Functional Inhibitors of Acid Sphingomyelinase (FIASMAs): A Novel Pharmacological Group of Drugs with Broad Clinical Applications

Johannes Kornhuber, Philipp Tripal, Martin Reichel, Christiane Mühle, Cosima Rhein, Markus Muehlbacher, Teja W. Groemer and Erich Gulbins[1]

Department of Psychiatry and Psychotherapy, University of Erlangen, [1]Department of Molecular Biology, University of Duisburg-Essen

Abstract
Acid sphingomyelinase (ASM) is an important lipid-metabolizing enzyme cleaving sphingomyelin to ceramide, mainly within lysosomes. Acid ceramidase (AC) further degrades ceramide to sphingosine which can then be phosphorylated to sphingosine-1-phosphate. Ceramide and its metabolite sphingosine-1-phosphate have been shown to antagonistically regulate apoptosis, cellular differentiation, proliferation and cell migration. Inhibitors of ASM or AC therefore hold promise for a number of new clinical therapies, e.g. for Alzheimer's disease and major depression on the one hand and cancer on the other. Inhibitors of ASM have been known for a long time. Cationic amphiphilic substances induce the detachment of ASM protein from inner lysosomal membranes with its consecutive inactivation, thereby working as functional inhibitors of ASM. We recently experimentally identified a large number of hitherto unknown functional inhibitors of ASM and determined specific physicochemical properties of such cationic amphiphilic substances that functionally inhibit ASM. We propose the acronym "FIASMA" (Functional Inhibitor of Acid SphingoMyelinAse) for members of this large group of compounds with a broad range of new clinical indications. FIASMAs differ markedly with respect to molecular structure and current clinical indication. Most of the available FIASMAs are licensed for medical use in humans, are minimally toxic and may therefore be applied for disease states associated with increased activity of ASM.

Acid sphingomyelinase and acid ceramidase

Acid sphingomyelinase (ASM, EC 3.1.4.12) is a lysosomal glycoprotein that catalyses the hydrolysis of sphingomyelin into ceramide and phosphorylcholine. Translocation of lysosomal ASM to the cell surface plays an important role during stress response [1]. CD95 ligands

Johannes Kornhuber, MD
Department of Psychiatry and Psychotherapy, University of Erlangen
Schwabachanlage 6, 91054 Erlangen (Germany)
Tel. +49-9131-853-4160, Fax +49-9131-853-4862
E-Mail Johannes.Kornhuber@uk-erlangen.de

and cytokines such as tumor necrosis factor-α, interleukin-1 and interferon-γ but also environmental stimuli including oxidative stress, reactive nitrogen species, ionizing radiation, ultraviolet-C radiation, heat shock and other agents of stress, injury or infections have been shown to stimulate ceramide production [2-16]. Ceramide consecutively leads to membrane reorganization involving membrane rafts [10, 17] and downstream signalling that may result in apoptosis. In addition to ASM, at least three other sphingomyelinases have been described in mammalian cells [18-20] that vary in their pH optimum, cofactor dependency and subcellular localization. Although these enzymes and an existing *de novo* synthesis pathway are alternative mechanisms for ceramide generation, activation of ASM is critical for at least some cellular responses, such as apoptosis induced by reactive nitrogen species [4], radiation [5] and CD95 [21].

Ceramide is further metabolized to sphingosine and sphingosine-1-phosphate by acid ceramidase (AC, EC 3.5.1.23) and sphingosine kinases. The specific activity of AC was found to be low in comparison with ASM in different tissues including the human brain. AC appears to be more abundant in human placenta than ASM [22-33]. The relative abundance and specific activity of ASM versus AC in mammalian lysosomes is not currently known.

While the biological function of sphingosine is largely unknown, sphingosine-1-phosphate has been shown to be involved in cellular differentiation, proliferation and cell migration [34-38]. The balance between ceramide and sphingosine-1-phosphate is referred to as the "ceramide/sphingosine-1-phosphate rheostat" [39-41], which regulates a proper balance between cell death and growth (Fig. 1).

Altered activity of ASM in human disease states

ASM is best known for its involvement in Niemann-Pick disease, where a heritable deficiency of this enzyme leads to the emergence of a lysosomal storage disorder [42]. Pathologic reduction of ASM activity is caused by mutations in the ASM gene itself or in a gene that encodes NPC-1, a protein indirectly regulating ASM activity [43]. The severity of Niemann-Pick disease correlates with the decrease of ASM activity [44]. However, studies using cells derived from Niemann-Pick disease patients or from ASM knock-out mice revealed that a deficiency of this enzyme might also have beneficial con-

sequences. There is now increasing evidence that activation of ASM and ceramide accumulation play a central role in the development of common human diseases (for review see [45, 46]).

Potential clinical indications of ASM inhibitors

From a theoretical point of view, there are broad clinical applications for inhibitors of ASM. Agents that reduce ASM activity and thereby also ceramide levels tend to attenuate receptor-mediated apoptosis, stress stimuli-induced apoptosis as well as growth factor-deprivation-mediated apoptosis and promote cell proliferation [47-52]. Thus, ASM-inhibitors potentially have anti-apoptotic and neuroprotective effects and may therefore be used in the treatment of disorders such as brain ischemia, stroke [12, 53], ethanol-induced neuronal cell death [54, 55], Alzheimer's dementia [22, 56, 57], Parkinson's disease, Chorea Huntington, spinal cord injury [58], seizure disorder [59], glaucoma, and to protect against neurodegeneration occurring in multiple sclerosis. Furthermore, such drugs should prevent the radiation- and chemotherapy-induced lethal gastrointestinal syndrome [8], and should be helpful in the endotoxic shock syndrome [60], in severe sepsis [61] and in liver cell death and anaemia occurring in Wilson's disease [62]. The accumulation of cellular ceramide observed in cystic fibrosis [63] may be successfully prevented by functional inhibitors of ASM [64, 65](Gulbins *et al.*, this issue). ASM inhibition suppresses lipopolysaccharide-mediated release of inflammatory cytokines from macrophages [66] and blocks induction of matrix metalloproteinase-1 [67] indicating a possible preventive or therapeutic role for ASM-inhibitors in inflammatory bowel disease. ASM-dependent production of ceramide plays a key role in the generation of pulmonary edema in acute lung injury [68] as well as in lung emphysema [69]. Inhibitors of ASM might thus be of therapeutic value in acute and chronic lung injury. In a pilot study, increased ASM activity was found in patients suffering from major depression [70]. ASM-inhibitors might therefore contribute to antidepressant effects [71]. In addition, ASM-inhibitors might be helpful to attenuate morphine anti-nociceptive tolerance [72]. ASM is essential for infection with *Neisseria gonorrhoeae* [73]. Moreover, ASM is involved in the formation of atherosclerotic plaques [46, 74-76]. In summary, ASM inhibitors hold promise for a number of new clinical therapies [47-52, 70, 77].

Kornhuber/Tripal/Reichel/Mühle/Rhein/Muehlbacher/Groemer/Gulbins

Fig. 1. The "ceramide/sphingosine-1-phosphate rheostat". ASM catalyses the degradation of sphingomyelin to ceramide and thus mediates pro-apoptotic effects (red). AC further metabolizes ceramide to sphingosine which is phosphorylated to sphingosine-1-phosphate by sphingosine kinases. AC thus mediates anti-apoptotic effects (blue). FIASMAs (functional inhibitors of acid sphingomyelinase) induce functional inhibition of ASM. Some FIASMAs, like desipramine, may also functionally inhibit AC.
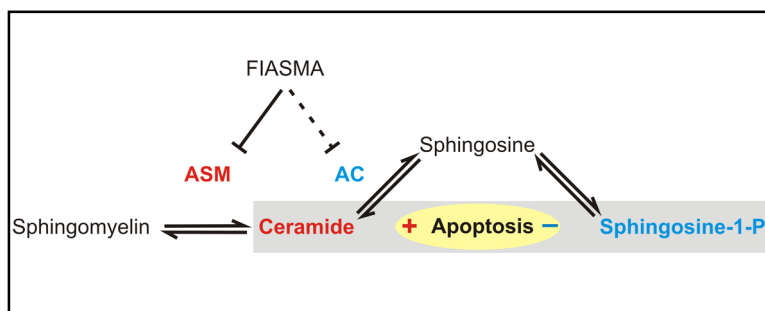
Currently available inhibitors of ASM

