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Review Article

Liposome encapsulated clodronate mediated elimination of pathogenic macrophages and microglia: A promising pharmacological regime to defuse cytokine storm in COVID-19

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ABSTRACT

The emergence of new SARS-CoV-2 variants continues to pose an enormous public health concern. The SARS-CoV-2 infection disrupted host immune response accounting for cytokine storm has been linked to multiorgan failure and mortality in a significant portion of positive cases. Abruptly activated macrophages have been identified as the key pathogenic determinant of cytokine storm in COVID-19. Besides, reactive microglia have been known to discharge a surplus amount of proinflammatory factors leading to neuropathogenic events in the brains of SARS-CoV-2 infected individuals. Considering the fact, depletion of activated macrophages and microglia could be proposed to eradicate the life-threatening cytokine storm in COVID-19. Clodronate, a non-nitrogenous bisphosphonate drug has been identified as a potent macrophage and microglial depleting agent. While recent advancement in the field of liposome encapsulation technology offers the most promising biological tool for drug delivery, liposome encapsulated clodronate has been reported to effectively target and induce prominent phagocytic cell death in activated macrophages and microglia compared to free clodronate molecules. Thus, in this review article, we emphasize that depletion of activated macrophages and microglial cells by administration of liposome encapsulated clodronate can be a potential therapeutic strategy to diminish the pathogenic cytokine storm and alleviate multiorgan failure in COVID-19. Moreover, recently developed COVID-19 vaccines appear to render the chronic activation of macrophages accounting for immunological dysregulation in some cases. Therefore, the use of liposome encapsulated clodronate can also be extended to the clinical management of unforeseen immunogenic reactions resulting from activated macrophages associated adverse effects of COVID-19 vaccines.

Contents

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1. Introduction

The ongoing outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) instigating the comorbid coronavirus disease 2019 (COVID-19) has become one of the leading causes of death worldwide within a short span of time [1]. Though different types of vaccines have been rapidly developed to attenuate the existing forms of SARS-CoV-2, the emergence of new viral variants represents a huge challenge to the health care system worldwide [2-5]. Thus, the establishment of innovative therapeutic strategies and repurposing of available pharmacological agents against the innumerable pathogenic consequences of SARS-CoV-2 remains crucial [6,7]. Notably, severe COVID-19 cases have been characterized by an overwhelming surge of cytokine levels in the circulation and in highly susceptible organs including the lungs and brain. Of note, the secretome of activated macrophages, mast cells, endothelial, epithelial cells and microglia appears to be the underlying basis of cytokine storm in COVID-19 [6-10]. Among various immunogenic cells, abruptly activated macrophages have been identified as a key pathogenic determinant of destructive pathogenic events in various organs including the lungs in COVID-19 [11,12]. Besides, SARS-CoV-2 mediated activation of microglia leads to neuroinflammation and destructive neuropathological events in the brain [13]. As a result, a significant portion of COVID-19 patients exhibit comorbid clinical complications like macrophage activation syndrome (MAS), acute respiratory distress syndrome (ARDS), destructive neurological deficits, neuroregenerative failure and dementia [7,8,14]. Considering the aforementioned facts, elimination of the cellular basis of cytokine storm could be a promising therapeutic strategy to combat COVID-19 [15-17]. Depletion of hyperactivated macrophages and microglia by a highly specific pharmacologic agent might provide a prompt and effective medical relief against COVID-19. Clodronate, a non-nitrogen-containing bisphosphonate has been recognized for its role in the depletion of phagocytic macrophages in cancer metastasis and bone diseases [18-20]. Besides, clodronate has also been known to diminish pathogenically activated microglia in the brain [21]. However, the use of free clodronate molecules in specifically targeting abruptly activated immune cells appears to be a highly challenging approach against COVID-19. The recent scientific advancement in the liposome encapsulation technology offers the most promising scientific tool for drug delivery. Notably, liposome encapsulated clodronate has been reported to effectively target and induce prominent phagocytic cell death in activated macrophages and microglial cells compared to free clodronate molecules. Thus, this review article describes the deleterious effects of activated macrophages and microglia mediated hyperinflammation in COVID-19 and describes conclusive experimental evidence to underpin the beneficial effects of liposome encapsulated clodronate in some human diseases. Further, this article strongly emphasizes the repurposing of liposome encapsulated clodronate and its therapeutic perspective in effectively mitigating cytokine storm through inducing phagocytosis in pathogenic macrophages and microglia in COVID-19.

