After one year of COVID-19 Pandemic and Hundreds of Suggested Drugs, Will Cathepsin L Inhibitors be the Solution?

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Abstract

Cysteine cathepsins are defined as lysosomal enzymes which are member of the papain family. Cysteine cathepsins (Cts) prevalently exist in whole organisms varying from prokaryotes to mammals and possess in their active site greatly conserved residue of cysteine. Cts are engaged in the digestion of cellular protein, activation of zymogen, and remodeling of extracellular matrix (ECM). Host cells are entered by SARS-CoV-2 via endocytosis. Cathepsin L and phosphatidylinositol 3-phosphate 5-kinase are crucial in terms of the endocytosis by cleaving the spike protein, which permits viral membrane fusion with endosomal membrane, and succeeded by the releasing of viral genome to the host cell. Thereby, inhibition of cathepsin L may be advantageous in terms of decreasing infection caused by SARS-CoV-2. Coordinate inhibition of multiple Cts and lysosomal function by different drugs and biological agents might be of value for some purposes such as parasite or viral infections and anti-neoplastic applications. It has been found that Zn^{2+} deficiency or dysregulation leads to an exaggerated activity of Cysteine cathepsin increasing the autoimmune/inflammatory response. At this purpose Zn^{2+} metal can be safely combined with a drug that increases the anti-proteolytic effect of endogenous Zn^{2+} lowering the excessive activity of some CysCts. Biguanide derivatives complex with Zn^{2+} have been found to be promising inhibitors of CysCts protease reactions. Molecular docking studies of Cathepsin L Inhibited by Metformin-Zn+2 complex have been performed showing two strong key interactions (Cys-25&His-163) and an extra H-bond with Asp-163 compared to the co-crystallized Zn^{+2} (PDB ID 4axl).

Keywords

COVID-19, Cathepsin L, SARS-CoV-2, Viral Fusion , Docking , Metformin , Zinc ,Quercetin , Bromelain ,Lactoferrin

1) Introduction

Cysteine cathepsins which are described as lysosomal enzymes are a member of the papain family. In a broad range of tumors the expression of cysteine cathepsins is dysregulated. Moreover, ample data asserted their engagement in the progression of cancer, metastasis, angiogenesis and drug resistance. In addition to that, they importantly contribute to some viral infections [1, 2]. In accordance to the active site of cathepsins which are lysosomal proteases, their subclassification can include aspartate, serine cathepsins and cysteine [1].

Cathepsins (Cts) are prevantely exist in whole organisms varying from prokaryotes to mammals and possess in their active site a greatly conserved cysteine residue . In term of the proteins degradation, they are fundamental in which they are incorporated in the lysosomes via phagocytosis, auto-phagocytosis and endocytosis[3]. In addition to that, their engagement presents in the digestion of cellular protein, activation of zymogen, and remodeling of extracellular matrix (ECM) [4, 5]. In accordance with physiological-related conditions, Cts are essential in term of tissue homeostasis maintenance, also their engagement presents in various processes namely differentiation, development, apoptosis and immune response[6]. Changes in activity, localization and expression of Cts have been linked to multiple pathological-related disorders, comprising progression of cancer [7, 8], Additionally ectopic expression of Cts is commonly correlated with poor prognosis[9].

The Cts family proteases comprises the subtypes B, C, F, H, K, L, O, S, V, W, and X[10] that mature in the lysosomes acidic environment. Additionally to the status of both redox and pH of the vicinity microenvironment, the biological inhibitors and activators are controlling their proteolytic activity, including growth factors, cytokines, endogenous inhibitors and collagen peptides [11, 12]. In accordance to the Cts family proteases proteolytic activity, further classification can be performed into exopeptidases (Cts C and X) or endopeptidases (F, O, S, K, V, L, and W), along with Cts H and B which possess both exopeptidase and endo activities[13]. The Cts specific contribution regarding phenomena of chemoresistance occurrence is presently at issue. CtsB and L overexpression was linked to the inactivation of drug increase and, most importantly, with trafficking of lysosome increase to the membrane of plasma and lysosomalcargo secretion [14], and consequently, knock-down of CTSL in cancer of ovarian cells SCOV3 led to apoptosis increase with induction of paclitaxel which is the most prevalent drug in ovarian cancer treatment [15], Moreover, Cts L contributed to the inhibition of the process of cell senescence in various lines of tumor cell, contributing a further protease-mediated drug target eradication mechanism (confining a drug within lysosomes) and resistance of drug [16-18]. Within this framework, Cts L inhibition led to increase of accumulation of Doxorubicin cell and a further convenient nuclear distribution of drug in the cells in spite of the expression of P-gp [18]. The inhibition of CstL is stabilized and improved the availableness of nuclear and cytoplasmic targets of drug including the receptor of estrogen-α, Bcr-Abl, topoisomerase-IIα, androgen receptor I and the histone deacetylase 1, leading to cellular