Direct inhibitors of ASM

A high throughput screening for direct ASM inhibitors was unsuccessful in finding tractable lead structures [78]. The rational development of compounds that block ASM by direct interaction with the enzyme is difficult, since the crystal structure of the enzyme is not available. Therefore, only few examples of inhibitors directly interacting with ASM are currently known. These substances include physiological inhibitors of ASM such as phosphatidyl-myo-inositol-3,4,5-triphosphate [79], L-α-phosphatidyl-D-myoinositol-3,5-biphosphate [80], compounds isolated from plants, such as α-mangostin [81, 82] and non-natural direct inhibitors of ASM, such as SMA-7 [66], AD2765 [83] and synthetic phosphoinositide analogues [84]. Several biphosphonates are potent and selective inhibitors of ASM [85], among them zoledronic acid, which is clinically used in the treatment of osteoporosis. It is unclear at present whether or not clinically-used biphosphonates work as ASM inhibitors *in vivo* and in therapeutic concentrations. Very recently, potent drug-like direct inhibitors of the ASM have been identified (C. Arenz, pers. communication). In contrast to functional inhibitors (see below) direct inhibitors do not need high lysosomal drug concentrations as a precondition for inhibition of ASM. This might have advantages and disadvantages in contrast to functional inhibitors of ASM. For further details on direct inhibitors of ASM see Arenz, this issue.

Functional inhibitors of ASM (FIASMAs)

Since the 1970s it has been shown that weak organic bases such as desipramine have the potential to inhibit the activity of ASM [70, 86-88]. It has been suggested that ASM is bound to intra-lysosomal membranes, thereby being protected against proteolytic inactivation. Desipramine and related drugs result in detachment of the ASM from the inner membrane [89] and its subsequent inactivation possibly by proteolytic degradation [90].
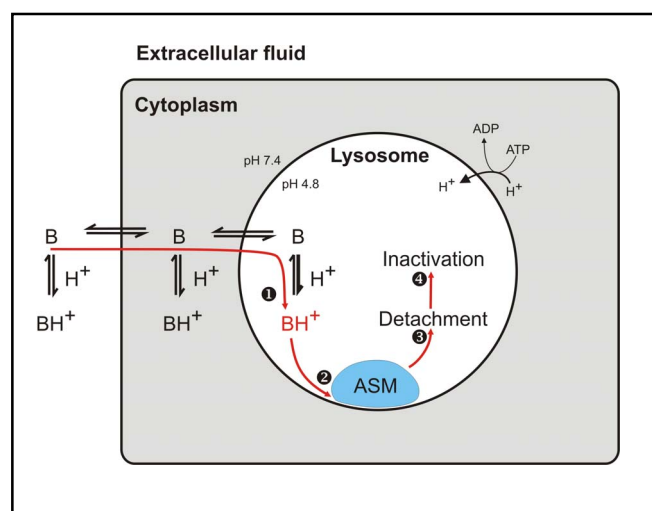

Fig. 2. Drug-induced functional inhibition of ASM is the result of lysosomal accumulation and detachment of ASM from the inner lysosomal membrane. The figure shows a schematic model of how FIASMAs (functional inhibitors of acid sphingomyelinase) and other lipophilic weak bases cumulate intra-lysosomally, thereby functionally inhibiting ASM. A low lysosomal pH is maintained by an ATP-driven proton pump. (1) Weak bases (B) cumulate in intracellular acidic compartments because the lysosomal membrane is much less permeable for the charged protonated bases (BH^+) compared to the uncharged form, a phenomenon called lysosomotropism. Substances with high logP- and high pKa-values are highly concentrated intra-lysosomally. The enzyme ASM is attached by electrostatic forces to the inner lysosomal membrane, thereby being protected against proteolysis. ASM is active in the membrane-bound form and degrades sphingomyelin to phosphorylcholine and ceramide. (2) High concentrations of the protonated bases disturb the binding of ASM to the inner lysosomal membrane and result in detachment of ASM (3) and subsequent inactivation (4), possibly involving proteolysis [89]. The figure is taken from Kornhuber *et al.* 2008 [91].

Weak bases, therefore, do not directly inhibit ASM, but result in a functional inhibition of ASM. We propose the acronym FIASMA (Functional Inhibitor of Acid SphingoMyelinAse) for a compound of this large group
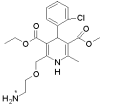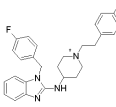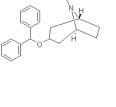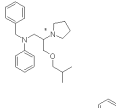
Table 1. Molecular structures of newly discovered FIASMAs (functional inhibitors of ASM) [91].
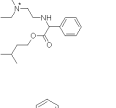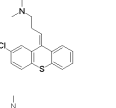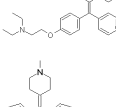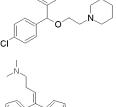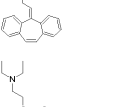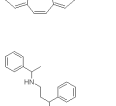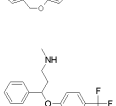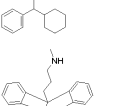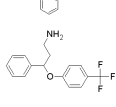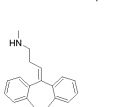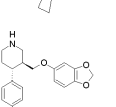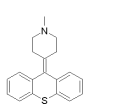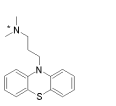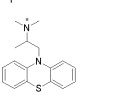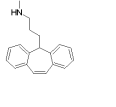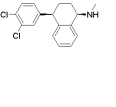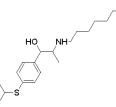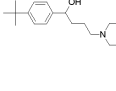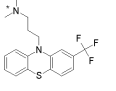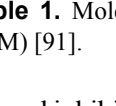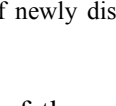
Amlodipine, Astemizole, Benztropine, Bepridil, Camylofin, Chlorprothixene, Clomiphene, Cloperastine, Cyclobenzaprine, Cyproheptadine, Doxepine, Drofenine, Fendiline, Fluoxetine, Maprotiline, Norfluoxetine, Nortriptyline, Paroxetine, Pimethixene, Promazine, Promethazine, Protriptyline, Sertraline, Suloctidil, Terfenadine, Triflupromazine

of drugs. According to this model, functional inhibition of ASM depends on high lysosomal concentrations of a weak basic drug (Fig. 2).

Recently, we identified several novel FIASMAs (for example dextromethorphan, fluoxetine, maprotilin, nortriptyline, orphenadrine, sertralin and triflupromazin, Table 1, [91]), most of them US Food and Drug Administration (FDA) approved known bioactive compounds, most likely to be minimally toxic and potentially rapidly available for clinical use. These novel FIASMAs enabled us to develop a qualitative property-activity relations model for functional inhibition of ASM by cationic amphiphilic compounds [91].

Inhibition of AC

Inhibition of AC might have anti-cancer effects [92-94]. Only few data are available for the pharmacological inhibition of AC. A number of direct AC inhibitors has been identified [92, 95-99]. These substances are lipid derivatives, which are active in cell culture models. Some of these drugs are also active *in vivo* [100]. A systematic investigation of drug-like non-lipid functional inhibitors of AC is completely lacking so far.

It has been suggested that ASM and AC together may form a tightly-associated complex which can be co-purified under *in vitro* conditions [25]. Whether or not such complexes exist under natural conditions is not presently known. There is parallel increase of AC and ASM activity in the brain of Alzheimer's disease patients which is a further hint for parallel regulation of AC and ASM [22]. On the other hand, the regulation by saposins differs between AC [101] and ASM [102], arguing for independent regulation of the two enzymes. The concentration of ceramide after application of lysosomotropic weak bases thus depends on the balance between inhibition of ASM and AC (Fig. 1). AC, similar to ASM, might be functionally inhibited by cationic amphiphilic agents like desipramine, chlorpromazine and chloroquine [103], resulting in an increased level of cellular ceramide, at least in the tumour cell lines investigated.

Kornhuber/Tripal/Reichel/Mühle/Rhein/Muehlbacher/Groemer/Gulbins

Properties of FIASMAs

Currently available FIASMAs have a number of interesting physicochemical, pharmacological and clinical properties.