2. An overview of the detrimental roles of activated macrophages and cytokine storm in COVID-19

Upon infection, the progression of SARS-CoV-2 mediated clinical manifestation followed by the host immune responses over the period has been categorized into three pathological phases namely, 1) early infection phase, 2) pulmonary phase, and 3) severe hyperinflammation phase [22]. The early stage of COVID-19 has been characterized by the recruitment and spread of SARS-CoV-2 in the epithelial and blood cells via the cognate and non-cognate receptors accounting for pathogenic changes in haematological parameters including lymphocytopenia and haemostatic dysfunctions [23]. The second stage has been characterized by replication of SARS-CoV-2 and localized inflammation in the lungs, leading to pneumonia and hypoxia [22]. As the viral load increases in the hyperinflammatory phase, the cellular components of the immune-system are drastically activated and emit a surplus amount of proinflammatory molecules accounting for cytokine storm [24]. The hyperinflammatory phase has been characterized by fatal ARDS, and multi organ injury and failure. The lungs, liver, gastrointestinal system, heart, kidney and the brain are known to be highly vulnerable to SARS-CoV-2 mediated cytokine storm (Fig. 1).

While SARS-CoV-2 can directly infect the organs through receptors present on the host cells, multiorgan defects take place as a result of the activation of macrophage and uncontrollable immune response. Among various organs, the lungs are the primary direct target of SARS-CoV-2 mediated pathogenesis. The activated alveolar macrophages-mediated hyperinflammation in the lungs has been associated with pulmonary edema and fibrosis in critically ill COVID-19 patients [25]. Next, a considerable portion of COVID-19 positive cases show altered levels of liver enzymes, indicating the dysfunction of the hepatic system. It has been demonstrated that activation of the hepatic resident macrophage population and the invasion of pathogenic circulating macrophages leads to severe liver damage in COVID-19. Moreover, histopathological observation of liver autopsies from COVID-19 victims indicated hepatic steatosis and prominent degeneration of hepatocytes [26]. Though COVID-19 has primarily been characterized by respiratory syndrome, convincing clinical data have clearly established the association between COVID-19 and heart injury. As pre-existing cardiovascular diseases have been considered as the potential risk factor of COVID-19 related death, it has become increasingly evident that SARS-CoV-2 infection leads to arrhythmia, coronary defects, venous thromboembolism and myocardial failure in the subjects regardless of any history of heart diseases [27]. Thus, cardiac dysfunction has been recognized as a life-threatening clinical feature of COVID-19. Besides, COVID-19 appears to affect the gastrointestinal tract as a significant portion of subjects with COVID-19 display dysbiosis and ulcerative colitis [28]. As the severity of the disease progresses, COVID-19 escalates kidney injury with obvious signs of hematuria and nephritis due to inflammatory reactions in the glomerular compartments of the kidneys [29]. Further, neuroimaging and post-mortem brain studies have clearly indicated that SARS-CoV-2 infection leads to various neurological deficits and mental illnesses due to abrupt onset of neuroinflammation [8,14]. As the unregulated activation of macrophages has been identified as a key cellular basis of hyperinflammation, the pathogenic events resulting from SARS-CoV-2 infection in various organs appears to be propagated via abnormally activated circulating or tissue resident macrophages. Recent reports revealed that activated microglia can interfere with neuroregenerative properties of the brain and induce neurocognitive impairments in COVID-19 [7,8,14] (Fig. 1).

Macrophages are the subset of myeloid cell lineage that differentiates from the bone marrow-derived monocytes in the blood. Macrophages are responsible for a wide range of immunological functions

