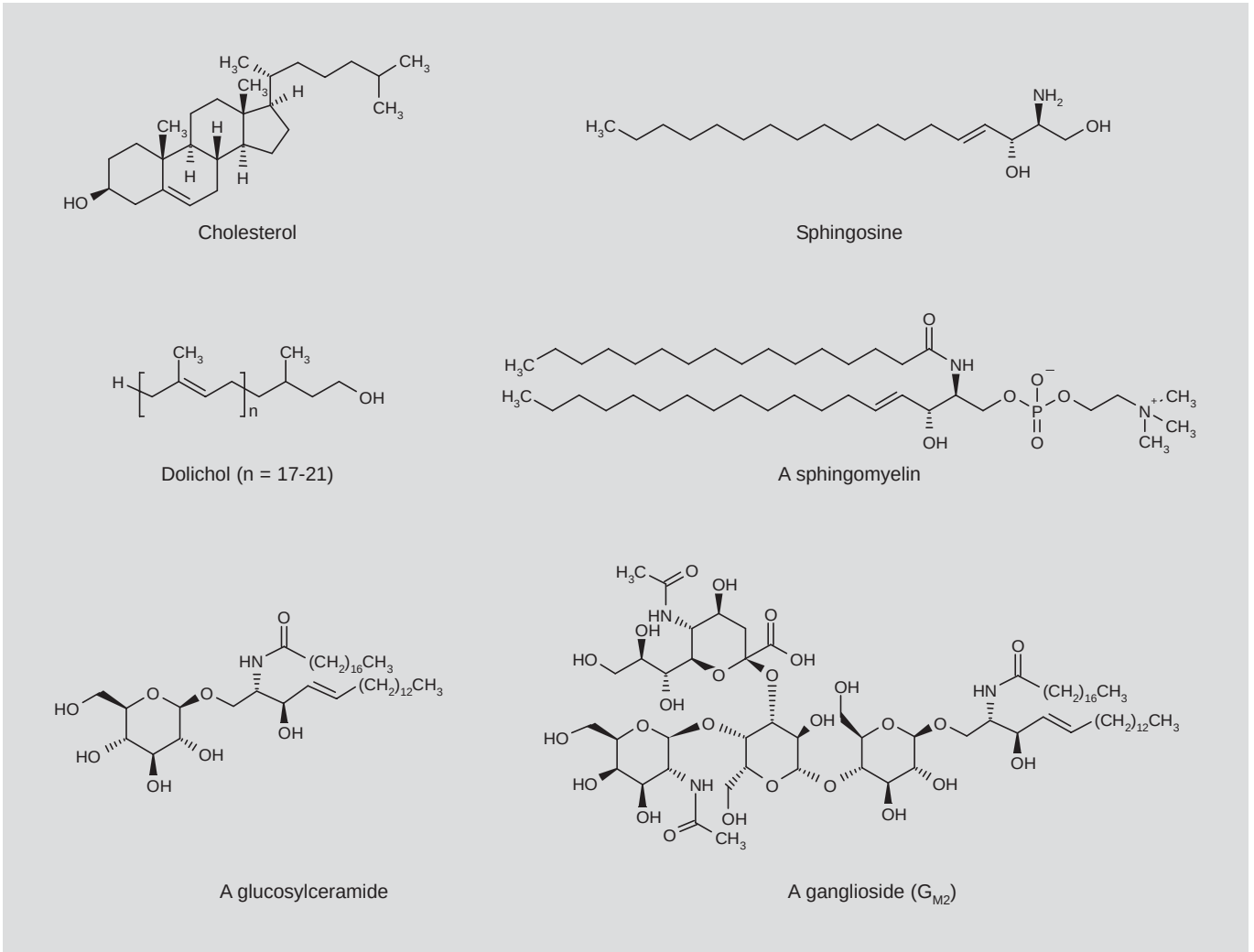

Figure 1. Examples of lipids that accumulate or are misregulated in NPC cells.

of neurofibrillary tangles, reminiscent of but distinct from those seen in Alzheimer's disease (19-22). Both apoptotic and autophagic neurological cell death occurs (23). The juvenile form of NPC is typically diagnosed in children aged 5-10 years when impaired physical and mental development is noted (18). These patients usually die in their early teenage years. Also seen is an early-onset, rapidly progressing form of NPC in neonates. Much less common is a slower progressing form in adults; a patient as old as 68 years has been reported (24).

MODELS AND MARKERS

Animal models have been employed to study the basis and progression of NPC and to investigate possible therapies. Most commonly used is the BALB/c npc^{nih} mouse (25), but a cat model (26) is also available. A previously studied dog model is no longer available (27). The cat model is considered to be superior to the mouse model due to greater similarity to progression of the human disease and larger brain size facilitating the study of neurological effects (28), but the cat model is considerably more expensive to employ than the mouse model. A caveat is that concerns have been expressed about the translatability of results of mouse models of neurodegenerative disease to human disorders (29-31). An NPC-like phenotype can be pharmacologically induced in cultured fibroblasts using the cholesterol transport inhibitor U-18666A (3-[2-(diethylamino)ethoxy]-androst-5-en-17-one).

The most commonly used method to study the accumulation of cholesterol in NPC cells is filipin staining (32, 33). Filipin is a mixture of four highly conjugated, fluorescent macrolide antibiotics, with filipin III (Fig. 2) being the main component (34). It binds to free cholesterol and acts as a fluorescent probe of cholesterol accumulation and distribution by fluorescence microscopy. Some concerns have been expressed about the reliability of this method due to the instability of the filipin stain, the lack of linear response and the fact that the fluorescence of filipin can be quenched by a variety of other compounds (35, 36), thereby limiting the quantification of cholesterol and giving false-positives in screens for compounds that lower the cholesterol content of cells. Some of these concerns have been addressed through a secondary screen using the direct detection of cholesterol by gas chromatography.

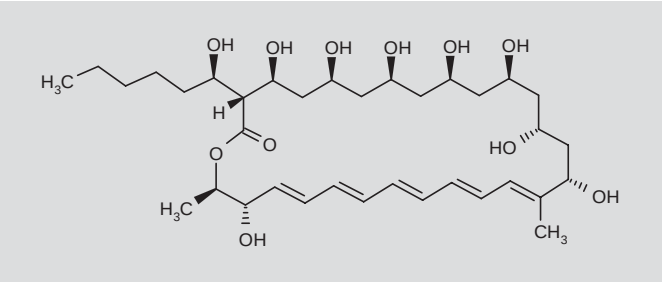

Figure 2. Filipin III.

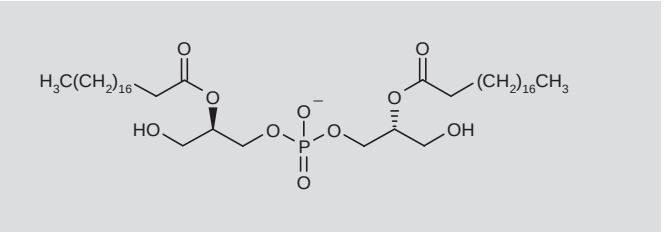

Figure 3. 2,2′-Bioleoyl lysobisphosphatidic acid, an example of a lysobisphosphatidic acid (LBPA).

GENETIC AND MOLECULAR BASIS OF THE DISEASE

NPC is correlated with mutations in either the *NPC1* or the *NPC2* gene coding for the corresponding NPC1 and NPC2 proteins (37). The *NPC1* gene has been found to be located on chromosome 18 (38), whereas *NPC2* is on chromosome 14 (39). Mutations in the *NPC1* gene account for 95% of all cases of the disease, whereas mutations in the *NPC2* gene account for the remaining 5% (40). Nonetheless, identical phenotypes arise from both sets of mutations.

