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Repurposing Functional inhibitors of Acid sphingomyelinase (FIASMA) drugs: an opportunity against SARS-CoV-2 infection ?

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Abstract

What is known and objective: SARS-CoV-2 outbreak is characterized by the absence of active compounds for the treatment. The purpose of this study was to present an hypothesis on the prophylactic and/or therapeutic use in SARS-CoV-2 infections of functional inhibitors of acid sphingomyelinase (FIASMA) that are mostly molecules approved drugs for various diseases.

Comment: We present and discuss the potential interest of FIASMA presenting a lysosomotropism that may explain in vitro activities against viral infections, and more specifically in infections mediated by coronaviruses the SARS-CoV-2. A successful in vitro to in vivo translation of FIASMA requires a particular attention to drug pharmacokinetics (dosing regimen, drug-drug interactions) and to viral kinetics.

What is new and conclusion: The drug repurposing approach with the hope to bring patients effective treatment faster has gathered much attention in the SARS-COV-2 pandemic era. The current knowledge suggests that FIASMA, as small molecules having a lysosomotropism and clinically approved indications in various diseases, should be paid more attention for their repurposing as potential drugs against SARS-COV-2.

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1 - What is known and objective

The current situation of the SARS-CoV-2 outbreak poses major therapeutic problems given the absence to date of a specific antiviral agent to treat this infection, decrease viral shedding and subsequent transmission. Numerous clinical trials are in progress with different disease severity at enrollment with a high majority of studies for treatment purpose compared to prevention purpose (1,628 trials versus 289 trials consulted on october, 28 2020, cf Living mapping and living systematic review of Covid-19 studies at <https://covid-nma.com/dataviz>). Efforts have to be maintained since it is likely that we will have to coexist in the future with pathogenic coronavirus type viruses (SARS-Cov-2 or other strains). Moreover, initial trials in the search for effective therapy have led somewhat disappointing results (1-2).

Given the difficulties and costs in rapidly bringing up treatments with a favorable benefit-risk balance, in particular with the aim of large-scale treatment either for treatment of patient population or for prevention in healthy subjects, it seems relevant to investigate whether - among the drugs marketed to date - some of them could have a therapeutic or a prophylactic potential. This so-called “drug repurposing” strategy is based on different methodological approaches, and to date the most successful examples of drug reuse have not involved a systematic approach but rather serendipity (3). In the repositioning strategy the first step which consists in identifying the candidate molecule (s) for a given indication is critical. The potential approaches are either experimental and / or computational (3). Among these, the use of new data sources - including clinical data repositories - offer new opportunities.

Some organic molecules, including currently marketed drugs, have the potential to functionally inhibit, from reversible and additive manner, the activity of acid sphingomyelinase (ASM) which is a lysosomal glycoprotein involved in a wide range of disorders (4) including virus infections. These molecules identified by the acronym FIASMA (i.e. functional inhibitors of acid sphingomyelinase) have the potential to inhibit this enzyme, and therefore disrupt the entry of viruses into cells. The purpose of this study was to present an hypothesis on the prophylactic and/or therapeutic use of FIASMAs (Functional Inhibitors of ASM) in SARS-CoV-2 infections through a literature search in PubMed, and medRxiv.org preprint server for Health Sciences.

2 – Comment

2.1 - FIASMA’s (Table 1)

Acid sphingomyelinase (ASM) is a lysosomal glycoprotein anchored to the inner lysosomal membrane which is translocated to the external leaflet of the cell membrane under specific stimuli where it catalyzes the hydrolysis of sphingomyelin into ceramide and phosphorylcholine. Shingomyelin is the most abundant sphingolipid component of the mammalian plasma membrane where it is associated with cholesterol to form lipid rafts. ASM inhibitors work either by direct inhibition or by functional inhibition (FIASMA). Unlike FIASMA, there are few direct inhibitors, by these including biphosphonates, in particular

zoledronic acid (5). Functional inhibitors of ASM (FIASMA's) are cationic amphiphilic molecules relatively heterogeneous in terms of chemical structure but which have the characteristic of being polycyclic molecules, with at least 1 basic nitrogen atom ($pK_a > 4$ which corresponds to a partially protonated functional group at acidic pH) and moderate to high lipophilicity ($\log P > 3$) (6).