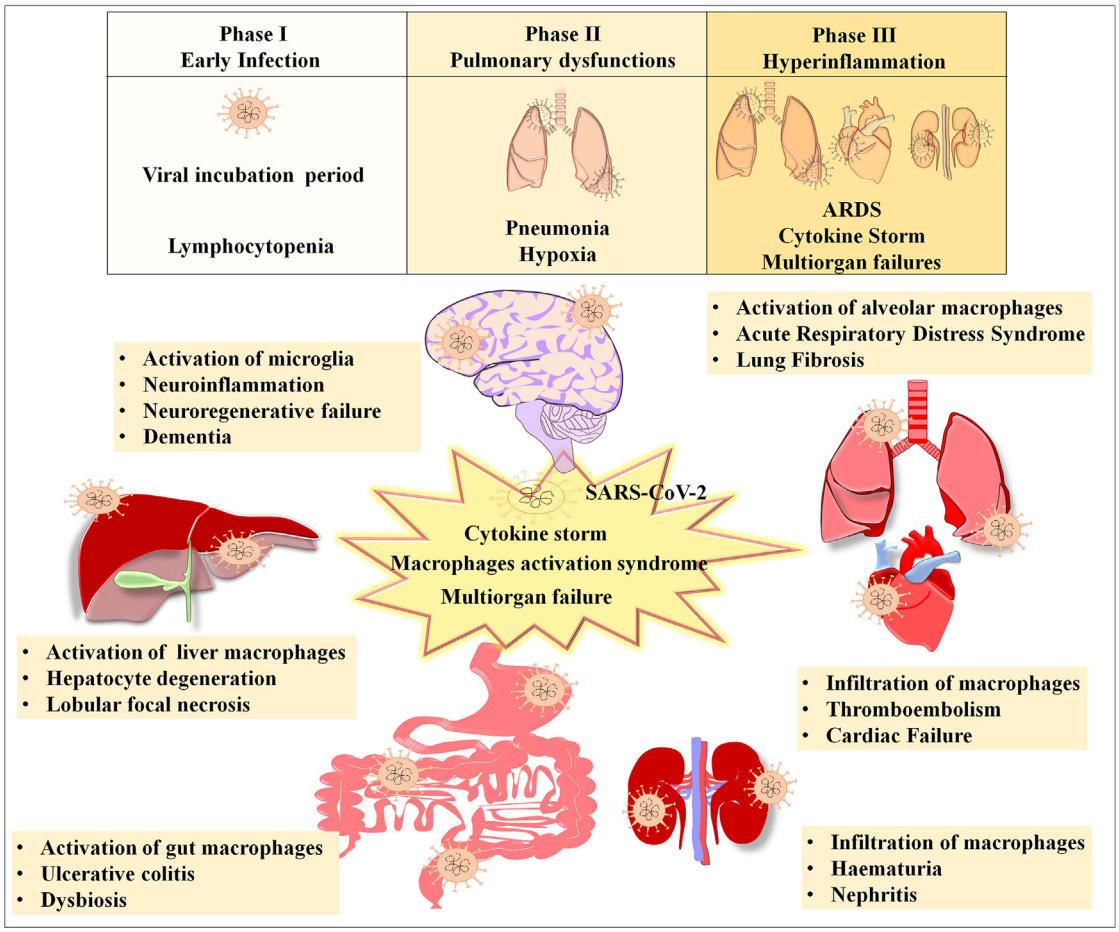

Fig. 1. Graphical representation of the key pathological hallmarks associated with activation of respective macrophages in various organs. The figure highlights SARS-CoV-2 mediated activation of macrophages, cytokine storm and clinical complications in lungs, liver, gastrointestinal tract, kidney and heart. Besides, the figure also indicates the SARS-CoV-2 mediated activation of microglial cells leading to neuroinflammation, regenerative failure in the brain and dementia.

including antigen presentation, tissue remodeling and phagocytosis [30]. Macrophages are normally found in an inactivated state throughout the body as various cellular entities such as histiocytes in lymph nodes, Kupffer cells in the liver, Hofbauer cells in the placenta, alveolar macrophages in pulmonary alveoli, osteoclast in the bone, and microglia in the brain [31] (Fig. 1). The molecular stimulants released during the interaction between any sort of antigen and the host cells can provoke the activation of macrophages. The inflammasomes induced activation of the natural killer (NK) cells, T-cell mediated cytotoxicity and pyroptosis have been recognized as the major pathogenic events associated with activated macrophages [32]. Notably, the macrophages undergoing pyroptotic cell death specifically release large amounts of IL-1α, IL-1β, IL-18 and high mobility group box protein (HMGB)-1 [33]. Certain inflammatory cytokines that originated from macrophages, such as TNF-α and IL-1, can lead to thrombosis [34]. The macrophages that receive inflammatory signals recruit T-cells into the site of inflammation and feedback inflammatory signals from T-cells further activate macrophages, thereby leading to cytokine storm [35].