The NPC1 protein is a large 1,278-residue, membrane-bound protein having 13 transmembrane domains and located in the membranes of late endosomes and lysosomes (41). On the other hand, the NPC2 protein is a much smaller, 130-residue, soluble lysosomal protein (42). Two x-ray crystal structures of bovine NPC2 have been reported (pdb codes 1nep and 2hka; 43, 44). Despite extensive investigations, the specific functions of these proteins are not well understood, although a general agreement is developing that functional forms of both proteins are required for normal lipid transport, since a deficiency in either one leads to the NPC phenotype (45-55). Without proper transport of lipids from the lysosomes to other cellular destinations, the abnormal lysosomal accumulation of lipids that characterizes NPC arises. An emerging picture is that a cooperative mechanism exists involving both NPC1 and NPC2 in the transfer of cholesterol to lipid bilayers for transport from lysosomes (56, 57). NPC1 has a sterol-sensing domain analogous to other proteins involved in cholesterol processing (41), and binding of sterols to NPC1 has been demonstrated experimentally (58-60). Likewise, binding of sterols to NPC2 has been investigated (59, 61) and has been confirmed by protein x-ray crystallography (44). Evidence has also been provided that NPC2 interacts directly with membranes to deliver cholesterol, where it may then be transferred to NPC1. This transfer is enhanced by another lysosomal lipid, a lyso-bisphosphatidic acid (Fig. 3) (62, 63).

Further details are required to explain the roles of the NPC1 and NPC2 proteins in the transport of cholesterol, as well as perhaps other lipids (64), and to explain the basis of the NPC phenotype. Over 250 point mutations have been identified throughout the several domains of the NPC1 protein. Whether all of these mutations could directly alter functions such as cholesterol binding is far from clear. There is evidence that some of these mutations may be functional to varying extents, but that misfolding errors lead to missorting, defective trafficking and degradation of the protein in the ER prior to transport to the lysosomal membranes as the normal site of NPC1 localization (65, 66).

Another topic of extensive research for several years has been the identification of the toxic causative agent(s) of the neurodegeneration and other pathological effects seen in NPC, with the focus being especially on whether free cholesterol (7, 67-75) or another of the accumulating lipids, such as a sphingolipid, is the principal factor (76-78). Closely coupled to this issue has been the question of which lipid class initiates the accumulation, whether it be cholesterol (50, 79), sphingolipids, or others (80), followed by secondary accumulation of the other lipid types. These issues have yet to be resolved, as there is evidence supporting each argument. A recent paper reports a study of the correlation between sphingosine accumulation and disruption of calcium distribution required for normal cellular function (81).

SMALL-MOLECULE THERAPEUTIC STRATEGIES

The investigation of possible treatments for NPC has been the subject of several reviews (1, 82-84), including the general reviews of NPC cited above (1-12). At this time, there is no fully effective treatment, let alone a cure for NPC, despite years of effort devoted to finding a suitable therapy based upon small-molecule drugs, modern molecular biological methods and tissue transplantation. Some therapies have been or are under investigation that ameliorate or slow the progression of the disease, but their efficacy is limited. The present review summarizes the recent developments in this very active research area, with particular emphasis on small-molecule treatments organized according to the targeted biochemical pathways.

In the general area of small-molecule drug discovery, traditional medicinal chemistry methods based largely upon trial-and-error testing of compounds are still heavily used, although there is an ever-increasing effort to implement methods of rational drug design based upon identification of cellular targets. Without a firm understanding of the molecular basis of NPC, as summarized above, the required knowledge of potential targets is missing, and therefore the rational design of a small-molecule drug is not yet possible. Once a specific, therapeutically relevant protein or other cellular target does become known through further basic research, then the power of modern computer-aided molecular design (CAMD) may be brought to bear on the problem of drug design. Until that point is reached, other strategies for discovering drugs for NPC must be pursued. These alternative approaches may be hypothesis-driven based on phenotypical cellular function, or they may be based on large-scale screening of compound collections, or so-called compound libraries. A short-term goal in this field is to find a small-molecule treatment by these approaches, whereas a longer-term goal is to develop a curative treatment based upon molecular biological methods such as gene replacement therapy. Tissue transplant does not appear to be an effective strategy.

The previous investigations of small-molecule therapies summarized below are based first upon hypothesis-driven targeting of cellular function and then upon compound screening and other approaches.

Sterol pathway targeting

In the earlier years of NPC research, when the disease was mainly believed to be a cholesterol-based disorder, a number of logical approaches were investigated in attempts to correct the cellular cholesterol overload (1). In one study, 25 human NPC patients were treated with various combinations of the cholesterol-lowering agents (Fig. 4) lovastatin, cholestyramine (a basic anion-exchange resin consisting of a styrene/divinylbenzene copolymer bearing quaternary ammonium chloride groups) and nicotinic acid, as well as dimethyl sulfoxide (DMSO). The first three drugs, but not DMSO, either individually or in combination, lowered liver and plasma cholesterol levels (85). In another study, a 9-month-old patient treated with lovastatin and cholestryamine also showed reduced lipid levels and normal neurodevelopmental measures over 19 months (86). The additional cholesterol-lowering drugs nifedipine and probucol similarly reduced cholesterol in the liver in an NPC mouse model (87). U-18666A is a cholesterol transport-inhibiting agent that is often used to induce an NPC-like phenotype, including apoptotic neuron cell death, and this effect is reduced by administration of pravastatin (88). Although these studies have shown initially promising signs of cholesterol reduction and other positive indications, none of these drug treatments has led to long-term improvements with respect to the onset of neurological symptoms. Likewise, controlled diets to reduce cholesterol have not been successful (82). When investigated in the NPC cat model, neurodegeneration was not altered, and cholesterol storage levels were not reduced significantly (89). In contrast, when neonatal NPC mice were treated with the squalene synthase inhibitor CP-340868, a reduction in cholesterol synthesis in the brain, cholesterol accumulation in neurons and astrogliosis (abnormal astrocyte proliferation) was observed. In addition there was a reduction in galactolipids such as galactoside G_{M3} in the brain, which may inhibit normal myelin maturation (74).

Agents that inhibit intestinal absorption of dietary and biliary cholesterol and other lipids have also been investigated. Cholestyramine is one such agent (see above). Ezetimibe (Zetia™; Fig. 5), which has been used to treat fatty liver disease and hyperlipidemia (84, 90), blocks cholesterol absorption by interfering with the function of the Niemann-Pick C1-like transporter protein (NPC1L1) (91). When studied in NPC mice, ezetimibe reduced liver cholesterol levels and provided evidence that the cell damage seen in NPC is correlated with the free cholesterol content of lysosomes and late endosomes (70).