The internal wall of the lysosomes is negatively charged due to the abundant presence of phospholipids, and the positively charged proteins form electrostatic interactions with this internal wall of the lysosome which protects them from lysosomal degradation; ASM is thus attached to the lysosomal membrane. FIASMA's which are lipophilic molecules integrate into the lysosomal membrane with their lipophilic part, and present their positively charged part in the lysosomal lumen, which leads to detachment of the ASM from the lysosomal membrane and its subsequent proteolytic degradation in the lysosomal lumen. Hence, ASM activating stimuli cannot lead to the translocation of ASM to the external leaflet of the cell membrane, leading to perturbation in the downstream of the signaling cascade and in lipid raft formation (Figure 1).

However, there remains residual ASM activity necessary for cell viability. There are significant differences in intra-lysosomal capture speed depending on lipophilicity ($\log P$) and degree of ionization (pK_a) (from minutes to hours in cell culture systems). Indeed, desipramine, fluoxetine, maprotiline, paroxetine and protriptyline have rapid lysosomal capture (<30 min) with moderate lysosomal accumulation (lysosome / extracellular concentration ratio <100: 1) (6). Drugs known for their significant FIASMA's properties (residual activity <50% at a concentration of 10 μM) have been described (7-10) and are listed in Table 1.

The interest in FIASMA drugs relies on their potential interest in treating pathologies in which there is an increased ASM activity such as neuro-psychiatric pathologies, and in particular in major depression (11) where the inhibition of ASM by modifying the sphingomyelin-ceramide balance could contribute significantly to their mechanism of action (12, 13). Other diseases include ischemic strokes (14), alzheimer's disease (15) and chronic obstructive pulmonary disease (16) as well as intra-cellular infections (17). In addition, the production of ceramide via ASM plays a key role in the production of pulmonary edema in acute pulmonary insufficiency, and ASM inhibitors may be of therapeutic value in diseases with chronic and acute pulmonary injury (6).

2.2 - FIASMA's and viral infections

Viruses usually take benefit of the endocytosis mechanisms to penetrate in the cytosol of cells, and different mechanisms of internalization are involved. These are clathrin-mediated endocytosis, macropinocytosis, caveolar/lipid raft-mediated endocytosis, as well as several less well characterized clathrin- and caveolin/lipid raft-independent mechanisms (18). Viruses using lipid raft-dependent pathways depends on cholesterol, lipid rafts and a complex signaling pathway and use glycosphingolipids as receptors.

The role of ASM inhibition in intracellular infectious pathologies, and in particular by enveloped viruses, is due to the fact that the life cycle of enveloped RNA viruses (e.g., Sars-Cov-2) is critically dependent of the lipid biosynthesis of the host (19). Binding and entry of

the virus involves interactions between the envelope's viral glycoproteins and cellular receptors concentrated in micro-domains rich in sphingolipids present on the membranes of host cells, and the sphingolipid itself is able to serve as an alternative receptor for certain viruses (i.e., HIV). The subsequent infection leads to a reorganization of cell membranes and lipid metabolism to support the production of new viral particles (19). Beyond the role in the entry of the virus, sphingolipids also play an important role in modulating the replication of the virus and in shaping the innate antiviral immune response (20).

More specifically, the role of FIASMA's in infection by intracellular bacteria has been shown (17), and ASM activity has been found essential for optimal cycle progression of intracellular bacterial pathogens. This study established the importance of ASM for infection by several intracellular bacteria and suggested FIASMAs (namely desipramine, amitriptyline, and nortriptyline) as potential therapies for diseases caused by intracellular pathogens. The role of sphingomyelin and ASM in the intra-cellular penetration of an alpha-herpes virus has been shown (21). Imipramine and amitriptyline (FIASMA's) have reduced cellular infection with porcine alphaherpesvirus pseudorabies virus (PRV) by intracellular sphingomyelin depletion. However, not all herpes viruses were dependent on sphingomyelin and ASM for cell infection.

Several drugs, not considered as FIASMA drugs, may likely act via intracellular sequestration and increase in endosomal and/or lysosomal pH to explain their antiviral activity. Azithromycin, as well as chloroquine and hydroxychloroquine, may share this mechanism of action, and act in a synergistic manner (22). A screen of 2,600 approved drugs with further evaluation of antiviral activity using in vitro virus-like particle entry assay and a murine model of EBOLA disease showed the cationic molecules had strong in vitro and in vivo antiviral activities (23). Viral entry assays showed that these compounds blocked a late stage of viral entry. The drugs with protective ability included sertraline and bepridil which are known as FIASMA's. Hence, drug targeting the lysosomes (lysosomotropic drugs) may have different mechanisms of action at the lysosomal level. The role of the Niemann-Pick C1 (NPC1) protein as an essential entry receptor was studied in a mouse model of EBOLA disease and showed that imipramine inhibited the entry of Ebola virus (EBOV) in vitro and protected against EBOV infection in a mouse model (24). Additionally, the increase in intra-lysosomal pH may impair the activity of cathepsin-L that is a key protease for membrane fusion as shown for SARS-CoV-2 (25).