Structural and physicochemical properties
Structural diversity. Using Tanimoto-coefficients based on 2D-fingerprints [104] we found that FIASMAs are structurally very diverse (Kornhuber *et al.*, unpublished), meaning that functional inhibition of ASM does not require very specific structural preconditions. FIASMAs include for example mono-, bi-, tri- and tetracyclic compounds (Table 1). The clinician has, therefore, the possibility to choose among a large structural variety of compounds, which is important when aiming to avoid drug interactions and unwanted side-effects.

Specific physicochemical properties. Instead of special structural prerequisites, functional inhibition of ASM requires specific physicochemical characteristics of compounds, resulting in high intra-lysosomal concentrations and partition of the drug into the inner leaf of the lysosomal membrane (Fig 2). All of the hitherto identified FIASMAs possess at least one basic nitrogen atom, have a moderate to high logP-value and most of them have a molecular weight below 500. Bupivacain, domperidone, droperidol and fluspirilene are lipophilic weak bases. Nevertheless, they do not inhibit ASM [91] (Kornhuber *et al.*, unpublished), meaning that not all lipophilic weak bases are FIASMAs. However, there are other specific physicochemical preconditions, like a high sum of partial charges at nitrogen atoms, that allow a highly accurate qualitative *in silico* prediction of functional inhibition of ASM (Kornhuber *et al.*, unpublished). A consequence of these physicochemical characteristics is the fact that FIASMAs more frequently violate the Lipinski-Rule-of-5 [105] than compounds without effect on ASM (Kornhuber *et al.*, unpublished).

Pharmacokinetic and pharmacodynamic properties
Favourable ADME properties. To be an effective drug, a compound must be not only active against a target, but also possess appropriate ADME (Absorption, Distribution, Metabolism, and Excretion) properties. Most of the available FIASMAs have favourable ADME properties. All of these drugs are orally active and many of them cross the blood-brain barrier (Kornhuber *et al.*, unpublished). Many FIASMAs may therefore be used for treatment or prevention of diseases of the central nervous system.

Large differences in lysosomal uptake characteristics. The cellular uptake kinetics differ markedly between the FIASMAs. In cell culture systems, FIASMAs enter the cells and lysosomes within minutes to many hours, depending on the logP- and pKa-values. These experimental findings are supported by a single cell simulation [106]. In human patients, the time to reach plateau values in tissue is even much longer (see below). When looking at the group of experimentally investigated FIASMAs, there is no significant correlation between calculated lysosomal concentration of a FIASMA and residual ASM activity. The following FIASMAs have a fast lysosomal uptake kinetic (equilibrium within 30 min) and a moderate lysosomal accumulation (accumulation ratio lysosome:extracellular < 100:1) according to the single cell model [106]: benztropine, desipramine, fluoxetine, maprotiline, paroxetine, protriptyline.

Long time to reach tissue plateau concentrations in humans. Plateau concentrations of some of the FIASMAs may only be reached after prolonged application in humans [107]. The reason for the long time required to reach plateau tissue concentrations in humans compared to *in vitro* cell culture models is probably the low ratio between the amounts of drug administered and the total volume of the storage compartment.

High apparent volume of distribution. The special physicochemical properties of FIASMAs, namely weak basicity and high lipophilicity, result in extensive tissue binding, which is evidenced by the high apparent volume of distribution of these drugs in humans [77, 108].

Biological and behavioural effects mediated via ASM. In animal and cell culture experiments, there is a high degree of congruence between behavioural or biological effects induced by FIASMAs or genetic deficiency of ASM [62, 63, 73, 109-114]. This indicates that ASM is a critical mediator of the biological or behavioural effects of FIASMAs.

Active in therapeutic concentrations. Increasing concentrations of FIASMAs result in decreasing ASM activity following sigmoidal concentration-effect curves. Several FIASMAs inhibit the ASM in a concentration range that is therapeutically achieved during common pharmacotherapy in human patients (Tripal *et al.*, unpublished).

Active across different cell types and in vivo. Functional inhibition of ASM obviously occurs largely independent of the cell type. We found a significant correlation between the inhibitory effects on ASM of 27 drugs

in H4 and PC12 cells (r=0.72; P < 0.001) [91]. Those FIASMAs identified to inhibit ASM using a cell culture model [91] were also found to inhibit ASM *in vivo* [64].

Residual basal ASM activity. FIASMAs do not induce complete degradation of ASM when applied *in vitro* [70] or *in vivo* [64]. Instead, FIASMAs leave a residual basal ASM activity. It is unclear at present whether or not this residual ASM activity is located within lysosomes. However, this residual basal ASM activity may be important for cell viability and might explain why patients under treatment with FIASMAs do not show symptoms of Niemann-Pick-disease apart from phospholipidosis.

No habituation. ASM activity remains low, even after prolonged administration of FIASMAs [70], meaning that there is no habituation effect.

Reversible inhibition of ASM. After withdrawal of FIASMAs in cell culture models, ASM activity returns to control levels within 3 days [70].

No rebound effect. There is no increased ASM activity after discontinuation of FIASMAs [70].

Additive effect. Co-application of structurally diverse FIASMAs result in an additive functional inhibition of ASM, arguing for action of these compounds on the same molecular target (Kornhuber *et al.*, unpublished).

Bimodal distribution. When applying 10 µM drug concentrations to a cell culture system, we observed a bimodal distribution of functional inhibition of ASM, meaning that a drug either acts as a FIASMA (reduction to less than 50% ASM activity compared to controls) or does not act as a FIASMA, with only a few intermediate compounds (Kornhuber *et al.*, unpublished). In agreement with this observation, the newly identified FIASMAs reduce ASM activity to a similar level when tested *in vivo* [64]. The bimodal distribution occurring with 10 µM drug concentrations also indicates, that the IC50 values of most FIASMAs are below 10 µM.

Specificity to inhibit different sphingomyelinases. Functional inhibition of ASM requires high lysosomal drug concentrations [89], which are achieved by the selective accumulation of the substances in acidic compartments (acid trapping) [106]. High lysosomal drug concentrations result in detachment of ASM from inner lysosomal membranes [89] (Fig. 2). This is probably the reason why FIASMAs do not inhibit secreted sphingomyelinase, neutral or alkaline sphingomyelinase [64, 78]. This also explains, why desipramine inhibits ASM only in intact cell, but not in cell lysates [87]. The FIASMA NB6 [115] did not reduce basal activity of secreted sphingomyelinase *in vivo*, but did abolish the endotoxin-induced increase in plasma sphingomyelinase activity [61]. Thus, reduced

Lysosomal enzymes inhibited by cationic amphiphilic drugs		
Acid sphingomyelinase	↓ [89;91]	
Acid ceramidase	↓ [103]	
Lysosomal acid lipase	↓ [132]	= [86]
Phospholipase A and C	↓ [133-135]	
Lysosomal enzymes not inhibited by cationic amphiphilic drugs		
Neuraminidase	↑ [132]	
LAMP1	↑ [103]	
Cathepsin B	= [103]	
Cathepsin L	↑ [103]	
β-N-Acetylhexosaminidase	= [86;132;136;137]	↑ [88;138]
α-Galactosidase	= [136]	↑ [88;138]
β-Galactosidase	= [136;137]	↑ [88;138]
Cerebroside-β-galactosidase	= [137]	
Cerebroside-β-glucosidase	= [136;137]	↑ [88]
α-Glucosidase	↑ [88]	
β-Glucosidase	↑ [138]	
β-Glucuronidase	↑ [88;138]	
α-Mannosidase	↑ [88]	
Arylsulfatase A	= [86;132;136;137]	↑ [138]
Arylsulfatase B	= [86]	↑ [138]
α-Fucosidase	= [132]	↑ [138]
Acid phosphatase	= [132;137]	↑ [88;138]
Phosphodiesterase	= [88]	
β-Xylosidase	↑ [138]	
α-Mannosidase	↑ [138]	

Table 2. Experimental data on the effect of FIASMAs (functional inhibitors of ASM) on other lysosomal enzymes. ↓ Reduced enzyme activity; = unchanged enzyme activity; ↑ enhanced enzyme activity. There are several limitations of this compilation of the literature. The data are not based on identical methods, instead different cell culture systems, different FIASMAs and different experimental conditions were used. The names of the enzymes were taken from the original literature and may differ from modern nomenclature. It is evident from the experimental data that FIASMAs inhibit only few lysosomal enzymes, while the activity of the majority of enzymes is not inhibited.

activity of ASM within acidic intracellular compartments induced by FIASMAs may secondarily result in reduced activity of secreted sphingomyelinase.