Cyclodextrins, such as 2-hydroxypropyl-β-cyclodextrin, are able to bind cholesterol and have been shown to lower cholesterol absorption and liver cholesterol content (92). Furthermore, cyclodextrin administration reduces neuronal (Purkinje) cell death and increases the lifespan of NPC mice (93). When a single dose of 2-hydroxypropyl-β-cyclodextrin is given to NPC mice at the age of 7 days (corresponding to prenatal development in humans), lysosomal concentrations of free cholesterol are lowered, esterified cholesterol is increased and cholesterol synthesis is reduced, along with corre-

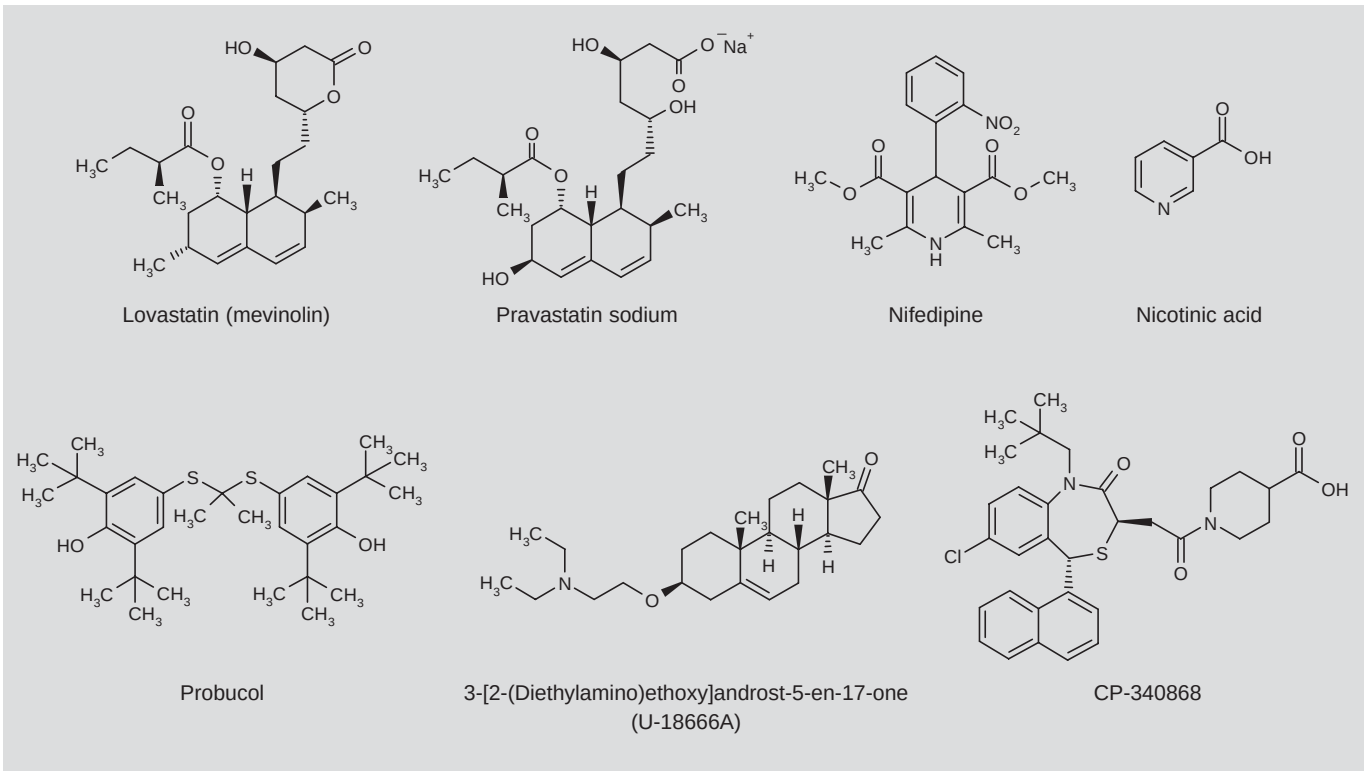

Figure 4. Cholesterol-lowering agents and the cholesterol transport inhibitor U-18666A.

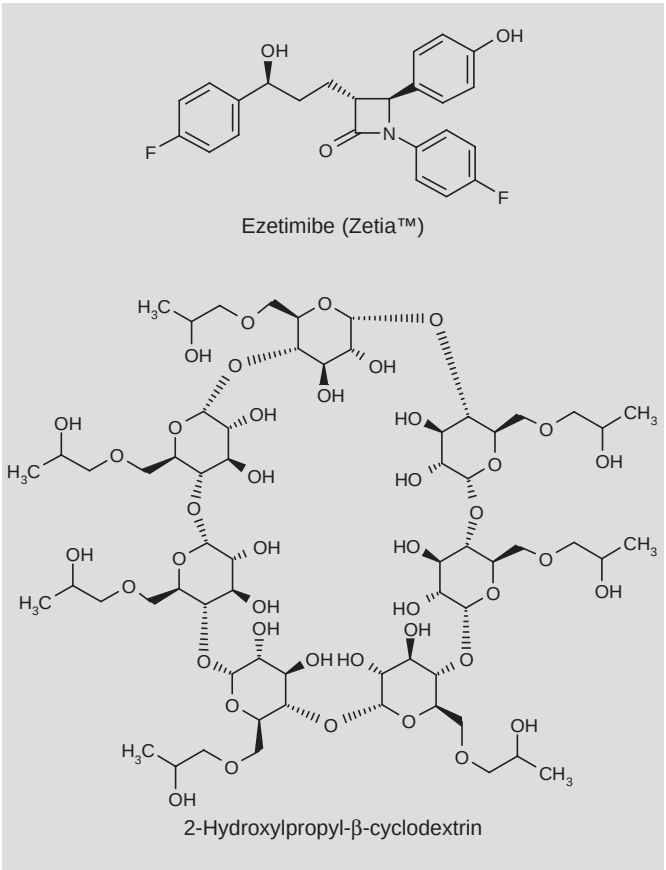

Figure 5. Cholesterol absorption inhibitors.

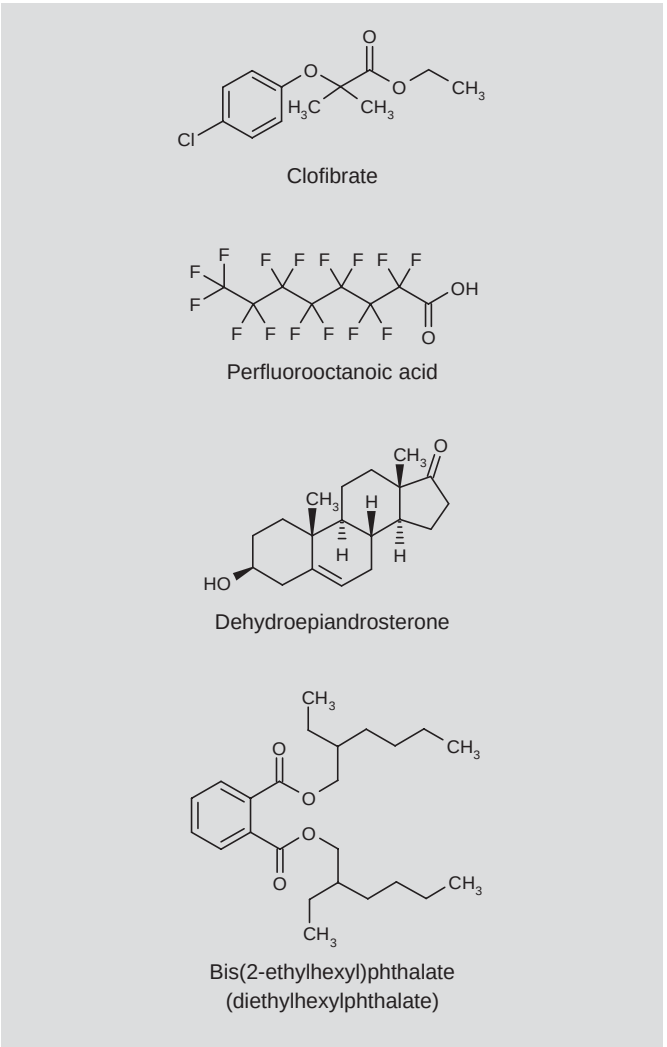

Figure 6. Peroxisomal inducers.

sponding effects on the cholesterol regulatory pathway, decreased expression of inflammatory factors in the liver and brain, and an increase in average lifespan to approximately 120 days compared to 84 days for untreated mice (94). The cyclodextrin may induce these effects by substituting for mutant NPC1 protein and transferring cholesterol to the NPC2 protein to re-establish normal cholesterol trafficking (95). The use of cyclodextrins as cholesterol-mobilizing agents has been suggested as a component of combination therapy for NPC based upon improvement of lipid trafficking (56). This suggestion stems from studies of combination therapies in fibroblasts derived from Gaucher's disease, another lysosomal storage disorder (96).