Very recently, as part of a high throughput screening using a genomic approach to reposition old molecules (DrugBank target database), amitriptyline has shown a reduction in cell death in a model of A549 infected human lung carcinoma cells by the lethal avian influenza A H5N1 virus (26). In a mouse model infected with H5N1, amitriptyline showed a reduction in the number of infiltrating cells, decreased the score of pulmonary lesions on the basis of pulmonary histopathology, and reduced pulmonary edema and improved the survival of mice infected with the H5N1 virus. Analysis of RNA-seq data from the lung tissues of mice infected with H5N1 shows that the genes significantly influenced by amitriptyline were mainly grouped in those corresponding to immune responses, neurophysiological processes and processes related to apoptosis.

As a whole, drugs that target the lysosomes (lysosomotropic drugs) and especially FIASMA's alter its functions and create an unfavorable host cell environment for viruses.

2.3 - FIASMA's and coronaviruses (Table 2)

As mentioned above, different mechanisms of internalization are used by viruses to penetrate in the cytosol of cells, and lipid-raft mediated endocytosis (27) and clathrin-mediated pathway (28) have been identified in the Coronaviridae suggesting potential target for drugs acting at the internalization step (i.e., fusion and entry). However, targeting the late compartments of the endocytic pathway after delivery of the SARS-CoV genome in the cytoplasm should also be considered as potential target for disruption of the replication, as shown with amiodarone (29). Hence different targets are to be considered against SARS-CoV-2, and those targeting directly the virus may be considered as most effective (30) than targeting the host cells. However, targeting the biology of the host cells, instead of targeting the biology of the virus, has the advantage to be less influenced by genetic variations (much less frequent in host cells compared to the viruses (e.g., mutations)). As lysosomotropic drugs with specific activity, attention should be paid to FIASMA's interfering via ASM inhibition to lipid-raft mediated endocytosis, and for some by interfering with other steps in the endocytic pathways.

In a docking study using two crystal structures from SARS-CoV main protease (M^{pro}), 4 drugs including the FIASMA drug promazine were identified used as templates for the rational design of anti-SARS proteinase drugs (31). However, negative results with promazine were obtained using in vitro and in vivo (mouse lung model) methods (32).

High-throughput screening through a library of current or previously FDA-approved drugs made it possible to identify on cell culture systems several molecules having antiviral activity against MERS-CoV and/or SARS-CoV viruses (33). Among the 27 compounds identified as active against both MERS-CoV and SARS-CoV, 9 of them were FIASMAs (i.e., emetine, tamoxifen, benztropine, fluphenazine, promethazine, astemizole, chlorpromazine, trifluorpromazine, clomipramine). A library screening of 348 FDA-approved drugs for anti-MERS-CoV activity in cell culture identified 4 compounds (chloroquine, chlorpromazine, loperamide and lopinavir) with activity in the low micromolar range (34). These drugs also inhibited the replication of SARS coronavirus and human coronavirus 229E. Among this set of compounds two of them (i.e., chlorpromazine and loperamide) are FIASMAs. In a mechanistic study on drugs. Taking into account previous results (33-34), as part of a mechanistic study on drug ability to block virus entry, the ability of a panel of drugs including 7 FIASMA's (i.e., chlorpromazine, promethazine, fluphenazine, astemizole, trifluorpromazine, clomipramine and tamoxifen) to inhibit MERS-CoV was confirmed (35).

Very recently, several studies have been directed towards the search of drugs potentially inhibiting Sars-CoV-2. SARS-CoV-2 proteins have been cloned, tagged and expressed in human cells and the human proteins associated with each have been identified (36). SARS-CoV-2 human protein-protein interactions were identified ($n = 332$) and 66 of them were targeted by 69 compounds (including 29 FDA-approved drugs) whose antiviral activity was investigated using 2 viral assays. Among this panel of drugs clinically usable for targeting the human proteins, two are FIASMAs (i.e., clemastine and cloperastine).