Specificity to inhibit other lysosomal hydrolases. Recent proteomic analyses reveal that the lysosome contains at least 60 soluble luminal proteins [116]. Most of these proteins are enzymes and some of them might, similar to ASM, be attached to the inner lysosomal surface by electrostatic forces. Therefore, there is concern that FIASMAs may act in an unspecific way, functionally inhibiting a large number of lysosomal hydrolases. However, the experimental data do not support this view. As shown in table 2, FIASMAs do not exert a general effect

Kornhuber/Tripal/Reichel/Mühle/Rhein/Muehlbacher/Groemer/Gulbins

on lysosomal hydrolases. Apart from ASM, only a few other hydrolases are inhibited by FIASMAs, namely AC, acid lipase and phospholipase A and C. Inhibitory effects on AC have been obtained with desipramine, chlorpromazine and chloroquine [103]. However, preliminary studies indicate that treatment with amitriptyline or fluoxetine reduces the concentration of ceramide in the hippocampus of experimental animals (Gulbins *et al.*, unpublished). These discrepant results may either indicate that the effects of weak basic lysosomotropic drugs differ between *in vitro* and *in vivo* models, or that desipramine inhibits ASM and AC to a similar extent, while amitriptyline and fluoxetine display preferential inhibition of ASM over AC. It is also possible that the cancer cells used by Elojeimy *et al.* [103] have an altered sphingolipid metabolism, where FIASMAs like desipramine inhibit AC.

Lack of specificity with respect to other molecular drug targets. Most FIASMAs were originally developed to target enzymes, receptors or transporters, such as the histamine receptor or the serotonin transporter. FIASMAs are therefore present in many pharmacological groups. When selecting a single FIASMA for clinical use, its pharmacological effects apart from inhibition of ASM have to be carefully considered. In future, it would be interesting to design FIASMAs with negligible effects on other molecular targets.

Clinical applicability

Approved for clinical use in humans. Most of the FIASMAs are licensed for medical use and most of them are listed in the ATC drug classification system recommended by the World Health Organization [117, 118]. Furthermore, many of the FIASMAs are approved by the FDA for medical application in humans [119]. These substances possess low toxicity and long-term clinical experience with their use is available. Some of these drugs have been in use for the last five decades. This suggests the potential for rapid advancement into preclinical and/or clinical trials. FIASMAs may therefore be well-suited for prolonged application in the treatment of chronic diseases or for long-time prevention of diseases.

Enriched in licenced drugs as compared to natural products. Using virtual *in silico* screening we found that FIASMAs occur in about 6% of licensed drugs for medical use in humans (ATC drug classification system), but only in about 1% of natural products ("pure natural products" [120]) (Kornhuber *et al.*, unpublished).

Enrichment in only few major drug classes. Analysis of the ATC drug classification system [117, 118] with

an *in silico* virtual screening approach (Kornhuber *et al.*, unpublished) revealed that FIASMAs are scattered across many drug classes, however very unevenly. We found a significant enrichment of FIASMAs in only a few major drug classes of the ATC system, including antihistamines for systemic use (drug class R06) and psychoanaleptics (N06) (Kornhuber *et al.*, unpublished).

Phospholipidosis has only minor functional consequences. Although many of the FIASMAs induce phospholipidosis in cell cultures and animal models [121-123], experimental studies have failed to definitively show that the presence of phospholipidosis induced by FIASMAs is detrimental to the organism [124]. Most of these drugs are well tolerated by patients even after long-term treatment.

While inhibitors directly targeting ASM with high specificity and with high potency have advantages as research tools, such properties are not necessarily associated with good clinical effects. There are many examples of advantageous clinical effects of unspecific ("dirty") and/or moderate affinity and/or indirectly acting drugs compared to directly acting drugs with high specificity for and potency against a certain target. For example, the "dirty" neuroleptic drug clozapine has superior anti-schizophrenic properties and a lower rate of extrapyramidal side effects compared to more specific or high-affinity dopamine receptor antagonists [125, 126]. Low-affinity NMDA glutamate receptor antagonists like memantine or amantadine have a superior clinical profile in comparison with more specific high-affinity NMDA receptors antagonists like PCP or MK-801 [127]. Indirect inhibition of NMDA receptors by flupirtine via activation of voltage-independent potassium channels is clinically effective and well tolerated [128]. Thus, neither unspecific action nor indirect action nor low affinity of therapeutic drugs is necessarily a disadvantage in the clinical setting.

There is currently no alternative to FIASMAs when aiming to reduce activity of ASM in human patients. It will still take many years for the development and safety evaluation of specific direct inhibitors of ASM. Only then will it be possible to judge and compare the relative advantages of direct and functional inhibitors of ASM with each other.

Many compounds which were recently identified as FIASMAs [91] have been clinically available for many years. The results of previous clinical studies with such drugs may now be reinterpreted in the context of the pathophysiology of ceramide signalling. This is

illustrated here by findings that the antidepressant drug fluoxetine tends to reduce the formation of new brain lesions in patients with multiple sclerosis [129-131]. Fluoxetine works as a FIASMA [91]. We therefore assume that the beneficial clinical effect of fluoxetine in multiple sclerosis may be related to functional inhibition of ASM and should therefore be replicable with other FIASMAs, but not with other antidepressant drugs lacking effects on ASM. Clinical studies aimed at inhibiting ASM have only recently been performed. The first study with a rational basis to treat a human disease, i.e. cystic fibrosis, with FIASMAs was performed by the Gulbins group [65].

Conclusion

Functional inhibitors of acid sphingomyelinase (FIASMAs) represent a new and large group of compounds with broad clinical applications, mainly for cytoprotective, antiapoptotic and anti-inflammatory indications. Since most of the currently known FIASMAs are licensed for medical use in humans, the way is now open to proceed with clinical studies aiming to treat ASM-associated disease conditions.

Abbreviations

AC (acid ceramidase); ADME (Absorption Distribution Metabolism Excretion); ASM (acid sphingomyelinase); ATC system (Anatomical Therapeutic Chemical system); FDA (US Food and Drug Administration); FIASMA (functional inhibitor of acid sphingomyelinase); NMDA (N-methyl-D-aspartate); PCP (phenyl-cyclohexyl-piperidine).

Acknowledgements

References

1   Jin S, Yi F, Zhang F, Poklis JL, Li PL: Lysosomal targeting and trafficking of acid sphingomyelinase to lipid raft platforms in coronary endothelial cells. Arterioscler Thromb Vasc Biol 2008;28:2056-2062.

2   Hannun YA, Luberto C: Ceramide in the eukaryotic stress response. Trends Cell Biol 2000;10:73-80.

3   Charruyer A, Grazide S, Bezombes C, Müller S, Laurent G, Jaffrézou JP: UV-C light induces raft-associated acid sphingomyelinase and JNK activation and translocation independently on a nuclear signal. J Biol Chem 2005;280:19196-19204.

4   Castillo SS, Levy M, Thaikoottathil JV, Goldkorn T: Reactive nitrogen and oxygen species activate different sphingomyelinases to induce apoptosis in airway epithelial cells. Exp Cell Res 2007;313:2680-2686.

5   Santana P, Peña LA, Haimovitz-Friedman A, Martin S, Green D, McLoughlin M, Cordon-Cardo C, Schuchman EH, Fuks Z, Kolesnick R: Acid sphingomyelinase-deficient human lymphoblasts and mice are defective in radiation-induced apoptosis. Cell 1996;86:189-199.

6   Morita Y, Perez GI, Paris F, Miranda SR, Ehleiter D, Haimovitz-Friedman A, Fuks Z, Xie Z, Reed JC, Schuchman EH, Kolesnick RN, Tilly JL: Oocyte apoptosis is suppressed by disruption of the acid sphingomyelinase gene or by sphingosine-1-phosphate therapy. Nat Med 2000;6:1109-1114.