There is evidence of a peroxisomal deficiency in NPC that may contribute to improper cholesterol processing. This observation has led to the investigation of the peroxisomal inducers clofibrate, perfluorooctanoic acid, dehydroepiandrosterone and diethylhexylphthalate (Fig. 6). When clofibrate and perfluorooctanoic acid were tested in NPC mice, peroxisomal oxidation of cholesterol increased, resulting in a reduction of cellular cholesterol levels (97). However, upon treatment of human NPC fibroblasts with clofibrate, increased cellular cholesterol content was observed (98). The abnormal dolichol levels seen in NPC are not affected by peroxisomal inducers. In NPC mouse liver, peroxisomal inducers increase the level of dolichol *O*-acyltransferase, which catalyzes dolichol esterification, but a peroxisomal inducer such as clofibrate does not increase the amount of dolichol esters. These results suggest that dolichol and/or fatty acids are not properly transported in NPC cells as part of the lipid transport defect (99).

Therapeutic administration of allopregnanolone and other neurosteroids has been heavily investigated (Fig. 7). Allopregnanolone is required for normal neuronal growth and survival and brain development, but is deficient in NPC due to age-correlated decreases in the expression of enzymes in neurosteroid biosynthetic pathways (83, 100). When allopregnanolone is used in NPC mice, with or without 2-hydroxypropyl-β-cyclodetrin (93), neurodegeneration and demyelination are delayed, neuron survival is improved, ganglioside accumulation is reduced and lifespan is significantly increased, but early postnatal administration is required to elicit the most pronounced effects (101, 102). Interestingly, allopregnanolone, its enantiomer *ent*-allopregnanolone and the synthetic oxysterol T-0901317 (93) all exhibited identical effects in NPC mice. The efficacy of T-0901317 is improved when used in combination with allopregnanolone (52). The neuroprotective effect of these compounds correlates with their role as pregnane X receptor (PXR) activators but not with $GABA_A$ receptor activation; among these

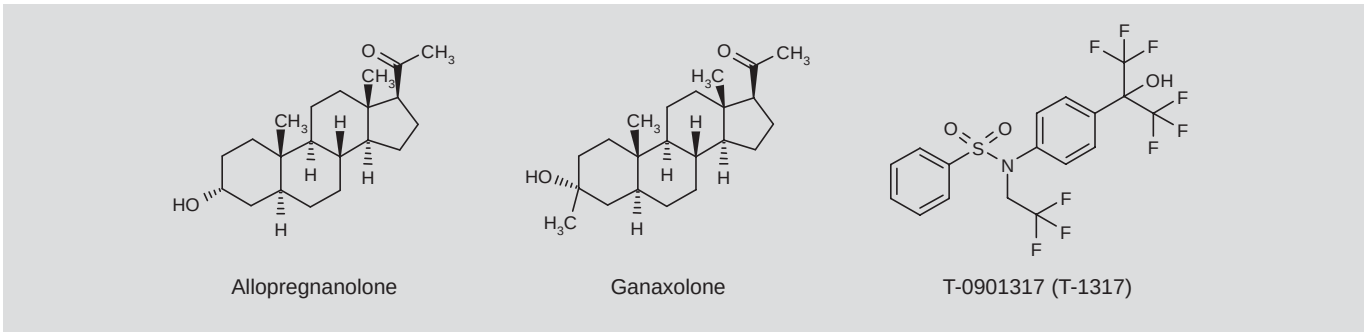

Figure 7. Neurosteroids.

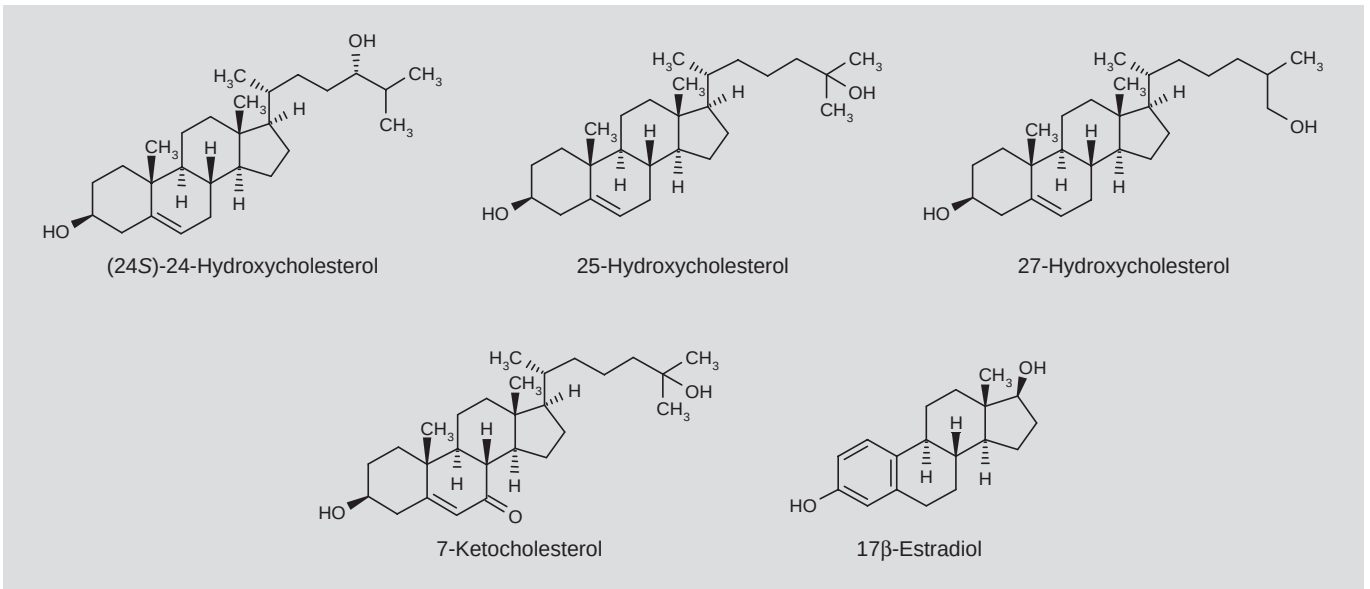

Figure 8. Oxysterols.

compounds, *ent*-allopregnanolone does not have this activity, but they are all PXR activators (103). An x-ray crystal structure of the T-0901317/PXR complex has been obtained (104). The synthetic $GABA_A$-activating neurosteroid ganaxolone produced similar but weaker neurological effects (105). Allopregnanolone has been shown to reduce levels of reactive oxygen species, lipid peroxidation and peroxide-induced apoptosis in human NPC fibroblasts or *NPC1* knockdown cells, which suggests correction of an abnormal cellular redox state as a therapeutic strategy (106). These effects are most pronounced when treatment is initiated at a very early postnatal age, but become less effective with later initiation of treatment.