response increase for various therapeutics drugs (tamoxifen, doxorubicin, trichostatin A, flutamide, and imatinib) [18]. Much work continues to exist to be performed in the utilize of Cts inhibitors in opposition to neoplasia but also cysteinyl cathepsins are produced by viruses, bacteria, parasites and fungi with a diversity of functions, thus making suggestion of another probable utilize of inhibitors[19]. Host cell CysCts are also engaged in some important viruses infectivity, e.g., Ebola [20], SARS-CoV-2 as well (principally) is currently renowned that it accesses cells via endocytosis. Phosphatidylinositol 3-phosphate 5-kinase and cathepsin L are crucial for the endocytosis[21]. In resemblance to SARS-CoV, succeeding SARS-CoV-2 endocytosis, is cathepsin L cleaves protein S, that permits viral membrane fusion along with the endosomal membrane, and, consequently, release of viral genome into the host cell occurs. Thus, inhibition of cathepsin L may be advantageous in decreasing SARS-CoV [22] and SARS-CoV-2 infection [21].

Cysteinyl cathepsin inhibitors can possess "poly-therapeutic" actions in opposition to low-level inflammation in combination with autoimmunity. The low-level inflammation and autoimmunity combination has a contribution to various age-linked diseases of unrelated reasons. Several Cts in diverse types of cell underlie multiple processes necessary for inflammation and autoimmunity, e.g. , response of cytokine, processing of lysosomal antigen, extracellular degradation, serine protease zymogens activation, etc. Amid several fine studies, a few milestones emerge for their perspicuity on Cts in inflammation requirements[23] and autoimmunity[24]. Coordinate multiple Cts and lysosomal function inhibition by different drugs and biological agents as Zn^{2+}, Biguanides and Lactoferrin…etc might be of value for some purposes such as parasite ot viral infections, and Anti-neoplastic implementations[25].