A protein-protein interaction network containing the genes co-expressed with the angiotensin converting enzyme 2, a main cell receptor of SARS-CoV and SARS-CoV-2, has been set up (37). These authors found that 36 drugs were associated with the gene network, including one FIASMA (i.e., amiodarone). In a screen of 3,000 FDA-approved drugs against SARS-CoV, tested in vitro on Vero cells, 24 drugs were retained including two FIASMAs (i.e.,

loperamide and cepharanthine) (38). Using a systems pharmacology-based network medicine platform, the quantification of interplay between the human coronaviruses interactome and drugs targets found 16 potential drugs including four FIASMAS (i.e., paroxetine, melatonin, quinacrine, carvedilol) (39). Four studies using docking, in vitro or in vivo studies reported that emetine could be a potential inhibitor of SARS-CoV-2 (40-43). Using artificial intelligence technology to identify marketed drugs with potential activity against SARS-CoV-2, the FIASMA drug clofazimine was identified, in a subset of 8 drugs, as a potential candidate based on in vitro cell activities against a feline coronavirus (44).

Studies currently in a review process, have evaluated either in silico (45) or in vitro (46) the potential of approved drugs. A docking was carried out with the papain-like protease (PLpro) of SARS-CoV-2 using a panel (n = 2,525) of molecules approved by the FDA (45) in order to find inhibitors. This work made it possible to select those who had the best affinity including chloroquine, formoterol and a FIASMA (i.e., amitriptyline). In an in vitro study, a subset of 17 drugs with activity as inhibitors of SARS-CoV-2 were identified (46). This subset of drugs included 5 FIASMAS (i.e., fluphenazine, chlorpromazine, clomipramine, promethazine, and emetine), and also highlighted chloroquine derivatives (e.g., hydroxychloroquine) as potential SARS-CoV-2 antiviral drugs. About 30 FDA approved drugs have been selected by computational analysis to inhibit the SARS-CoV-2 main protease (M^{pro}) (47). Among 7 drugs, the 3 more potent were ebastine and 2 FIASMA's (i.e., pimozide and bepridil). Using quantitative high-throughput screen coupled with artificial intelligence based machine learning from a library of 1,425 FDA-approved drugs, a subset of 16 compounds with in vitro antiviral effects were identified including amiodarone as a FIASMA drug (48). In a cell culture model 3 FIASMAS (amiodarone, fluoxetine, and imipramine) inhibited the entry and propagation of SARS-CoV-2 without cytotoxic adverse effects and had also antiviral activity against two currently circulating influenza A virus subtypes (49). In the study, fluoxetine was associated with increased cholesterol storage as well as dysregulated acidification. A very recent paper, showed that amitriptyline, desipramine, escitalopram, fluoxetine, imipramine, maprotiline, and sertraline displayed an almost complete *ex vivo* inhibition of the infection of human epithelial cells and different human cell lines with SARS-CoV-2 and pp-VSV-SARS-CoV-2 spike particles (50).

Based on a potential prophylactic activity of the anti-psychotic chlorpromazine against SARS-CoV-2, and as a result of a lower prevalence of COVID-19 infections in psychiatric patients (4%) comparatively to health care professionals (14%), suggestions of a prophylactic effect of with psychoactive compounds have emerged (51-53), and a clinical trial (i.e., The reCoVery study) is ongoing. The antiviral activity of chlorpromazine involves the inhibition of clathrin-mediated endocytosis and, once again, the status of FIASMA was not mentioned.

As a whole, numerous in vitro or in vivo study have been identified 28 FIASMA's as potential antiviral drug candidates against SARS-COV, MERS-COV or SARS-COV-2. In this subset of drugs, 6 can be identified as active against both the 3 coronaviruses (chlorpromazine, clomipramine, emetine, fluphenazine, loperamide, promethazine, see Table 2). It should be noticed that there are many unknowns in SARS-CoV-2 cell penetration. Apart from caveolar/lipid raft-mediated endocytosis, clathrin-mediated endocytosis and micropinocytosis potentially involved in cell entry may be modulated by some drugs with FIASMA properties such as chlorpromazine, sertraline and promethazine as inhibitors of

clathrin-mediated endocytosis, and imipramine as inhibitors of micropinocytosis (54). Furthermore, the endocytic mechanisms for coronaviruses can vary along the level in the respiratory tract. Nasal epithelial cells express a wide variety of endocytic markers while pneumocytes have a more restricted pattern with micropinocytosis as a predominant endocytic pathway (54). Type II alveolar pneumocytes as preferential targets of SARS-CoV-2 should explain the late alveolar damage observed in some patients.

2.4 - In vitro-to-in vivo translation

Solid evidence of antiviral activity based on in vitro and on pre-clinical studies is a prerequisite to further studies in humans. However, in vivo translation of antiviral activity requires that the drugs have suitable profiles of drug exposure (concentration and duration) at the site(s) of action. Hence, drug pharmacokinetics and drug regimens are essential parameters for a successful translation.