Various oxysterols (Fig. 8) have been studied for NPC therapy based on the roles these compounds play in regulating cholesterol homeostasis and based on their impaired biosynthesis in NPC (8, 49). One hypothesis is that the NPC proteins are involved in the intracellular transport of free cholesterol to sites of oxidation to produce the oxysterols 25- and 27-hydroxycholesterol. These compounds activate liver X receptor (LXR) transcription factors that regulate sterol regulatory element-binding protein (SREBP) and ATP-binding cassette transporter 1 (ABCA1) involved in intracellular trafficking and the control of cholesterol biosynthesis and the efflux of cholesterol and phospholipids from cells (107-109). A consequence of this flaw in the generation of oxysterols is that NPC cells do not respond properly to LDL-derived cholesterol loading, and the normal regulation of cholesterol levels does not occur (49). Another role in cholesterol homeostasis is suggested by oxidation of cholesterol to oxysterols such as (24*S*)-24-hydroxycholesterol in the brain, and subsequent secretion to provide a pathway for return of excess cholesterol to the liver (46, 110).

Treatment of mutant *NPC1* human fibroblast cells with 25-hydroxycholesterol or 7-ketocholesterol reduces lysosomal cholesterol and increases cholesterol in the ER, which is a normal destination of cholesterol in the homeostasis pathway (111). Administration of 25- and 27-hydroxycholesterol to either mutant *NPC1* or mutant *NPC2* human fibroblast cells also normalizes LDL receptor activity, which is of importance in cholesterol homeostasis (46). Treatment of neonatal mutant *NPC1* mice with 17β-estradiol, the levels of which are reduced in the brain and astrocytes of this mutant strain, results in a delay of neurological symptoms, improved survival of Purkinje

cells and extended lifespan (71). Another observation is that the NPC1 protein binds oxysterols even more strongly than cholesterol (58). Taken together, these data suggest that oxysterols in combination with cholesterol-binding agents such as cyclodextrins may serve to correct the cholesterol-trafficking problems in NPC (56).

Treatment of mutant *NPC1* or mutant *NPC2* human fibroblast cells with the synthetic LXR ligand T-0901317 (see Figure 7) or the nuclear retinoid X receptor (RXR) ligand 9-*cis*-retinoic acid (Fig. 9) increases ABCA1 expression, promotes cellular efflux of cholesterol and lowers lysosomal/endosomal cholesterol (49, 112). The activation of ABCA1 therefore provides a possible compensatory pathway to bypass mutant NPC proteins (112). In normal cells, T-0901317 reduces cholesterol in neurons, which is correlated with an increase in levels of the cholesterol chaperone protein apolipoprotein E (apoE) and an increase in the activity of HMG-CoA reductase, which catalyzes the rate-limiting step of cholesterol biosynthesis (113). The use of T-0901317 in NPC mice resulted in lowered cholesterol levels in the brain, decreased nerve cell inflammation, improved neurological function, decreased loss of Purkinje cells and increased lifespan (114). In a separate study, the synthetic RXR ligand bexarotene was found to have potential for protecting against atherosclerosis by improving cholesterol distribution (115), a finding which suggests that this agent should also be investigated in NPC.

Sphingolipid pathway targeting

Based on the knowledge that sphingolipids are major components of the abnormal lysosomal lipid accumulation that occurs in NPC, many potential therapeutic strategies have been directed at the regulatory pathways for this lipid class. Most prominent has been miglustat (*n*-butyldeoxynojirimycin, NB-DNJ, Zavesca) and the closely related *n*-butyldeoxygalactonojirimycin (NB-DGJ; Fig. 10). These compounds are glucosylceramide synthase inhibitors in the glycosphingolipid biosynthesis pathway. The use of agents that reduce the biosynthesis of glucosylceramides, which are precursors of more complex glycosphingolipids, is often called substrate reduction therapy in the context of lysosomal storage disorders (13, 116-118). Due to its ability to cross the blood–brain barrier, miglustat may therefore be suitable for the treatment of neurological diseases. Miglustat first received European Union (EMEA) and U.S. FDA approval for the treatment of Gaucher's disease (119, 120) and has been evaluated in long-term human trials for NPC and Tay-Sachs disease. In early 2009, miglustat was approved for the treatment of NPC in the European Union (121).

Exposure of mutant *NPC1* Chinese hamster ovary (CHO) cells to miglustat reverses abnormal lipid trafficking (76). When NPC mice and cats are treated with miglustat, neurological symptoms are delayed, ganglioside accumulation is reduced and lifespan is increased by 1-2 months (50, 122). In human NPC patients, glycosphingolipid levels are reduced, correction of lipid trafficking is seen and improvements in brain function, swallowing, eye movement, body movement, hearing, cognition, attention, and liver and spleen function are observed (123-127). As compared to young children, patients diagnosed in late childhood or as juveniles or adults show greater benefits of miglustat treatment. Patients have tolerated treatment well in these trials, and beneficial effects persist throughout trial periods lasting as long as 66 months. Side effects include diarrhea, flatulence, nausea, abdominal pain, abdominal distension, decreased appetite, weight loss and tremor (126, 128-130).

The pharmacokinetics of the drug have also been studied (131). At least some of the positive effects of miglustat appear to be limited in duration, with progression of dementia and body motion problems seen over several months of treatment (132). The favorable effects that are seen for miglustat on neurological symptoms have been used as one of the arguments that sphingolipid accumulation is the basis of the cell toxicity that occurs in NPC.

A number of other compounds that function in the sphingolipid pathway have been investigated from various perspectives. Several lipophilic adamantyl-substituted analogues of miglustat have been synthesized and compared directly with the parent compound. The adamantyl derivative depicted in Figure 10 has the highest potency of these analogues as a glucosylceramide synthase inhibitor and is more potent than miglustat itself. These compounds have been suggested as treatments for sphingolipid disorders such as Gaucher's disease, but have not yet been studied in NPC (133). The additional compounds NB6, D-609 and AD-2765 have been studied in other sphingolipid pathway contexts (77, 134-136) and may be of interest in future NPC investigations.

In one of the cellular studies that suggested that sphingosine accumulation is the initiating factor in NPC, sphingosine storage in acidic compartments (lysosomes) was correlated with reduced calcium levels in these compartments and altered calcium homeostasis, which in turn led to accumulation of sphingomyelin, glycosphingolipid and cholesterol. Treatment of mutant *NPC1* mouse glial cells with curcumin, which increases calcium levels in the cytosol, where it is required by calcium-dependent proteins involved in cellular trafficking, corrects the lipid accumulation defect. In NPC mice, curcumin leads to improved weight gain, improved body movement, slower disease progression and longer lifespan. Curcumin is the bright yellow natural component of the curry spice turmeric. A side effect of its use in mice is that the animals become noticeably yellow in appearance. Thapsigargin treatment of mutant *NPC1* CHO cells likewise demonstrated a lipid correction effect, but it was relatively toxic compared to curcumin. Correction of the lipid phenotype and lysosomal calcium levels in mutant *NPC1* human fibroblast cells was also observed upon reduction of sphingosine with the serine C-palmitoyltransferase inhibitor myriocin (ISP-1) (81).

Apoptosis inhibitors

Studies of the neurodegenerative effects in NPC have suggested possible therapeutic roles for antiapoptotic agents (137, 138). The apoptosis-promoting c-ABL/p73 system is expressed in the brain of NPC mice. Upon treatment with the well-known c-ABL inhibitor imatinib (Gleevec®; Fig. 11), the mice exhibit improved Purkinje cell survival, reduced apoptosis in the brain, reduced neurological symptoms, improved body movement and longer lifespan (139). These results are in contrast with studies of minocycline and the Bcl-2 protein, which are known to inhibit caspase-1 activation in the apoptotic pathway, but which do not lead to neurological improvement in NPC mice (140). A study of the protection of neurons from apoptotic cell death by taurine, a very simple inhibitor of caspase-9, is suggestive of further studies of antiapoptotic agents in NPC (69).