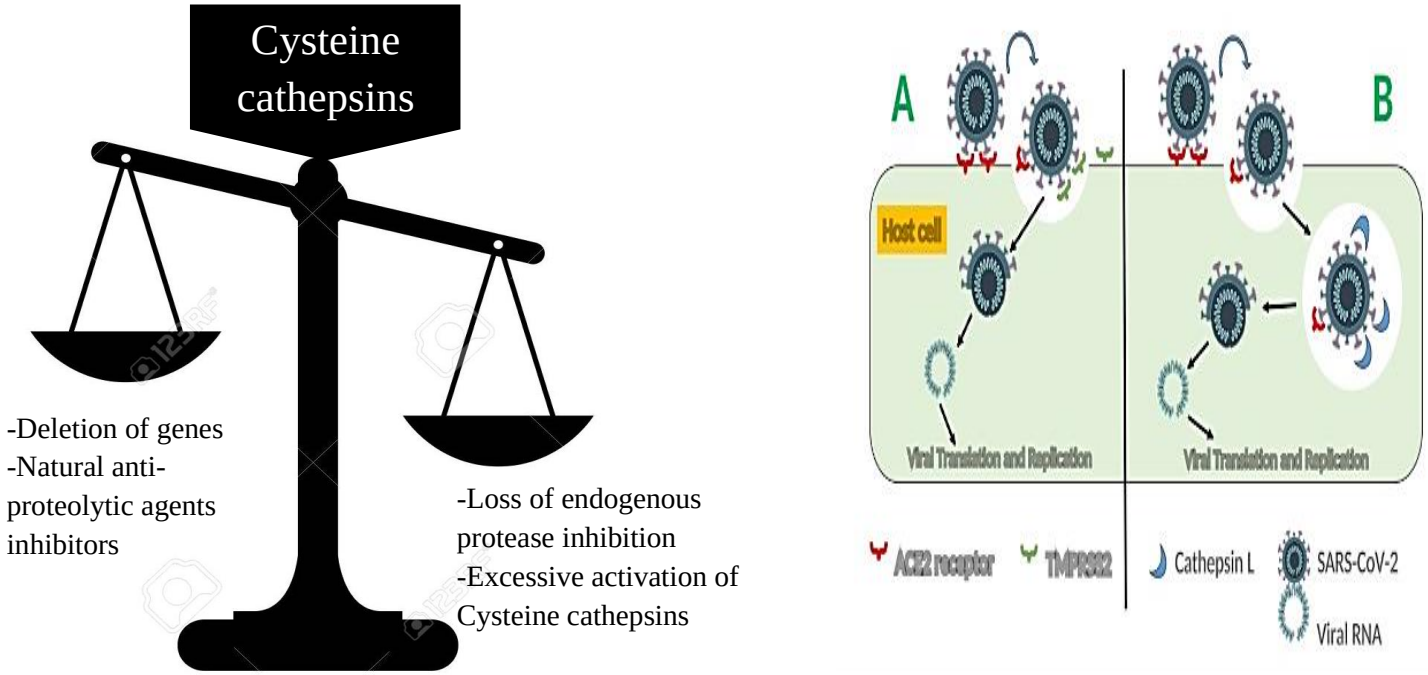


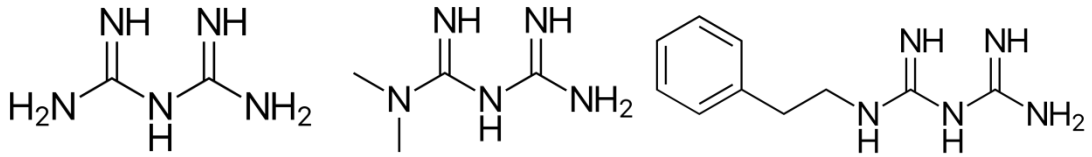
Fig. 1 Equilibrium between activators of cysteine vs anti-proteolytic agents inhibitors and its impact on the pathogenic process [116]

Fig. 2

2) Cysteinyl cathepsin L inhibitors:

1) Zinc: (Zn^{2+}) was figured out to be a specifically strong in term of multiple caspases inhibition[26]. Consequently, it was figured that multiple caspases inhibition is conducted by mediation of Zn^{2+}-binding allosteric exosites which are distant from their catalytic mechanisms[27]. Also, specific viral proteases have Zn^{2+}-binding exosites[28]. Calpain (a cysteine protease) undergoes inhibition by Zn^{2+} and activation by deficiency of Zn^{2+} [29]. It is renowned that the Zn^{2+} interacts with the E–F hand structure which presents in certain papain-like viral proteases and calpains. Nonwithstanding that the 20S proteasome is a serine protease, the Zn^{2+}-binding site has been recorded to possess a high affinity; the previously mentioned site of binding and the catalytic mechanism are assumably separate from each other[30]. Reportedly, Zn^{2+} is responsible for the induction of inhibitory dissociation of the subunits of drosophila proteasome [31]. The majority of carboxyl proteases are not inhibited in large manner by the intensity of interactional cell Zn^{2+}, and metallo-proteases demand Zn^{2+} for the purpose of activity, and in case a drug can raise the anti-proteolytic impact of endogenous Zn^{2+} in a safe manner, this might decrease the excessive rates of reaction of some or all Cts comprising viral or parasite proteases inhibition. Biguanide (guanylguanidine) creates complexes with Zn^{2+} and other metal cations. It has been discovered that specific derivatives of biguanide are interactional inhibitors of Zn^{2+} of protease reactions in examination of enzyme and a lot of degradation of cellular protein in the bioassay of perfused tissue [32]. Furthermore, it has been assessed that Zn^{2+} deficiency or dysregulation are causing mammalian inflammation and autoimmunity[33]. An association between dysregulation of (a) Zn^{2+} in diverse types of cells and compartments, (b) one or more exaggerated activity of CysCts, and (c) increased autoimmune/inflammatory processes appears to be probable. Such a relationship provides several therapeutic intervention points[33].