FIASMA's are lipophilic basic amine drugs and as such have a very high volume of distribution. Indeed, steady-state volume of distribution (V_{dss}) of amitriptyline and of chlorpromazine is 14.4 L per kg (55) and 8.9 L per kg (56). As lipophilic drugs, they are highly metabolized with significant first-pass effect. Indeed, the systemic clearance of amitriptyline and chlorpromazine is quite high (0.9 L/min and 1.3 L/min), and they have a low and variable oral bioavailability: ranging from 4 to 38 % for chlorpromazine (56) and from 33 to 62 % for amitriptyline (55). Even though they have a high volume of distribution, as a result of a high clearance, their apparent elimination half-life of is not too long (approx. 11 h and 18.5 h for amitriptyline and chlorpromazine, respectively). However, reaching steady-state to obtain a maximal effect would require a delay (approximately 7-times the half-life) that may prove unsuitable in an epidemic context either for prevention or for a curative use. Alternatively, a loading dose may be used to reach more rapidly the steady state provided that the tolerance profile is not a limiting factor. Furthermore, as illustrated for these two prototypic drugs, the variable oral bioavailability is a factor of inter-individual that should be considered.

Other variability factors that may impact dosing such renal and hepatic impairment as well as drug-drug interactions with concomitant medications should also be considered with these drug candidates as lipophilic drugs usually have significant first-pass effect. Furthermore, a particular attention should be paid to FIASMA interacting with P-gp either as substrate and/or as inhibitor (Table 2). P-gp is present in the lysosomal membrane (57) and P-gp substrates may have a higher lysosomal distribution compared to those driven only by passive diffusion (58). Hence, co-administration of strong P-gp inhibitor (e.g., clarithromycin, itraconazole, verapamil and some HIV protease inhibitor) with FIASMAs may decrease their lysosomal accumulation. Such feature should be considered as part of drug combination therapy in the management of SARS-CoV-2 infection.

As part of prophylaxis use in individual contacts, knowledge of the pharmacokinetics in different sub-populations (including pediatrics and elderly people) should be estimated. Besides drug pharmacokinetics, viral kinetics has to be considered to optimize dosing

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3 regimens given the limited window to initiate an antiviral therapy in a curative setting. The
4 viral profile kinetics (i.e., time to peak viral load and duration of viral shedding) varies among
5 viruses and is not yet well defined for SARS-CoV-2. These elements are mandatory for the
6 definition of the initiation and of the duration of an antiviral treatment (59).
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8 These elements highlight the fact that drug pharmacokinetics and viral kinetics has to be
9 known with precision in order not to be lost in translation.
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12 **2.5 – Framework of future investigations**
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14 Firstly, retrospective investigations on COVID-19 confirmed studied could be conducted to
15 explore the prevalence and the potential effects of FIASMAs. Indeed, it seems worthwhile to
16 investigate if patients infected with Sars-Cov-2 virus and exposed to FIASMA's and have a
17 more favorable evolution of the disease. Indeed, a recent retrospective study (59) on 65
18 inpatients tested positive for SARS-CoV-2 and treated or not by calcium channel blockers
19 (CCB, i.e., nifedipine or amlodipine as a FIASMA) for hypertension found that patients
20 treated by CCB were significantly more likely to survive than those not treated by CCB (50 %
21 survived in the CCB group versus 14.6 % survived in the No-CCB group). As a retrospective
22 study involving few patients, these data have to be considered with caution. Moreover, a
23 submitted study (61) has explored the association between antidepressant use and the risk
24 of intubation or death in inpatients with COVID-19. Significant association was reported
25 between the use of any antidepressant and reduced risk of intubation or death. When
26 individual antidepressant was taken into account, significant associations were found for
27 escitalopram, fluoxetine and venlafaxine. Among the 3 antidepressants one was a FIASMA
28 (fluoxetine). Data available in the clinical hospital data warehouses may help us testing this
29 assumption and to identify marketed drugs to be repositioned. For example, in the Erasme
30 hospital from Brussels, 370 (27 %) of the 1373 hospital admission for COVID-19 (March to
31 October 2020) took at least one FIASMA. Alternatively, in the Rennes University Hospital, the
32 prevalence of FIASMA in patients admitted for COVID-19 was 8.5 % (55 on 642 until May 5,
33 2020).
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35 Secondly, pharmaco-epidemiological studies could be carried out exploring in general
36 population if the chronic prescription of particular FIASMAs would be associated with a low
37 prevalence of severe symptoms of the COVID-19 infection.
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39 Thirdly, all the compound identified as FIASMAs could be tested in vitro to explore their
40 activity against SARS-COV-2.
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42 Fourthly, the FIASMAs having either a protective effect in general population or a significant
43 activity in vitro could be tested in vivo using notably randomized double blinded study
44 against placebo.
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51 **3 - What is new and conclusion**
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53 The drug repurposing approach with the hope to bring patients effective treatment faster
54 has gathered much attention in the SARS-COV-2 pandemic era. The current knowledge
55 suggests that FIASMAs, as small molecules having a lysosomotropism and clinically approved
56 indications in various diseases, should be paid more attention for their repurposing as
57 potential drugs against SARS-COV-2. We strongly suggest to explore the potential protective
58 effect of the FIASMAs in subjects treated chronically for various diseases, and in case of
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positive results to test the corresponding FIASMA in off-label therapy using notably large-sized, randomized and double-blinded controlled clinical trials.