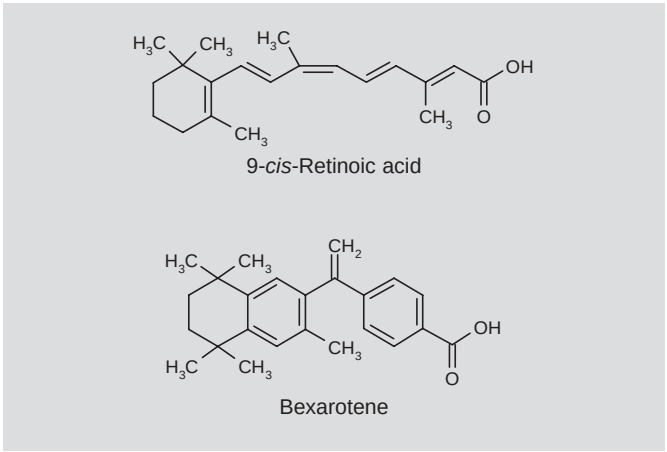

Figure 9. Nuclear retinoid X receptor (RXR) ligands.

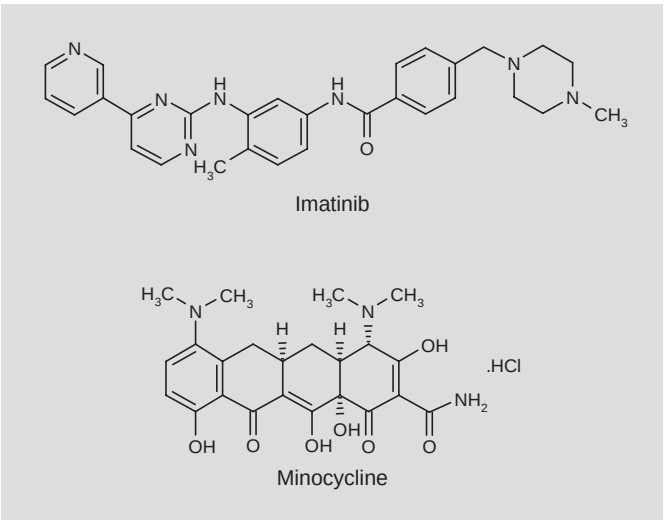

Figure 11. Apoptosis inhibitors.

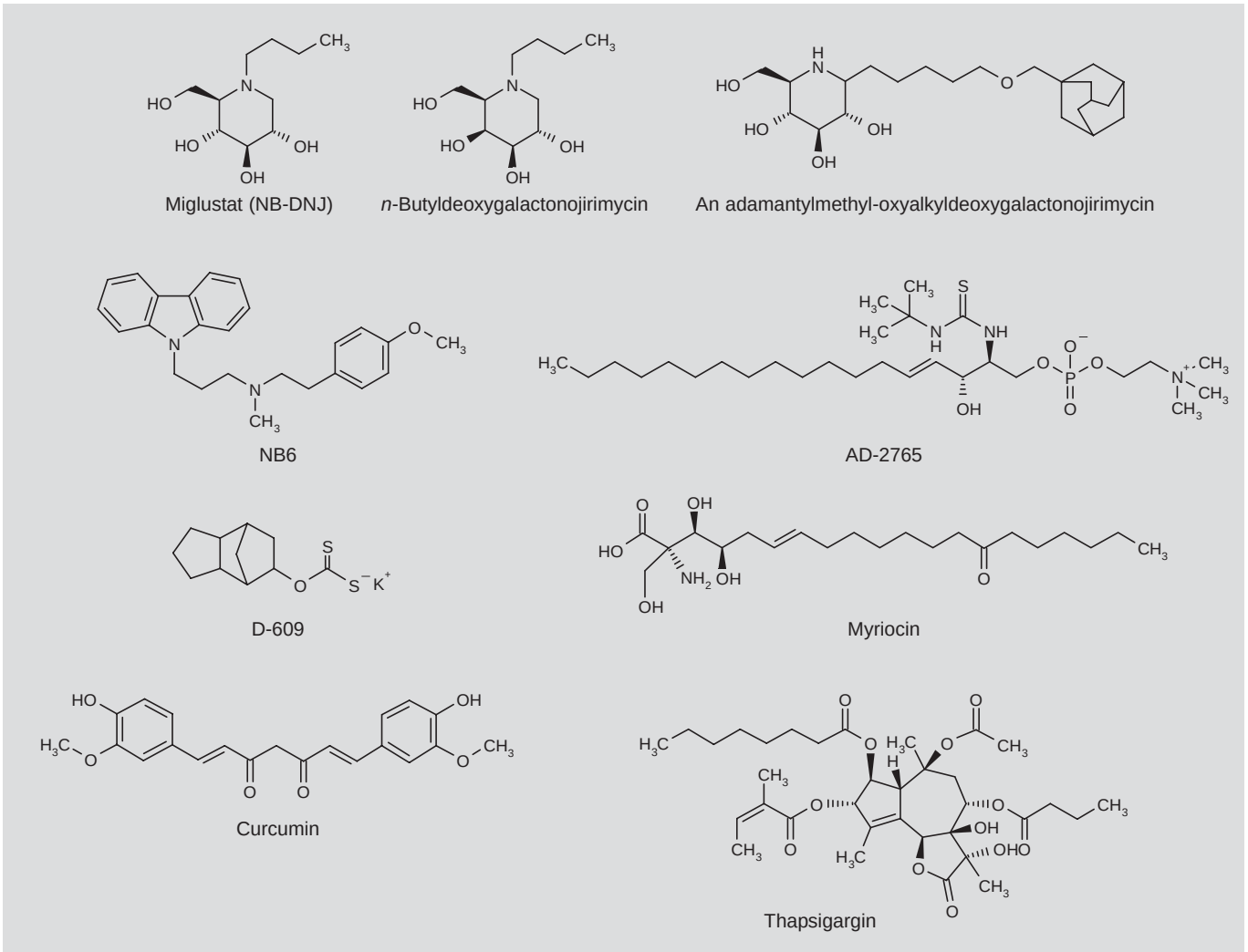

Figure 10. Sphingolipid pathway-targeting agents.

Neurodegeneration inhibitors and neurotrophic factors

Compounds that function as neurodegeneration inhibitors, neurotrophic factors or neurogenesis agents (Fig. 12) have been considered for the treatment of many neurodegenerative diseases, such as Alzheimer's disease (141, 142). Although NPC has not been included in most of these studies, these compounds may have worthwhile potential for future studies in this context as well. As one example, N^{ω}-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase (NOS) inhibitor, has been investigated due to the increased production of NO in neural stem cells in NPC mice (143). NO is associated with a reduced self-renewal ability of neural stem cells, neurodegeneration and activation of both glycogen synthase kinase 3β (GSK-3β) and capsase-3. Treatment of NPC mice with L-NAME restored neural stem cell self-renewal and inhibited GSK-3β and caspase-3. These results suggest NO regulation as a therapeutic target for NPC. GSK-3 has been widely investigated as a potential therapeutic target for neurodegenerative diseases in general (144). A few of the recently studied inhibitors include lithium ion (145, 146), SB-415286 and analogues (147-149), and cimetidine (150).