2) Biguanides: the Cts inhibitory effect of Zn^{2+} is mediated by interactions with series of compounds known as Biguanides (Fig 1) as Phenformin and Metformin (oral hypoglycemic agent and was used in the 1940s for treatment of Influenza virus[34]) and Cts proteases by formation of ‘‘Zn^{2+} sandwich’’ in between different derivatives of biguanide and the cysteinyl cathepsins active site regions. Biguanide forms complexes of bidentate Zn^{2+} via the two imino nitrogens, and also the Cysteinyl Cathepsin possess bidentate affinity for Zn^{2+} consisting of Cys(thiolate)- His(imidazole) active region [25]. The partners of imino biguanide nitrogens and the thiolate-imidazole catalytic are capable of forming complex of mixed type with Zn^{2+} which is centrally coordinated in a reversible manner i.e. a ‘‘sandwich of drug- Zn^{2+}-protease’’and water accessibility to the Zn^{2+} 2 uninhabited sites, and The potency of inhibition is determined by forces of attractiion vs. repulsiveness which stabilize the metal sandwich and the Electrostac Atraction, Hydrogen Bonds, Forces of Van der Waals, Hydrophobic Binding and Pi Interacons of the Biguanide Substituent Moieties and the Cysteinyl Cathepsin Subsites[25, 32].



(a) (b) (c)

Fig. 3 structure of a- biguanide, b- Metformin and c- Phenformin [116]

3) Bromelain and Lactoferrin: Inhibitor of bromelain VI [BI-VI; 5.89 kDa; heavy chain (H, 41 residues amino acid) and light chain (L, 11 residues of amino acid) that is a peptide exist in stem of pineapple acts as cathepsin L inhibitor (Ki 0.2×10^{-6} M) in a selective manner and at limited degree trypsin [35].On contrary, Lactoferrin is a protein exists at a lot of contents of human milk than these of cow's milk. It leads to inhibition of cathepsin L in a strong manner. The lactoferrin IC50 in opposition to cathepsin L was 10^{-7} M. Meanwhile, the synthetic peptide IC50 that aims at active site of cathepsin L was 10^{-5} M. Remarkably, lactoferrin has no role in inhibiton of both cathepsin H and cathepsin B[36]. Consequenty, this might permit cathepsin L fine targeting for impediment of internalization of SARS-CoV-2, meanwhile averting potential endangers to the cells. Both Bromelain and lactoferrin -VI, that manifest high selectiveness to cathepsin L, comprise multiple bonds of disulfide, adjusted in core of protein. As inhibition of cathepsin L is proceeded by thiol chemistry that provide much more preferentially cathepsin L inhibition instead of other cathepsins [2].

4) Vitamin D: which is produced within skin providing an adequate vitamin D source. Vitamin D subsequent metabolism to its active metabolite $1,25(OH)_2D_3$ [37]., qRT-PCR affirmed a transcriptomic analysis, revealed that expression of cathepsin L undergoes inhibition by $1,25(OH)_2D_3$ in MDA-MB-231 cells of breast cancer [38]. The previously mentioned protease has been associated with malignancy promotion in several types of cancer and viral infections and the inhibition of this protrease via $1,25(OH)_2D_3$ could be a part of the antitumor action of $1,25(OH)_2D_3$ in cancer of breast[39].

5) Hydroxychloroquine and Chloroquine: Hydroxychloroquine and Chloroquine are aminoquinolines which are commonly prescribed as treatment for rheumatic diseases and malaria, namely rheumatoid arthritis and systemic lupus erythematosus. The increase of endosomal pH one of the renowned cloroquine actions, that are capable to result in inhibition of activation of CatL , and subsequently protein S activation in the endosomes would be diminished [40], also Chloroquine interfere with ACE2 receptors glycosylation, an effect that is capable to cause impairment to the attachment of SARS-CoV particles to attachment of ACE2 [41-43].