Author Contributions : All authors performed the literature search and wrote the manuscript.

Conflict of interest :

All the authors (PLC & GL) disclose any financial and personal relationships with other people or organizations that could inappropriately influence their work.

Figure 1. Mechanisms of action of functional inhibitors of acid sphingomyelinase (ASM).

Left : ASM is anchored to the inner leaflet of the lysosomal membrane by electrostatic forces so that the enzyme is protected from proteolytic degradation. Specific stimuli allow the translocation of ASM from the inner lysosome to the external leaflet of the cell where ASM catalyzes the hydrolysis of sphingomyelin into ceramide and phosphorylcholine. Shingomyelin is the most abundant sphingolipid component of the mammalian plasma membrane where it is associated with cholesterol to form lipid rafts.

Right : FIASMA are cationic drugs with lipophilic properties that diffuse in the lysosome by passive diffusion and potentially via an additional mechanism using ABCB1 transporter located on the lysosomal membrane. These drugs become protonated in the intra-lysosomal acidic environment and increase the intra-lysosomal pH so that ASM is detached from the inner leaflet of the lysosomal membrane, and is further degraded by proteolysis. ASM translocation is no longer effective and the formation of lipid rafts is altered. Hence, different mechanisms of internalization used by viruses (i.e., fusion and entry) to penetrate in the cytosol of cells are altered.

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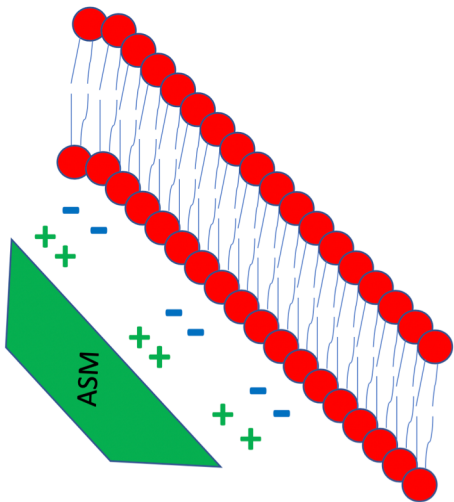
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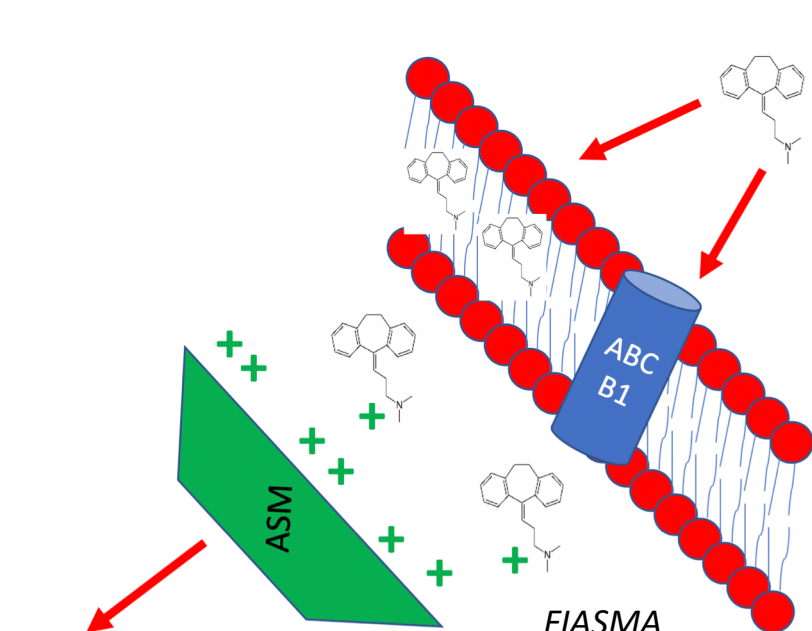
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Functional acid sphingomyelinase (ASM) anchored to the inner lysosomal membrane via electrostatic forces.