(*R*)-Roscovitine, aloisine and indirubin have been examined briefly in the context of being inhibitiors of the cyclin-dependent kinase CDK5, which is overexpressed in NPC and is associated with neurodegenerative pathways in this and other diseases (151, 152), thus pointing towards future therapeutic studies of these compounds. Jiadifenin and synthetic analogues (153), nankakurines (154) and tricycloillicinone (155) are examples of neurotrophic factors that have been studied recently in the broader context of neurodegenerative diseases, and neurodazine is a recently identified neurogenesis agent (156).

Histone deacetylase inhibitors

Many additional classes of compounds have been explored in miscellaneous approaches to developing NPC therapies. Among these compounds are histone deacetylase (HDAC) inhibitors (Fig. 13), which in recent years have been recognized for their importance as small-molecule regulators of gene expression in the growing field of epigenetics (157). HDAC inhibitors have been studied especially heavily for the treatment of cancers, with vorinostat (suberoylanilide hydroxamic acid, SAHA, Zolinza®) being the first such agent approved by the FDA for the treatment of cutaneous T-cell lymphoma patients (158). However, there are also considerable data indicating the therapeutic potential of HDAC inhibitors for many noncancerous diseases, including several rare diseases and neurological disorders (159, 160). Among the simplest but least potent HDAC inhibitors are butyric acid, 4-phenylbutyric acid and valproic acid, whereas vorinostat, curcumin (see Figure 10) (161) and trichostatin A (TSA) have much greater activities. In some general non-

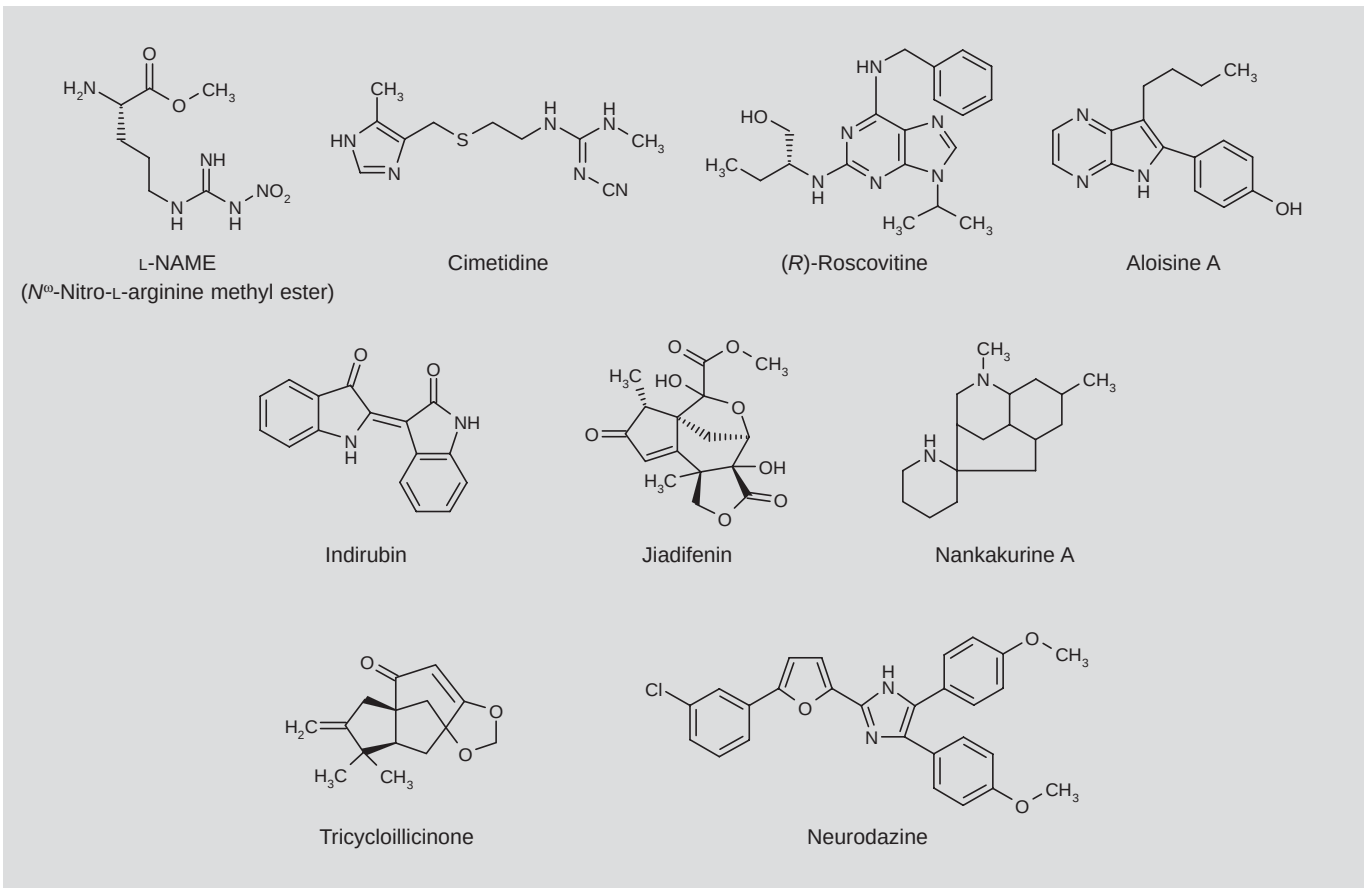

Figure 12. Neurodegeneration inhibitors, neurotrophic factors and a neurogenesis agent.

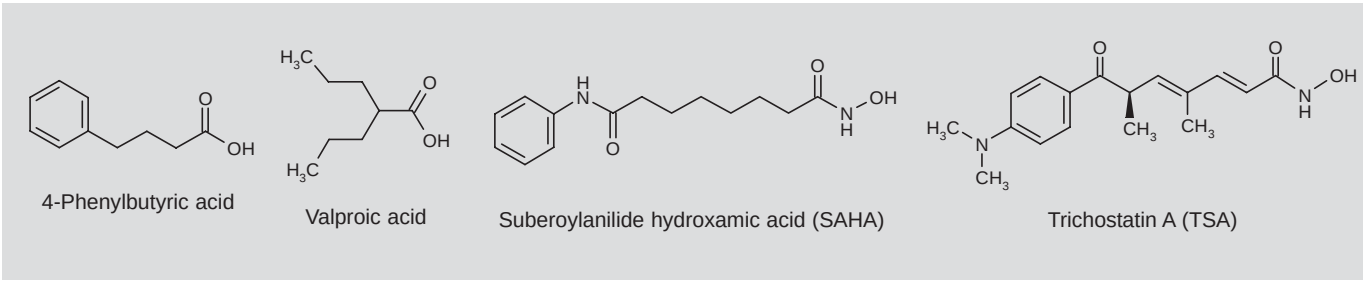

Figure 13. Histone deacetylase (HDAC) inhibitors.