7   Peña LA, Fuks Z, Kolesnick RN: Radiation-induced apoptosis of endothelial cells in the murine central nervous system: protection by fibroblast growth factor and sphingomyelinase deficiency. Cancer Res 2000;60:321-327.

8   Paris F, Fuks Z, Kang A, Capodieci P, Juan G, Ehleiter D, Haimovitz-Friedman A, Cordon-Cardo C, Kolesnick R: Endothelial apoptosis as the primary lesion initiating intestinal radiation damage in mice. Science 2001;293:293-297.

9   Garcia-Barros M, Paris F, Cordon-Cardo C, Lyden D, Rafii S, Haimovitz-Friedman A, Fuks Z, Kolesnick R: Tumor response to radiotherapy regulated by endothelial cell apoptosis. Science 2003;300:1155-1159.

10 Grassmé H, Jekle A, Riehle A, Schwarz H, Berger J, Sandhoff K, Kolesnick R, Gulbins E: CD95 signaling via ceramide-rich membrane rafts. J Biol Chem 2001;276:20589-20596.

11 Grassmé H, Jendrossek V, Riehle A, von Kürthy G, Berger J, Schwarz H, Weller M, Kolesnick R, Gulbins E: Host defense against Pseudomonas aeruginosa requires ceramide-rich membrane rafts. Nat Med 2003;9:322-330.

12 Yu ZF, Nikolova-Karakashian M, Zhou D, Cheng G, Schuchman EH, Mattson MP: Pivotal role for acidic sphingomyelinase in cerebral ischemia-induced ceramide and cytokine production, and neuronal apoptosis. J Mol Neurosci 2000;15:85-97.

13 Zhang Y, Mattjus P, Schmid PC, Dong Z, Zhong S, Ma WY, Brown RE, Bode AM, Schmid HHO, Dong Z: Involvement of the acid sphingomyelinase pathway in UVA-induced apoptosis. J Biol Chem 2001;276:11775-11782.

14 Gulbins E, Bissonnette R, Mahboubi A, Martin S, Nishioka W, Brunner T, Baier G, Baier-Bitterlich G, Byrd C, Lang F, Kolesnick R, Altman A, Green D: FAS-induced apoptosis is mediated via a ceramide-initiated RAS signaling pathway. Immunity 1995;2:341-351.

15 Chung HS, Park SR, Choi EK, Park HJ, Griffin RJ, Song CW, Park H: Role of sphingomyelin-MAPKs pathway in heat-induced apoptosis. Exp Mol Med 2003;35:181-188.

16 Kolesnick RN: 1,2-Diacylglycerols but not phorbol esters stimulate sphingomyelin hydrolysis in GH3 pituitary cells. J Biol Chem 1987;262:16759-16762.

17 Megha, London E: Ceramide selectively displaces cholesterol from ordered lipid domains (rafts): Implications for lipid raft structure and function. J Biol Chem 2004;279:9997-10004.

18 Nyberg L, Duan R-D, Axelson J, Nilsson A: Identification of an alkaline sphingomyelinase activity in human bile. Biochim Biophys Acta 1996;1300:42-48.

19 Liu B, Hassler DF, Smith GK, Weaver K, Hannun YA: Purification and characterization of a membrane bound neutral pH optimum magnesium-dependent and phosphatidylserine-stimulated sphingomyelinase from rat brain. J Biol Chem 1998;273:34472-34479.

20 Karakashian AA, Giltiay NV, Smith GM, Nikolova-Karakashian MN: Expression of neutral sphingomyelinase-2 (NSMase-2) in primary rat hepatocytes modulates IL-b-induced JNK activation. FASEB J 2004;18:968-970.

21 Kirschnek S, Paris F, Weller M, Grassmé H, Ferlinz K, Riehle A, Fuks Z, Kolesnick R, Gulbins E: CD95-mediated apoptosis in vivo involves acid sphingomyelinase. J Biol Chem 2000;275:27316-27323.

22 He X, Huang Y, Li B, Gong CX, Schuchman EH: Deregulation of sphingolipid metabolism in Alzheimer's disease. Neurobiol Aging 2008.

23 Schuchman EH, Suchi M, Takahashi T, Sandhoff K, Desnick RJ: Human acid sphingomyelinase. Isolation, nucleotide sequence and expression of the full-length and alternatively spliced cDNAs. J Biol Chem 1991;266:8531-8539.

24 Qiu H, Edmunds T, Baker-Malcolm J, Karey KP, Estes S, Schwarz C, Hughes H, Van Patten SM: Activation of human acid sphingomyelinase through modification or deletion of C-terminal cysteine. J Biol Chem 2003;278:32744-32752.

25 He X, Okino N, Dhami R, Dagan A, Gatt S, Schulze H, Sandhoff K, Schuchman EH: Purification and characterization of recombinant, human acid ceramidase. Catalytic reactions and interactions with acid sphingomyelinase. J Biol Chem 2003;278:32978-32986.

26 Schulze H, Schepers U, Sandhoff K: Overexpression and mass spectrometry analysis of mature human acid ceramidase. Biol Chem 2007;388:1333-1343.

27 Al BJM, Tiffany CW, Gomes de Mesquita DS, Moser HW, Tager JM, Schram AW: Properties of acid ceramidase from human spleen. Biochim Biophys Acta 1989;1004:245-251.

28 Bartelsen O, Lansmann S, Nettersheim M, Lemm T, Ferlinz K, Sandhoff K: Expression of recombinant human acid sphingomyelinase in insect Sf21 cells: purification, processing and enzymatic characterization. J Biotechnol 1998;63:29-40.

29 Bernardo K, Hurwitz R, Zenk T, Desnick RJ, Ferlinz K, Schuchman EH, Sandhoff K: Purification, characterization, and biosynthesis of human acid ceramidase. J Biol Chem 1995;270:11098-11102.

30 Lansmann S, Ferlinz K, Hurwitz R, Bartelsen O, Glombitza G, Sandhoff K: Purification of acid sphingomyelinase from human placenta: Characterization and N-terminal sequence. FEBS Lett 1996;399:227-231.

31 Linke T, Lansmann S, Sandhoff K: Purification of acid ceramidase from human placenta. Methods Enzymol 2000;311:201-207.

32 Quintern LE, Weitz G, Nehrkorn H, Tager JM, Schram AW, Sandhoff K: Acid sphingomyelinase from human urine: purification and characterization. Biochim Biophys Acta 1987;922:323-336.

33 Tayama M, Soeda S, Kishimoto Y, Martin BM, Callahan JW, Hiraiwa M, O'Brien JS: Effect of saposins on acid sphingomyelinase. Biochem J 1993;290:401-404.

34 Schwarz A, Futerman AH: Distinct roles for ceramide and glucosylceramide at different stages of neuronal growth. J Neurosci 1997;17:2929-2938.

35 Futerman AH, Hannun YA: The complex life of simple sphingolipids. EMBO Rep 2004;5:777-782.

36 Hobson JP, Rosenfeldt HM, Barak LS, Olivera A, Poulton S, Caron MG, Milstien S, Spiegel S: Role of the sphingosine-1-phosphate receptor EDG-1 in PDGF-induced cell motility. Science 2001;291:1800-1803.

37 Spiegel S, Milstien S: Sphingosine 1-phosphate, a key cell signaling molecule. J Biol Chem 2002;277:25851-25854.

38 Toman RE, Payne SG, Watterson KR, Maceyka M, Lee NH, Milstien S, Bigbee JW, Spiegel S: Differential transactivation of sphingosine-1-phosphate receptors modulates NGF-induced neurite extension. J Cell Biol 2004;166:381-392.

39 Spiegel S, Merrill AH, Jr.: Sphingolipid metabolism and cell growth regulation. FASEB J 1996;10:1388-1397.

40 Cuvillier O, Pirianov G, Kleuser B, Vanek PG, Coso OA, Gutkind S, Spiegel S: Suppression of ceramide-mediated programmed cell death by sphingosine-1-phosphate. Nature 1996;381:800-803.

41 Spiegel S, Cuvillier O, Edsall LC, Kohama T, Menzeleev R, Olah Z, Olivera A, Pirianov G, Thomas DM, Tu Z, Van B, Jr., Wang F: Sphingosine-1-phosphate in cell growth and cell death. Ann N Y Acad Sci 1998;845:11-18.