ASM membrane detachment and further inactivation

FIASMA interference with ASM membrane binding

FIASMA :
Functional inhibitors of acid sphingomyelinase (ASM)

FIASMA diffusion in the lysosome via passive diffusion or transporter-mediated diffusion

Table 1 : Functional inhibitors of acid sphingomyelinase with their ATC classification (functional inhibition is defined by a reduction to ASM activity below 50 % at a 10 μ M concentration, Kornhuber et al. 2013 and Hoehn et al. 2016).

| Denomination | ATC-5 | ATC-4 |
|-----------------|---------|---|
| Alverine | A03AX08 | Drugs for functional gastrointestinal disorders |
| Amiodarone | C01BD01 | Class 1 and 3 antiarrhythmic drugs |
| Amitriptyline | N06AA09 | Antidepressant drugs |
| Amlodipine | C08CA01 | Selective calcium channel blockers |
| Astemizole | R06AX11 | Antihistamines for systemic use |
| Benztropine | N04AC01 | Antiparkinson drugs |
| Bepridil | C08EA02 | Non-selective calcium channel blockers |
| Camylofine | A03AA03 | Drugs for functional gastrointestinal disorders |
| Carvedilol | C07AG02 | Beta blocking agents |
| Cepharanthine | (1) | |
| Chlorpromazine | N05AA01 | Antipsychotic drugs |
| Chlorprothixene | N05AF03 | Antipsychotic drugs |
| Cinnarizine | N07CA02 | Antivertigo preparations |
| Clemastin | R06AA04 | Antihistamines for systemic use |
| Clofazimine | J04BA01 | Drugs for treatment of lepra |
| Clomiphene | G03GB02 | Gonadotropins and other ovulation stimulants |
| Clomipramine | N06AA04 | Antidepressant drugs |
| Cloperastine | R05DB21 | Cough suppressants |
| Cyclobenzaprine | M03BX08 | Muscle relaxants, centrally acting agents |
| Cyproheptadine | A15AA01 | Appetite stimulants |
| Desipramine | N06AA01 | Antidepressant drugs |
| Desloratadine | R06AX27 | Antihistamines for systemic use |
| Dicyclomine | A03AA07 | Drugs for functional gastrointestinal disorders |

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| 1 | | | |
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| 3 | Dilazep | C01DX10 | Vasodilators used in cardiac diseases |
| 4 | | | |
| 5 | Doxepin | N06AA12 | Antidepressant drugs |
| 6 | | | |
| 7 | Emetine | P01AX02 | Antiprotozoal |
| 8 | | | |
| 9 | Ethopropazine | N04AA05 | Anticholinergic agents for Parkinson |
| 10 | | | |
| 11 | Fendiline | C08EA01 | Calcium channel blockers |
| 12 | | | |
| 13 | Flunarizine | N07CA03 | Antivertigo preparations |
| 14 | | | |
| 15 | Fluoxetine | N06AB03 | Antidepressant drugs |
| 16 | | | |
| 17 | Flupenthixol | N05AF01 | Antipsychotic drugs |
| 18 | | | |
| 19 | Fluphenazine | N05AB02 | Antipsychotic drugs |
| 20 | | | |
| 21 | Fluvoxamine | N06AB08 | Antidepressant drugs |
| 22 | | | |
| 23 | Hydroxyzine | N05BB01 | Anxiolytic drugs |
| 24 | | | |
| 25 | Imipramine | N06AA02 | Antidepressant drugs |
| 26 | | | |
| 27 | Lofepamine | N06AA07 | Antidepressant drugs |
| 28 | | | |
| 29 | Loperamid | A07DA03 | Antipropulsives |
| 30 | | | |
| 31 | Loratadine | R06AX13 | Antihistamines for systemic use |
| 32 | | | |
| 33 | Maprotiline | N06AA21 | Antidepressant drugs |
| 34 | | | |
| 35 | Mebeverine | A03AA04 | Drugs for functional bowel disorders |
| 36 | | | |
| 37 | Mebhydroline | R06AX15 | Antihistamines for systemic use |
| 38 | | | |
| 39 | Melatonine | N05CH01 | Hypnotics and sedatives drugs |
| 40 | | | |
| 41 | Mibefradil | C08CX01 | Selective calcium channel blockers |
| 42 | | | |
| 43 | Nortriptyline | N06AA10 | Antidepressant drugs |
| 44 | | | |
| 45 | Paroxetine | N06AB05 | Antidepressant drugs |
| 46 | | | |
| 47 | Penfluridol | N05AG03 | Antipsychotic drugs |
| 48 | | | |
| 49 | Perhexiline | C08EX02 | Non-selective calcium channel blockers |
| 50 | | | |
| 51 | Perphenazine | N05AB03 | Antipsychotic drugs |
| 52 | | | |
| 53 | Pimethixene | R06AX23 | Antihistamines for systemic use |
| 54 | | | |
| 55 | Pimozide | N05AG02 | Antipsychotic drugs |
| 56 | | | |
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|-----------------|---------|---|
| Promazin | N05AA03 | Antipsychotic drugs |
| Promethazin | D04AA10 | Antipruritics, including antihistamines, anesthetics... |
| Protriptyline | N06AA11 | Antidepressant drugs |
| Quinacrine | P01AX05 | Antiprotozoal drugs |
| Sertindole | N05AE03 | Antipsychotic drugs |
| Sertraline | N06AB06 | Antidepressant drugs |
| Suloctidil | C04AX19 | Peripheral vasodilators |
| Tamoxifen | L02BA01 | Hormone antagonists and related agents |
| Terfenadine | R06AX12 | Antihistamines for systemic use |
| Thioridazin | N05AC02 | Antipsychotic drugs |
| Trifluoperazine | N05AB06 | Antipsychotic drugs |
| Triflupromazine | N05AA05 | Antipsychotic drugs |
| Trimipramine | N06AA06 | Antidepressant drugs |