6) Heparin: The spike protein of SARS-CoV-2 has an affinity to bind with heparin and its derivates and, consequently, inhibit viral infection [44]. In addition to that, it was

discovered that glycosaminoglycans bind to the site of cleavage of spike S1/S2 proteolytic (CatL) [45]. Considering that CatL is fundamental in term of viral invasion[46-49] and representation of heparin as CatL activity inhibitor by enhancing serpin inhibition of the CatL [50], the observed antiviral features for heparin could be associated with = impaired S1/S2 proteolytic throughout the time of SARS-CoV-2 cell invasion [51].

7) Ivermectin: According to the fact which states that proteolytical cleavage of S protein of SARS-CoV-2 virus occurs via type-II transmembrane serine protease (TMPRSS2) into subunits of S1 and S2 [52]. Ivermectin revealed high affinity of binding to the viral S protein and also surface receptors of the human cell ACE-2 and TMPRSS2. It has been found that ivermectin is docked between the ACE2 receptor and viral spike [53]. S protein activation via TMPRSS2 is capable of making activity of cathepsin L and low pH level unneeded for the fusion of viral envelope with the endosomal membrane [54]. Ivermectin molecular docking along with TMPRSS2 proposed an essential ivermectin role in terms of inhibition of the virus entry to the host cell, potentially by endosomal pH increase. In addition to that, it effectively binds to both serine-type protease or main protease (Mpro) and papain-like protease (PLpro) of SARS-CoV-2; thereby, it may also contribute to orevention of the viral polyproteins post-translational processing[55-60].

8) Statins: Legumain (asparaginyl endopeptidase) is defined as cysteine protease which is principally confined to the lysosomes and was characterized for the first time in mammals in 1997 [61], and its over-expression is correlated with instability of atherosclerotic plaque and malignancy of cancer [62, 63].It was found that legumain plays a role in cathepsin B and L maturation process [64]. Lately, it has been reported that legumain mRNA down-regulation in macrophages is ascribed to atorvastatin and also cathepsin L activity decrease in patients who were treated from statin with aortic aneurysms [65, 66]. In addition to that, it has been reported that extracellular glucose regulate the cysteine proteases cathepsin B, D, L and S activity in murine macrophage-like J774A.1 cells and human monocytes [67]. This, together along with the findings that simvastatin declined metabolism of glucose in myotubes and the reported legumain mRNA down-regulation by atorvastatin seen in monocytes [66].

9) Dexamethasone: Dexamethasone is responsible for inhibition of cathepsins B and L [68, 69] and is capable of being used with patient who develop a severe respiratory disorder and demands support of oxygen as this drug has already been represented to decrease mortality in this specific clinical situation [70]. Oral secretolytic agent **Bromhexine hydrochloride** is also taken into consideration as a probable candidate to add to such combinations which revealed inhibitory activity in opposition to TMPRSS2 and it is generally safe to use [71]. In addition to that, glycopeptide antibiotic **Teicoplanin** usage in hospitalized patients who evolve pneumonia as an outcome of secondary infections of bacteria since this antibiotic was represented as an inhibitor of cathepsin L [72, 73] and thereby might be advantageous in controlling viral infection as well.

10) Quercetin: it is defined as cysteine proteinases (cathepsins) from leishmania spp. Are propitious molecular targets in opposition to leishmaniasis. leishmania Mexicana cathepsin l is fundamental in the life span of parasite and of a crucial importance in mammals virulence factor. Natural products which were observed to show activity of antileishmanial manner were screened as an integral part of our continuous work which is dedicated to design inhibitors against the L. meexicana cathepsin L-like Rcpb2.8 amid them, tetrahydrorobustaflavone, agathisflavone, 3-oxo-urs-12-en-28-oic acid, and quercetin revealed obvious activity of inhibition on Rcpb2.8 along with IC50 values varying from 0.43 to 18.03 μM. (74). Quercetin is a powerful inhibitor of cathepsin with an IC50 in the low micromolar range [75]. Quercetin is a renowned and easily obtainable supplement and, thereby, it must be taken into consideration as an adjuvant in order to weaken or prevent such viral infections course. An elevated endosomal pH leads to inhibition of Cathepsin B/L activity and, thereby, chloroquine treatment will be capable of working [76]. A clinical trial of quercetin has been conducted on COVID-19 patients.(NCT04468139).