42 Brady RO, Kanfer JN, Mock MB, Fredrickson DS: The metabolism of sphingomyelin. II. Evidence of an enzymatic deficiency in Niemann-Pick disease. Proc Natl Acad Sci U S A 1966;55:366-369.

43 Carstea ED, Morris JA, Coleman KG, Loftus SK, Zhang D, Cummings C, Gu J, Rosenfeld MA, Pavan WJ, Krizman DB, Nagle J, Polymeropoulos MH, Sturley SL, Ioannou YA, Higgins ME, Comly M, Cooney A, Brown A, Kaneski CR, Blanchette-Mackie EJ, Dwyer NK, Neufeld EB, Chang T-Y, Liscum L, Strauss JF, III, Ohno K, Zeigler M, Carmi R, Sokol J, Markie D, O'Neill RR, van Diggelen OP, Elleder M, Patterson MC, Brady RO, Vanier MT, Pentchev PG, Tagle DA: Niemann-Pick C1 disease gene: Homology to mediators of cholesterol homeostasis. Science 1997;277:228-231.

44 Schuchman EH, Miranda SRP: Niemann-Pick disease: Mutation update, genotype/phenotype correlations, and prospects for genetic testing. Genet Test 1997;1:13-19.

45 Uhlig S, Gulbins E: Sphingolipids in the lungs. Am J Respir Crit Care Med 2008;178:1100-1114.

46 Smith EL, Schuchman EH: The unexpected role of acid sphingomyelinase in cell death and the pathophysiology of common diseases. FASEB J 2008;22:3419-3431.

47 Kolesnick R: The therapeutic potential of modulating the ceramide/sphingomyelin pathway. J Clin Invest 2002;110:3-8.

48 Gulbins E, Li PL: Physiological and pathophysiological aspects of ceramide. Am J Physiol Regul Integr Comp Physiol 2006;290:R11-R26.

49   Thevissen K, François IEJA, Winderickx J, Pannecouque C, Cammue BPA: Ceramide involvement in apoptosis and apoptotic diseases. Mini Rev Med Chem 2006;6:699-709.

50   Deigner HP, Haberkorn U, Kinscherf R: Apoptosis modulators in the therapy of neurodegenerative diseases. Expert Opin Investig Drugs 2000;9:747-764.

51   Pandey S, Murphy RF, Agrawal DK: Recent advances in the immunobiology of ceramide. Exp Mol Pathol 2007;82:298-309.

52   Raff MC, Barres BA, Burne JF, Coles HS, Ishizaki Y, Jacobson MD: Programmed cell death and the control of cell survival: lessons from the nervous system. Science 1993;262:659-700.

53   Altura BM, Gebrewold A, Zheng T, Altura BT: Sphingomyelinase and ceramide analogs induce vasoconstriction and leukocyte-endothelial interactions in cerebral venules in the intact rat brain: Insight into mechanisms and possible relation to brain injury and stroke. Brain Res Bull 2002;58:271-278.

54   Pascual M, Valles SL, Renau-Piqueras J, Guerri C: Ceramide pathways modulate ethanol-induced cell death in astrocytes. J Neurochem 2003;87:1535-1545.

55   Reichel M, Greiner E, Richter-Schmidinger T, Yedibela Ö, Tripal P, Jacobi A, Bleich S, Gulbins E, Kornhuber J: Increased acid sphingomyelinase activity in peripheral blood cells of acutely intoxicated patients with alcohol dependence. Alcohol Clin Exp Res 2010;34:46-50.

56   Malaplate-Armand C, Florent-Béchard S, Youssef I, Koziel V, Sponne I, Kriem B, Leininger-Muller B, Olivier JL, Oster T, Pillot T: Soluble oligomers of amyloid-b peptide induce neuronal apoptosis by activating a cPLA2-dependent sphingomyelinase-ceramide pathway. Neurobiol Dis 2006;23:178-189.

57   Han X, Holtzman M, McKeel DW, Jr., Kelley J, Morris JC: Substantial sulfatide deficiency and ceramide elevation in very early Alzheimer's disease: potential role in disease pathogenesis. J Neurochem 2002;82:809-818.

58   Cuzzocrea S, Deigner H-P, Genovese T, Mazzon E, Esposito E, Crisafulli C, Di PR, Bramanti P, Matuschak G, Salvemini D: Inhibition of ceramide biosynthesis ameliorates pathological consequences of spinal cord injury. Shock 2009;31:634-644.

59   Mikati MA, Zeinieh M, Habib RA, El HJ, Rahmeh A, El SM, Usta J, Dbaibo G: Changes in sphingomyelinases, ceramide, Bax, Bcl2, and caspase-3 during and after experimental status epilepticus. Epilepsy Res 2008;81:161-166.

60   Haimovitz-Friedman A, Cordon-Cardo C, Bayoumy S, Garzotto M, McLoughlin M, Gallily R, Edwards CK, III, Schuchman EH, Fuks Z, Kolesnick R: Lipopolysaccharide induces disseminated endothelial apoptosis requiring ceramide generation. J Exp Med 1997;186:1831-1841.

61   Claus RA, Bunck AC, Bockmeyer CL, Brunkhorst FM, Lösche W, Kinscherf R, Deigner H-P: Role of increased sphingomyelinase activity in apoptosis and organ failure of patients with severe sepsis. FASEB J 2005;19:1719-1721.

62   Lang PA, Schenck M, Nicolay JP, Becker JU, Kempe DS, Lupescu A, Koka S, Eisele K, Klarl BA, Rubben H, Schmid KW, Mann K, Hildenbrand S, Hefter H, Huber SM, Wieder T, Erhardt A, Haussinger D, Gulbins E, Lang F: Liver cell death and anemia in Wilson disease involve acid sphingomyelinase and ceramide. Nat Med 2007;13:164-170.

63   Teichgräber V, Ulrich M, Endlich N, Riethmüller J, Wilker B, de Oliveira-Munding CC, van Heeckeren AM, Barr ML, von Kürthy G, Schmid KW, Weller M, Tümmler B, Lang F, Grassme H, Döring G, Gulbins E: Ceramide accumulation mediates inflammation, cell death and infection susceptibility in cystic fibrosis. Nat Med 2008;14:382-391.

64   Becker KA, Riethmüller J, Lüth A, Döring G, Kleuser B, Gulbins E: Acid sphingomyelinase inhibitors normalize pulmonary ceramide and inflammation in cystic fibrosis. Am J Respir Cell Mol Biol 2009;in press.

65   Riethmüller J, Anthonysamy J, Serra E, Schwab M, Döring G, Gulbins E: Therapeutic efficacy and safety of amitriptyline in patients with cystic fibrosis. Cell Physiol Biochem 2009;24:65-72.

66   Sakata A, Ochiai T, Shimeno H, Hikishima S, Yokomatsu T, Shibuya S, Toda A, Eyanagi R, Soeda S: Acid sphingomyelinase inhibition suppresses lipopolysaccharide-mediated release of inflammatory cytokines from macrophages and protects against disease pathology in dextran sulphate sodium-induced colitis in mice. Immunology 2007;122:54-64.

67   Bauer J, Liebisch G, Hofmann C, Huy C, Schmitz G, Obermeier F, Bock J: Lipid alterations in experimental murine colitis: role of ceramide and imipramine for matrix metalloproteinase-1 expression. PLoS One 2009;4:e7197.

68   Göggel R, Winoto-Morbach S, Vielhaber G, Imai Y, Lindner K, Brade L, Brade H, Ehlers S, Slutsky AS, Schütze S, Gulbins E, Uhlig S: PAF-mediated pulmonary edema: a new role for acid sphingomyelinase and ceramide. Nat Med 2004;10:155-160.

69   Petrache I, Natarajan V, Zhen L, Medler TR, Richter AT, Cho C, Hubbard WC, Berdyshev EV, Tuder RM: Ceramide upregulation causes pulmonary cell apoptosis and emphysema-like disease in mice. Nat Med 2005;11:491-498.

70   Kornhuber J, Medlin A, Bleich S, Jendrossek V, Henkel AW, Wiltfang J, Gulbins E: High activity of acid sphingomyelinase in major depression. J Neural Transm 2005;112:1583-1590.

