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

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Desipramine • Lysosomotropism • Acid trapping • Acid sphingomyelinase • Acid ceramidase • Cationic amphiphilic drugs • Clinical use

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
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 consequences. 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
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 phosphatidylinositol-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]. 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...
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 triflupromazine, 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.

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Table 1. Molecular structures of newly discovered FIASMAs (functional inhibitors of ASM) [91].

<table>
<thead>
<tr>
<th>Amlodipine</th>
<th>Astemizole</th>
<th>Benztrpine</th>
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<tbody>
<tr>
<td>Bepridil</td>
<td>Camylofin</td>
<td>Chlor-prothixene</td>
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<tr>
<td>Clomiphene</td>
<td>Clope-rasline</td>
<td>Cyclo-benzaprine</td>
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<tr>
<td>Cypro-heptadine</td>
<td>Doxepine</td>
<td>Drofenine</td>
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<tr>
<td>Fendiline</td>
<td>Fluoxetine</td>
<td>Maprotiline</td>
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<tr>
<td>Nor-fluoxetine</td>
<td>Nor-triptiline</td>
<td>Paroxetine</td>
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<tr>
<td>Pimothixine</td>
<td>Promazine</td>
<td>Promethazine</td>
</tr>
<tr>
<td>Pro-triptiline</td>
<td>Sertraline</td>
<td>Sulocidil</td>
</tr>
<tr>
<td>Terfena-dine</td>
<td>Triflupromazine</td>
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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]: benztprine, 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 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

<table>
<thead>
<tr>
<th>Lyosomal enzymes inhibited by cationic amphiphilic drugs</th>
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<tbody>
<tr>
<td>Acid sphingomyelinase         ↓[69,91]</td>
</tr>
<tr>
<td>Acid ceramidase               ↓[103]</td>
</tr>
<tr>
<td>Lysosomal acid lipase         ↓[132] = [86]</td>
</tr>
<tr>
<td>Phospholipase A and C         ↓[133-136]</td>
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<tr>
<th>Lyosomal enzymes not inhibited by cationic amphiphilic drugs</th>
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<tr>
<td>Neuraminidase                   ↑[132]</td>
</tr>
<tr>
<td>LAMP1                           ↑[103]</td>
</tr>
<tr>
<td>Cathepin B                      ↑[103]</td>
</tr>
<tr>
<td>Cathepin L                      ↑[103]</td>
</tr>
<tr>
<td>β-N-Acetylhexosaminidase        = [86; 132; 136; 137] ↑[88; 138]</td>
</tr>
<tr>
<td>α-Galactosidase                 = [136] ↑[88; 138]</td>
</tr>
<tr>
<td>β-Galactosidase                 = [136; 137] ↑[88; 138]</td>
</tr>
<tr>
<td>Cerebroside β-galactosidase     = [137]</td>
</tr>
<tr>
<td>Cerebroside β-glucosidase       = [136; 137] ↑[86]</td>
</tr>
<tr>
<td>α-Glucoydase                    ↑[88]</td>
</tr>
<tr>
<td>β-Glucoydase                    ↑[136]</td>
</tr>
<tr>
<td>β-Glucuronidase                 ↑[88; 138]</td>
</tr>
<tr>
<td>α-Mannosidase                   ↑[88]</td>
</tr>
<tr>
<td>Arylsulfatase A                 = [86; 132; 136; 137] ↑[138]</td>
</tr>
<tr>
<td>Arylsulfatase B                 = [86] ↑[138]</td>
</tr>
<tr>
<td>α-Fucosidase                    = [132] ↑[136]</td>
</tr>
<tr>
<td>Acid phosphatase                = [132; 137] ↑[88; 138]</td>
</tr>
<tr>
<td>Phosphodiesterase              = [88]</td>
</tr>
<tr>
<td>α-Xylosidase                    ↑[136]</td>
</tr>
<tr>
<td>α-Mannosidase                   ↑[136]</td>
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</table>

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.

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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 display preferential inhibition of ASM have to be carefully considered. In future, it would be interesting to design FIASMAs with negligible effects on other molecular targets.

**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 licensed 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 psychoanaлектics (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**

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