Footnote : (1) Cepharanthine is only approved by Japan health authority in some indications

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Table 2 : Functional inhibitors of acid sphingomyelinase with activity against SARS-CoV, MERS-CoV and SARS-CoV-2 on *in silico*, or *in vitro* and in *ex vivo* models. Interaction with P-gp (substrate and/or inhibition) was retrieved from Metrabase a public cheminformatics and bioinformatics database for transporter data analysis (<http://www-metabase.ch.cam.ac.uk>).

| FIASMA | SARS-CoV | | MERS-CoV | | SARS-CoV-2 | | P-gp substrate | P-gp inhibitor |
|-----------------------|----------|--------------|----------|---------|------------|-------------|----------------|----------------|
| Amiodarone | □■ | (29,37) | | | □□■ | (37,48, 49) | o | + |
| Amitriptyline | | | | | □❖ | (45,50) | + | + |
| Astemizole | ■ | (33) | ■ ■ | (33,35) | | | + | + |
| Bepidil | | | | | ■ | (47) | + | + |
| Benztropine | ■ | (33) | ■ | (33) | | | o | o |
| Carvedilol | | | | | □ | (39) | o | + |
| Cepharanthine | | | | | ■ | (38) | + | + |
| Chlorpromazine | ■ ■ ■ | (28, 33, 34) | ■ ■ | (33,35) | ■ | (46) | + | + |
| Clemastine | | | | | □ | (36) | - | o |
| Clofazimine | | | | | ■ | (44) | o | + |
| Clomipramine | ■ | (33) | ■ ■ | (33,35) | ■ | (46) | + | + |
| Cloperastine | | | | | □ | (36) | o | o |
| Desipramine | | | | | ❖ | (50) | | |
| Emetine | ■ | (33) | ■ | (33) | □■ ■ ■ ■ | (40-43, 46) | + | + |
| Fluoxetine | | | | | ■❖ | (49, 50) | + | + |
| Fluphenazine | ■ | (33) | ■ ■ | (33,35) | ■ | 46 | o | + |
| Imipramine | | | | | ■❖ | (49,50) | + | + |
| Loperamide | ■ | (34) | ■ | (34) | ■ | (38) | + | + |
| Melatonin | | | | | □ | (39) | o | o |
| Maprotiline | | | | | ❖ | (50) | o | + |
| Paroxetine | | | | | □ | (39) | + | + |
| Pimozide | | | | | ■ | (47) | + | + |
| Promazine | □■† | (31,32) | | | | | - | + |
| Promethazine | ■ | (33) | ■ ■ | (33,35) | ■ | (46) | - | + |
| Quinacrine | | | | | □ | (39) | o | o |
| Sertraline | | | | | ❖ | (50) | + | + |
| Tamoxifene | ■ | (33) | ■ ■ | (33,35) | | | - | + |
| Triflupromazine | ■ | (33) | ■ ■ | (33,35) | | | + | + |

Foot note :

In bold : drugs active against the 3 coronaviruses

in silico (□), in vitro (■), *ex vivo* (❖) and negative result (†).

P-gp interaction : no information (o), positive information (+), negative information (-)