71   Kornhuber J, Reichel M, Tripal P, Groemer TW, Henkel AW, Mühle C, Gulbins E: The role of ceramide in major depressive disorder. Eur Arch Psychiatry Clin Neurosci 2009;259:S199-S204.

72   Ndengele MN, Cuzzocrea S, Masini E, Vinci MC, Esposito E, Muscoli C, Petrusca DN, Mollace V, Mazzon E, Li D, Petrache I, Matuschak GM, Salvemini D: Spinal ceramide modulates the development of morphine antinociceptive tolerance via peroxynitrite-mediated nitroxidative stress and neuroimmune activation. J Pharmacol Exp Ther 2009;329:64-75.

73   Grassmé H, Gulbins E, Brenner B, Ferlinz K, Sandhoff K, Harzer K, Lang F, Meyer TF: Acidic sphingomyelinase mediates entry of N. gonorrhoeae into nonphagocytic cells. Cell 1997;91:605-615.

74   Devlin CM, Leventhal AR, Kuriakose G, Schuchman EH, Williams KJ, Tabas I: Acid sphingomyelinase promotes lipoprotein retention within early atheromata and accelerates lesion progression. Arterioscler Thromb Vasc Biol 2008;28:1723-1730.

75   Augé N, Nègre-Salvayre A, Salvayre R, Levade T: Sphingomyelin metabolites in vascular cell signaling and atherogenesis. Prog Lipid Res 2000;39:207-229.

76   Marathe S, Kuriakose G, Williams KJ, Tabas I: Sphingomyelinase, an enzyme implicated in atherogenesis, is present in atherosclerotic lesions and binds to specific components of the subendothelial extracellular matrix. Arterioscler Thromb Vasc Biol 1999;19:2648-2658.

77   Kornhuber J, Retz W, Riederer P: Slow accumulation of psychotropic substances in the human brain. Relationship to therapeutic latency of neuroleptic and antidepressant drugs? J Neural Transm Suppl 1995;46:311-319.

78   Mintzer RJ, Appell KC, Cole A, Johns A, Pagila R, Polokoff MA, Tabas I, Snider RM, Meurer-Ogden JA: A novel high-throughput screening format to identify inhibitors of secreted acid sphingomyelinase. J Biomol Screen 2005;10:225-234.

79 Testai FD, Landek MA, Goswami R, Ahmed M, Dawson G: Acid sphingo-myelinase and inhibition by phosphate ion: role of inhibition by phosphatidyl-myo-inositol 3,4,5-triphosphate in oligodendrocyte cell signaling. J Neurochem 2004;89:636-644.

80 Kölzer M, Arenz C, Ferlinz K, Werth N, Schulze H, Klingenstein R, Sandhoff K: Phosphatidylinositol-3,5-bisphosphate is a potent and selective inhibitor of acid sphingomyelinase. Biol Chem 2003;384:1293-1298.

81 Okudaira C, Ikeda Y, Kondo S, Furuya S, Hirabayashi Y, Koyano T, Saito Y, Umezawa K: Inhibition of acidic sphingomyelinase by xanthone compounds isolated from Garcinia speciosa. J Enzyme Inhib 2000;15:129-138.

82 Hamada M, Iikubo K, Ishikawa Y, Ikeda A, Umezawa K, Nishiyama S: Biological activities of alpha-mangostin derivatives against acidic sphingomyelinase. Bioorg Med Chem Lett 2003;13:3151-3153.

83 Darroch PI, Dagan A, Granot T, He X, Gatt S, Schuchman EH: A lipid analogue that inhibits sphingomyelin hydrolysis and synthesis, increases ceramide, and leads to cell death. J Lipid Res 2005;46:2315-2324.

84 Roth AG, Redmer S, Arenz C: Potent inhibition of acid sphingomyelinase by phosphoinositide analogues. Chembiochem 2009;10:2367-2374.

85 Roth AG, Drescher D, Yang Y, Redmer S, Uhlig S, Arenz C: Potent and selective inhibition of acid sphingomyelinase by bisphosphonates. Angew Chem Int Ed Engl 2009;48:7560-7563.

86 Albouz S, Hauw JJ, Berwald-Netter Y, Boutry JM, Bourdon R, Baumann N: Tricyclic antidepressants induce sphingomyelinase deficiency in fibroblast and neuroblastoma cell cultures. Biomedicine 1981;35:218-220.

87 Yoshida Y, Arimoto K, Sato M, Sakuragawa N, Arima M, Satoyoshi E: Reduction of acid sphingomyelinase activity in human fibroblasts induced by AY-9944 and other cationic amphiphilic drugs. J Biochem (Tokyo) 1985;98:1669-1679.

88 Sakuragawa N, Sakuragawa M, Kuwabara T, Pentchev PG, Barranger JA, Brady RO: Niemann-Pick disease experimental model: sphingomyelinase reduction induced by AY-9944. Science 1977;196:317-319.

89 Kölzer M, Werth N, Sandhoff K: Interactions of acid sphingomyelinase and lipid bilayers in the presence of the tricyclic antidepressant desipramine. FEBS Lett 2004;559:96-98.

90 Hurwitz R, Ferlinz K, Sandhoff K: The tricyclic antidepressants desipramine causes proteolytic degradation of lyso-somal sphingomyelinase in human fibroblasts. Biol Chem Hoppe Seyler 1994;375:447-450.

91 Kornhuber J, Tripal P, Reichel M, Terfloth L, Bleich S, Wiltfang J, Gulbins E: Identification of new functional inhibitors of acid sphingomyelinase using a structure-property-activity relation model. J Med Chem 2008;51:219-237.

92 Holman DH, Turner LS, El-Zawahry A, Elojeimy S, Liu X, Bielawski J, Szulc ZM, Norris K, Zeidan YH, Hannun YA, Bielawska A, Norris JS: Lysosomotropic acid ceramidase inhibitor induces apoptosis in prostate cancer cells. Cancer Chemother Pharmacol 2008;61:231-242.

93 Liu X, Elojeimy S, Turner LS, Mahdy AE, Zeidan YH, Bielawska A, Bielawski J, Dong J-Y, El-Zawahry AM, Guo G-W, Hannun YA, Holman DH, Rubinchik S, Szulc Z, Keane TE, Tavassoli M, Norris JS: Acid ceramidase inhibition: a novel target for cancer therapy. Front Biosci 2008;13:2293-2298.

94 Zeidan YH, Jenkins RW, Korman JB, Liu X, Obeid LM, Norris JS, Hannun YA: Molecular targeting of acid ceramidase: Implications to cancer therapy. Curr Drug Targets 2008;9:653-661.

95 Grijalvo S, Bedia C, Triola G, Casas J, Llebaria A, Teixidó J, Rabal O, Levade T, Delgado A, Fabriàs G: Design, synthesis and activity as acid ceramidase inhibitors of 2-oxooctanoyl and N-oleoylethanolamine analogues. Chem Phys Lipids 2006;144:69-84.

96 Bielawska A, Bielawski J, Szulc ZM, Mayroo N, Liu X, Bai A, Elojeimy S, Rembiesa B, Pierce J, Norris JS, Hannun YA: Novel analogs of D-e-MAPP and B13. Part 2: Signature effects on bioactive sphingolipids. Bioorg Med Chem 2008;16:1032-1045.

97 Sugita M, Willians M, Dulaney JT, Moser HW: Ceramidase and ceramide synthesis in human kidney and cerebellum. Description of a new alkaline ceramidase. Biochim Biophys Acta 1975;398:125-131.

98 Bedia C, Casas J, Garcia V, Levade T, Fabriàs G: Synthesis of a novel ceramide analogue and its use in a high-throughput fluorogenic assay for ceramidases. Chembiochem 2007;8:642-648.

99 Bedia C, Canals D, Matabosch X, Harrak Y, Casas J, Llebaria A, Delgado A, Fabriás G: Cytotoxicity and acid ceramidase inhibitory activity of 2-substituted aminoethanol amides. Chem Phys Lipids 2008;156:33-40.

100 Nahas Z, Jiang Y, Zeidan YH, Bielawska A, Szulc Z, Devane L, Kalivas P, Hannun YA: Anti-ceramidase LCL385 acutely reduces BCL-2 expression in the hippocampus but is not associated with an increase of learned helplessness in rats. Behav Brain Res 2009;197:41-44.