11) **Melatonin**: In term of organism functions, melatonin is capable of stabilization and regulation of plenty of its functions. Recently, it has been shown by investigators that administration of melatonin in low quantity doses did not only led to stimulatory impact on organism immune reactions, but also credit goes to–antioxidant properties, as it perform neutralization to the detrimental impact of stress [77-79]. it retrieves the organism hormonal homeostasis [80,81]. This impact reliesin on the used doses, but first and foremost it relies on the exogenous melatonin administration time [82,83]. this illustration of the melatonin immunological function became the foundation for the implementation of this hormone in the therapeutic approaches of several diseases, first and foremost in immunotherapy of neoplasm [84,85-87]. lysosomal space has a critical contribution in the organism defensive processes, controls of endocrine glands secretion, liquidation and transportation of substances which are foreign and utilized cellular organelles [88,89]. It actively plays a role in adaptive organism reactions, and preserves the organism stability in various environmental circumstances. Our outcomes have indicated that melatonin had a significant impact on the investigated glycosidases activity (BGRD, NAG), PROTEOLYTIC ENZYMES (aap,Cath.D and L) and lysosomal acid lipase of the lysosomal compartment. Exogenous melatonin, administered for 7 and 14 days in dose of 20 mg/kg b.w declined the activities of all the examined lysosomal space enzymes in the kidney and liver of female and male mice [90].

12) Gallinamide A: Several natural marine products are strong proteases inhibitors, an essential drug target class in human diseases. Thereby, extracts of marine cyanobacteria were evaluated for inhibition role to cathepsin L of human. Here, we have revealed that gallinamide A inhibitis the cysteine protease cathepsin L of human in selective and potent manner [91]

13) Catfish: It was figured that in majority of the proteinase Cathepsin L cause degradation of myofibril protein in pacific whitening fish surimi [92] and cannot be removed by conventional bleaching [93]. The purification of natural protease inhibitors has been successfully achieved into cystatin from eggs of Glassfish [94], ovarian fluid from tilapia fish, eggs of salmon, skin of fish from cod and Atlantic salmon [95], plasma of chum salmon and as inhibitors of trypsin from eggs of skipjack tuna[96], Catfish (pangasius sp.) in addition to other creatures, represent a source of natural inhibitors and enzymes which are capable of existing within cells (intracellular) and are either outside the cell (extracellular) or attached to the membrane [97]. Inhibitor of cathepsin was obtained by extraction from the muscle of catfish in the phase of pre-rigor. The cathepsin enzyme is found within the lysosomes. Meanwhile the inhibitor is found at pre-rigor phase and in cytoplasm, inhibitor of cathepsin and cathepsin itself continue to be separated [98].

3) Discussion

The novel coronavirus SARS-CoV-2 has spread globally in 2020 causing the pandemic atypical pneumonia coronavirus disease with more than 128 million confirmed cases and more than 2 million deaths worldwide (WHO statistics 31 march 2021). Pharmacological investigation of SARS-CoV-2 infection considers the endocytic pathways through which the opportunistic virus take advantage of the host cell and enters in the human lung epithelium. This fusion process is the most important moment for the virus to start the synthesis of viral proteins and the replication mechanism. Recent studies conducted some insight into the cell biology of the SARS-CoV-2 human virus showing that some receptors found on host cells' membrane are substantial for SARS-CoV-2 fusion and endocytosis, including ACE2 (Angiotensin Converting Enzyme 2), TMPRSS2 (Transmembrane Serine Protease 2) and cathepsin L. ACE2 receptors have many biological roles, however, it has been linked with COVID-19 through its binding with the spike protein of SARS-CoV-2, which allow the virus to exposed to TMPRSS2 and cathepsin L enzymatic receptors, thus enhance the fusion and endocytosis process.