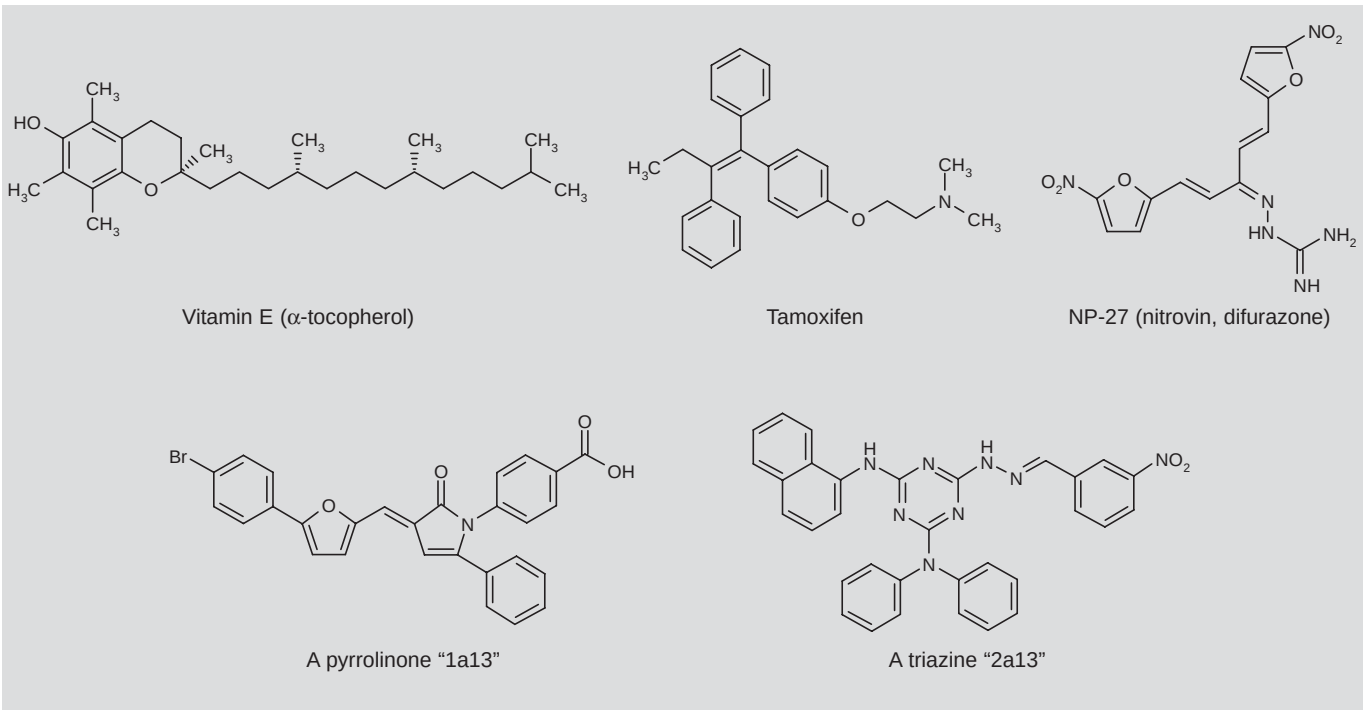

Figure 14. Other compounds investigated for NPC therapy.

NPC studies, TSA was found to promote regulation of cholesterol biosynthesis (162) and of cholesterol 24-hydroxylase (163). Studies of HDAC inhibitors specifically in NPC have been very limited to date. In one investigation, butyrate and TSA increased the expression of the *NPC1* gene in mouse adrenal tumor cells (164). The possible clinical relevance of this finding is that, in at least some cases of NPC, there may be small residual *NPC1* activity, which might be sufficient to delay disease onset upon upregulation (123). An alternative perspective is that increased expression of misfolded mutant NPC proteins or of proteins in compensatory pathways for lipid regulation could produce a therapeutic effect (56). Firmer indications are provided by a study of mutant *NPC1* mice whereby treatment with even the weak HDAC inhibitor valproic acid led to a reduction of cholesterol accumulation in neural stem cells, upregulation of neurotrophic genes and restoration of neuronal differentiation (165). The more potent vorinostat and TSA produced a very pronounced lowering of cholesterol accumulation in mutant *NPC1* CHO cells (166). A more comprehensive study of HDAC inhibitors in NPC is clearly in order.

Other classes of compounds

Additional compounds of miscellaneous structural classes have been the subject of various NPC studies, most of which have shown only limited if any therapeutic potential (Fig. 14). These compounds include vitamin E (α-tocopherol) and tamoxifen (167), and DMSO (168-171).

Further classes of compounds have been identified for potential use in NPC by screening of compound libraries, which is a common practice in drug discovery in general when therapeutic targets are not well understood for a given disease, in which case rational design or selection of drug candidates is not feasible. One such screen of over 40,000 compounds in mutant *NPC1* mouse ovarian granulosa cells led to NP-27 (nitrovin, difurazone), which restored cholesterol homeostasis by stimulating cholesterol transport and cholesterol esterification (172). Because NP-27 was found to be too toxic for additional studies, the investigators suggested the need to develop nontoxic analogues that retain cholesterol homeostasis activity. The mecha-

nism of action in this regard is not understood, but NP-27 was previously developed as a veterinary growth promoter used as a feed additive that affects aldosterone production and release (173).

In another library screening of over 20,000 compounds in mutant *NPC1* CHO cells, several compounds were found that lowered lysosomal cholesterol accumulation (174). The most potent compounds included a series of pyrrolinones (e.g., library accession no. 1a13; Fig. 14) and a series of triazines (e.g., library accession no. 2a13). The cellular basis for the observed activity is not understood, but interestingly, the pyrrolinone 1a13 had previously been found in an independent library screening to be an inhibitor of the glycosyltransferase murG, which functions in a key step of bacterial cell wall synthesis (175). There is no indication yet of whether a similar mode of action on sphingolipid or other lipid glycosylation may be involved in the effect seen for 1a13 in NPC cells. Also to be noted is a structural resemblance between 1a13 and the neurogenesis agent neurodazine (see Figure 12).

CONCLUSIONS AND FUTURE PROSPECTS

There is no demonstrated therapy providing an effective, long-term treatment and rescue of the NPC phenotype. Compounds such as miglustat, allopregnanolone, oxysterols and cyclodextrins show promise in slowing the progression of the disorder, but these and other compounds are still far from providing the type of treatment, let alone a cure, that is desired for this devastating disease.

Along with other drug development efforts, screening of compound libraries continues to be an ongoing activity in several laboratories. Whether these efforts will provide the desired treatment may perhaps not be as important as the discovery of active compounds that may serve as the basis for identifying new cellular targets. If therapeutic targets were to be identified by this type of screening or by other cell-based studies, then the power of modern rational drug design could be applied to NPC.

The general concept of proteostatic regulation has been suggested as a means of disease control based upon maintaining normal protein function (176). For example, compounds may exist that could serve as molecular chaperones to promote and maintain the normal folding of mutant NPC proteins. The suggestion has been made that oxysterols could serve in this capacity, since NPC1 binds to them more tightly than to cholesterol (56).

The development of a useful small-molecule therapy, no matter how effective, is unlikely to provide a true cure for a genetically based disease such as NPC. At best, such a therapy could be life-saving but would likely require life-long administration to offset the severe effects of the disease. Therefore, in a longer-range perspective other treatment strategies must also be considered. These alternatives include tissue or organ transplant (177-183), stem cell therapy (184-189), enzyme replacement or supplementation therapy (118) and gene replacement therapy to restore or improve the expression of NPC proteins or other proteins such as Rab GTPases affecting lipid pathways (190-203). Several versions of these approaches have been investigated with mixed results, not only for NPC but also for NPA, NPB, Gaucher's disease and other lipid storage disorders. As the required technology improves over time, gene replacement therapy could ideally provide an actual cure for NPC, but current general limitations on safe and effective gene delivery must first be overcome. Related issues also complicate the use of enzyme replacement or supplementation therapy, although a recent application of new technology is the use of nanocarriers to improve enzyme delivery (204). Until additional technological advances are made, small-molecule drug development will continue to be pursued, along with epigenetic therapy to regulate gene expression and the other strategies discussed in this review.

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