101 Linke T, Wilkening G, Sadeghlar F, Mozcall H, Bernardo K, Schuchman E, Sandhoff K: Interfacial regulation of acid ceramidase activity. Stimulation of ceramide degradation by lysosomal lipids and sphingolipid activator proteins. J Biol Chem 2001;276:5760-5768.

102 Linke T, Wilkening G, Lansmann S, Moczall H, Bartelsen O, Weisgerber J, Sandhoff K: Stimulation of acid sphingomyelinase activity by lysosomal lipids and sphingolipid activator proteins. Biol Chem 2001;382:283-290.

103 Elojeimy S, Holman DH, Liu X, El-Zawahry A, Villani M, Cheng JC, Mahdy A, Zeidan Y, Bielwaska A, Hannun YA, Norris JS: New insights on the use of desipramine as an inhibitor for acid ceramidase. FEBS Lett 2006;580:4751-4756.

104 Matter H: Selecting optimally diverse compounds from structure databases: a validation study of two-dimensional and three-dimensional molecular descriptors. J Med Chem 1997;40:1219-1229.

105 Lipinski CA, Lombardo F, Dominy BW, Feeney PJ: Experimental and computational approaches to estimate solubility and permeability in drug discovery and developmental settings. Adv Drug Deliv Rev 1997;23:3-25.

106 Trapp S, Rosania GR, Horobin RW, Kornhuber J: Quantitative modeling of selective lysosomal targeting for drug design. Eur Biophys J 2008;37:1317-1328.

107 Kornhuber J, Reichel M, Tripal P, Groemer TW, Henkel AW, Mühle C, Gulbins E: The role of ceramide in major depressive disorder. Eur Arch Psychiatry Clin Neurosci 2009;259:S199-S204.

108 Lombardo F, Obach RS, Shalaeva MY, Gao F: Prediction of human volume of distribution values for neutral and basic drugs. 2. Extended data set and leave-class-out statistics. J Med Chem 2004;47:1242-1250.

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

110 Brand V, Koka S, Lang C, Jendrossek V, Huber SM, Gulbins E, Lang F: Influence of amitriptyline on eryptosis, parasitemia and survival of Plasmodium berghei-infected mice. Cell Physiol Biochem 2008;22:405-412.

111 Grassmé H, Riehle A, Wilker B, Gulbins E: Rhinoviruses infect human epithelial cells via ceramide-enriched membrane platforms. J Biol Chem 2005;280:26256-26262.

112 Bauer J, Huy C, Brenmoehl J, Obermeier F, Bock J: Matrix metalloproteinase-1 expression induced by IL-1b requires acid sphingomyelinase. FEBS Lett 2009;583:915-920.

113 Llacuna L, Marí M, Garcia-Ruiz C, Fernandez-Checa JC, Morales A: Critical role of acidic sphingomyelinase in murine hepatic ischemia-reperfusion injury. Hepatology 2006;44:561-572.

114 Kato Y, Ozawa S, Tsukuda M, Kubota E, Miyazaki K, St-Pierre Y, Hata R: Acidic extracellular pH increases calcium influx-triggered phospholipase D activity along with acidic sphingomyelinase activation to induce matrix metalloproteinase-9 expression in mouse metastatic melanoma. FEBS J 2007;274:3171-3183.

115 Deigner H-P, Claus R, Bonaterra GA, Gehrke C, Bibak N, Blaess M, Cantz M, Metz J, Kinscherf R: Ceramide induces aSMase expression: implications for oxLDL-induced apoptosis. FASEB J 2001;15:807-814.

116 Lübke T, Lobel P, Sleat DE: Proteomics of the lysosome. Biochim Biophys Acta 2009;1793:625-635.

117 The selection and use of essential medicines. World Health Organ Tech Rep Ser 2004;920:1-127.

118 The WHO Collaborating Centre for Drug Statistics Methodology NIoPH. The ATC/DDD System. http://www.whocc.no/atcddd/

119 Approved Drug Products with Therapeutic Equivalence Evaluations, ed 28th edition. U.S. Department of Health and Human Services. Food and Drug Administration. Center for Drug Evaluation and Research. Office of Pharmaceutical Science. Office of Generic Drugs, 2008.

120 Micro Source Discovery. Pure Natural Products. www.msdiscovery.com/natprod.html

121 Anderson N, Borlak J: Drug-induced phospholipidosis. FEBS Lett 2006;580:5533-5540.

122 Lüllmann-Rauch R: Drug-induced lysosomal storage disorders. Front Biol 1979;48:49-130.

123 Xia Z, Ying G, Hansson AL, Karlsson H, Xie Y, Bergstrand A, DePierre JW, Nässberger L: Antidepressant-induced lipidosis with special reference to tricyclic compounds. Prog Neurobiol 2000;60:501-512.

124 Reasor MJ, Kacew S: Drug-induced phospholipidosis: Are there functional consequences? Exp Biol Med (Maywood) 2001;226:825-830.

125 Kapur S, Seeman P: Does fast dissociation from the dopamine D2 receptor explain the action of atypical antipsychotics? A new hypothesis. Am J Psychiatry 2001;158:360-369.

126 Davis JM, Chen N, Glick ID: A meta-analysis of the efficacy of second-generation antipsychotics. Arch Gen Psychiatry 2003;60:553-564.

127 Kornhuber J, Weller M: Psychotogenicity and N-methyl-D-aspartate receptor antagonism: implications for neuroprotective pharmacotherapy. Biol Psychiatry 1997;41:135-144.

128 Kornhuber J, Bleich S, Wiltfang J, Maler M, Parsons CG: Flupirtine shows functional NMDA receptor antagonism by enhancing Mg2+ block via activation of voltage independent potassium channels. J Neural Transm 1999;106:857-867.

129 Mostert JP, Admiraal-Behloul F, Hoogduin JM, Luyendijk J, Heersema DJ, van Buchem MA, De Keyser J: Effects of fluoxetine on disease activity in relapsing multiple sclerosis: a double-blind, placebo-controlled, exploratory study. J Neurol Neurosurg Psychiatry 2008;79:1027-1031.

130 Sijens PE, Mostert JP, Irwan R, Potze JH, Oudkerk M, De Keyser J.: Impact of fluoxetine on the human brain in multiple sclerosis as quantified by proton magnetic resonance spectroscopy and diffusion tensor imaging. Psychiatry Res: Neuroimaging 2008;164:274-282.

131 Flax JW, Gray J, Herbert J: Effect of fluoxetine on patients with multiple sclerosis. Am J Psychiatry 1991;148:1603.

132 Albouz S, Boutry JM, Dubois G, Bourdon R, Hauw JJ, Baumann N: Lipid and lysosomal enzymes in human fibroblasts cultured with perhexiline maleate. Naunyn Schmiedebergs Arch Pharmacol 1981;317:173-177.

133 Kubo M, Hostetler KY: Mechanism of cationic amphiphilic drug inhibition of purified lysosomal phospholipase A1. Biochemistry 1985;24:6515-6520.

134 Matsuzawa Y, Hostetler KY: Inhibition of lysosomal phospholipase A and phospholipase C by chloroquine and 4,4'-bis(diethylaminoethoxy)α,β-diethyldiphenylethane. J Biol Chem 1980;255:5190-5194.

135 Hostetler KY, Matsuzawa Y: Studies on the mechanism of drug-induced lipidosis. Cationic amphiphilic drug inhibition of lysosomal phospholipases A and C. Biochem Pharmacol 1981;30:1121-1126.

136 Jaffrézou JP, Herbert JM, Levade T, Gau MN, Chatelain P, Laurent G: Reversal of multidrug resistance by calcium channel blocker SR33557 without photoaffinity labeling of P-glycoprotein. J Biol Chem 1991;266:19858-19864.

137 Albouz S, Vanier MT, Hauw JJ, Le Saux F, Boutry JM, Baumann N: Effect of tricyclic antidepressants on sphingomyelinase and other sphingolipid hydrolases in C6 cultured glioma cells. Neurosci Lett 1983;36:311-315.

138 Igarashi M, Suzuki K, Chen SM: Changes in brain hydrolytic enzyme activities in rats treated with cholesterol biosynthesis inhibitor, AY9944. Brain Res 1975;90:97-114.