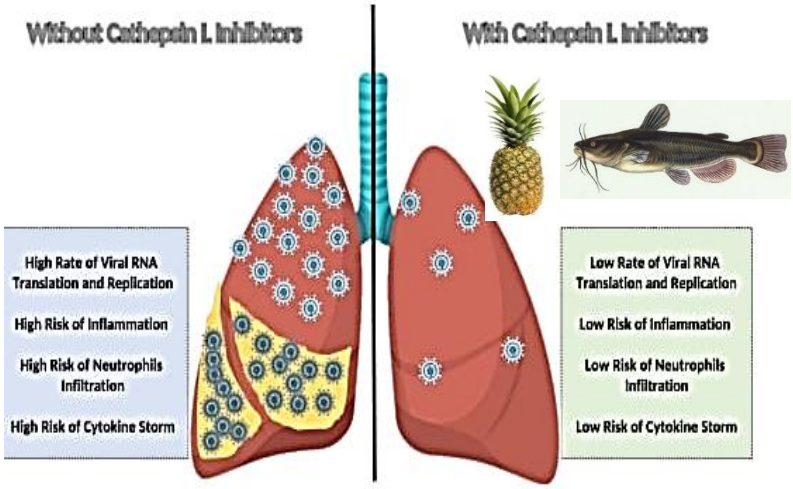


Fig. 4

Cathepsin L, a cysteine protease, tends to increase in chronic inflammation. It is expressed in all tissues and cells. It is responsible of proteolysis of protein antigens derived by pathogen endocytosis. Cysteine cathepsin inhibitors counteract the inflammatory response in combination with autoimmunity. Several studies demonstrates that multiple caspases inhibition are mediated by Zn^{2+}-binding allosteric exosites. It has been found that Zn^{2+} deficiency or dysregulation leads to an exaggerated activity of CysCts and increased autoimmune/inflammatory processes. Zn^{2+} metal can be safely combined with a drug that increases the anti-proteolytic effect of endogenous Zn^{2+} lowering the excessive activity of some CysCts. Biguanide derivatives complex with Zn^{2+} have been found to be promising inhibitors of CysCts protease reactions.

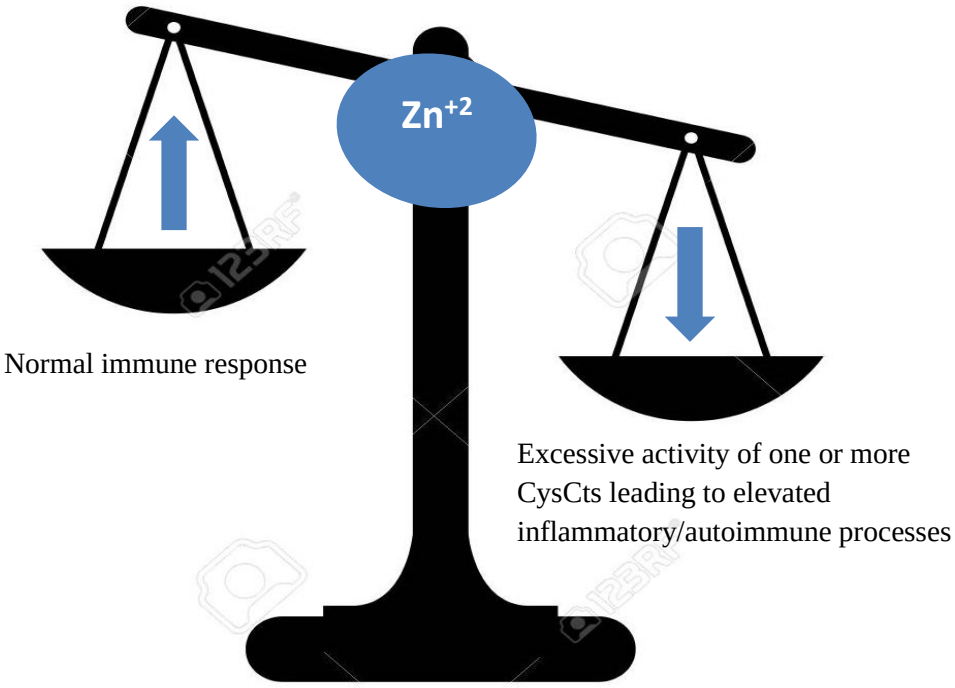


Fig. 5 : The effect of zinc on immune response

Although some drugs available nowadays have anti-cathepsin L activity like heparin, chloroquine, hydroxychloroquine, teicoplanin and amantadine, these drugs may exert anti-viral effect only when given at high doses (supra-therapeutic doses). Therefore, it is better to find or design a drug with higher potency and more selective against cathepsin L enzyme to ensure its efficiency and safety and when treating COVID-19 patients.

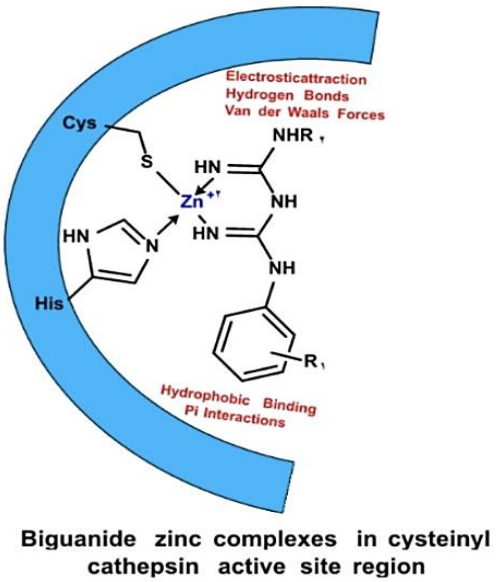
Another issue is the anticipated efficacy after inhibition of cathepsin L protease. As known, SARS-CoV-2 have more than one pathway to enter and infect human cells,

either via serine protease (TMPRSS2) or cysteine protease (cathepsin L). Blocking cathepsin L only will not probably be effective in reducing the viral RNA introduction to the host cells, in addition to its translation and replication process. This issue is the expected cause of unsuccessful treatment with some cathepsin L inhibitors (e.g. hydoxychloroquine) *in vivo*, despite its successfulness in eliminating SARS-CoV-2 *in vitro*. In this context, inhibition of both serine and cysteine proteases could result in more powerful efficacy in treating and protecting against COVID-19. Covid-19 requires the infection of pulmonary epithelial cells by cathepsin L (102) with upregulation of cathepsin L production via IL-6 (103).

4) Molecular Docking studies:

Metformin (Biguanide) through its two imino nitrogens forms a bidentate **Zn^{+2} complex** with the three amino acids **Cysteine-25 (thiolate), Histidine-163 (imidazole) and Aspartate-162** present at the active binding site of the Cathepsin L (Drug-Zn^{+2}-protease sandwich). Metformin-Zn^{+2} complex has the exact binding mode and interactions as the co-crystallized Zn^{+2} **(PDB ID 4axl)** because it forms the same two H-bonding with Cys-25 and His-163 as the co-crystallized ligand but with an additional H-bond with Asp-163.

The Cathepsin L protein was prepared by Autodock tool program and docking studies were performed using Pyrx and then visualized by Pymol.



Biguanide zinc complexes in cysteinyl cathepsin active site region

Fig. 6 Biguanide zinc complexes in cysteinyl cathepsin active site region

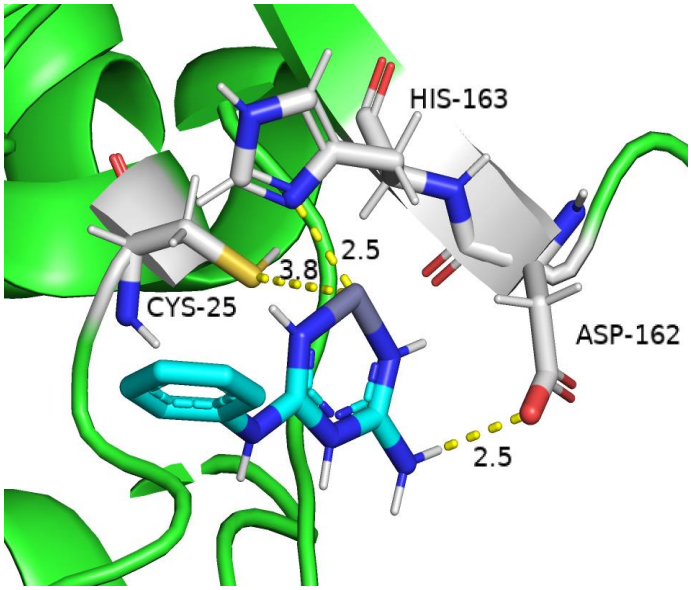


Fig. 7 Molecular docking of Cathepsin L Inhibited by Metformin-Zn^{+2} complex.

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