The SARS-CoV-2 infection causes activation of immune cells and elicits inflammatory responses in the host [36,37]. The abnormally activated immune cells discharge an exceedingly large amount of immunoregulatory factors accounting for cytokine storm in COVID-19 patients [10,38-41]. Notably, cytokine release syndrome (CRS) in COVID-19 has been associated with abnormal levels of interferon (IFN)-γ, interferon γ-induced protein 10 kDa (IP-10), macrophage inflammatory protein (MIP)-1a, transforming growth factor (TGF)-β, tumor necrosis factor (TNF)-α, granulocyte colony-stimulating factor (G-CSF), and interleukins such as IL-2, IL-6, IL-7 and IL-10 [37,42]. Among them, elevated levels of IL-6 have been known to exacerbate the pathogenic severity of COVID-19 through TNF-α mediated activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) signalling [6,43,44]. Besides, an abnormal level of TGF-β has been identified to be involved in pulmonary fibrosis in COVID-19 [25,45]. Moreover, prominent elevation of C-reactive protein (CRP), an inflammatory indicator associated with disease severity and fatality has also been reported in the serum samples of COVID-19 patients [46,47]. In addition, the SARS-CoV-2 infection leads to the overproduction of inflammatory chemokines such as monocyte chemoattractant protein-1 (MCP-1) and the chemokine (C-X-C motif) ligand (CXCL)-1, 5, 10 in the circulation of COVID-19 patients [48]. The activated forms of myeloid cells appear to be the potential source of proinflammatory chemokines and cytokines in COVID-19 [37]. Among various immunogenic cells, the activated macrophages have been regarded as critical pathogenic determinants in COVID-19 as it induces tissue lesion through the abundant cytokine release [49]. The high level of inflammatory mediators like ILs and TNF, originating from the activated macrophages have been known to provoke NF-kB and mitogen-activated protein kinase (MAPK) signalling cascades in COVID-19 [50].

Besides, microglia, the resident macrophages of the brain, are known to be associated with the neuroimmune responses upon infection, injury and neurological illnesses. A “two-hit hypothesis” claims that COVID-19 patients exhibit neuroinflammation due to

pre-activated microglia that were rendered by adverse experiences during their early life [51]. A cohort study by Schurink et al. reported the prominent signs for the activation of microglia with the formation of nodules in the post-mortem COVID-19 brains [52]. Thus, the establishment of treatment methods against the root cause of the hyperinflammatory condition has become highly mandatory for COVID-19.

3. Recapitulation of the key pharmacological agents proposed or attempted against COVID-19

Recently, many pharmacological agents have been examined and proposed to neutralize cytokine storm in COVID-19. Initially, convalescent plasma therapy (CPT) has been considered the most common therapy for COVID-19 [53]. However, CPT has been identified to have some risks of inducing allergic reactions, alveolar damage, and possible transmission of the human immunodeficiency virus (HIV) and hepatitis viruses (HBVs). Next, inhibitors of ACE-2 have also been considered as a preventive measure against COVID-19 as it blocks the SARS-CoV-2 entry into the host cells [54]. However, the use of ACE-2 inhibitors has been found to be associated with many adverse effects, interfering with key physiological functions [55]. As most of the inflammatory cytokines and their downstream signalling mediators have been linked to the activation of pathogenic NF-kB signalling, blockade of the NF-kB pathway using inhibitor of nuclear factor kappa-B kinase subunit (IKK)-β antagonists has been proposed to be effective against inflammation in COVID-19 [6]. IL-6-STAT3 blockers have also been proposed to ameliorate cytokine storm in COVID-19 [39]. High-dose methylprednisolone along with calcineurin inhibitors like cyclosporine, a T-cell blocking agent has been considered to neutralize the cytokine storm in COVID-19 [56,57]. A few reports suggest the use of non-steroidal anti-inflammatory drugs (NSAIDs) to block pro-inflammatory responses mediated by cyclo-oxygenase (COX) and prostaglandins in COVID-19 [58]. The use of biosurfactants for their anti-inflammatory roles, cationic and antioxidant properties have been suggested for treatment against COVID-19 [59]. Previous studies revealed that glycyrrhizic derivatives possess antiviral effects against the SARS-CoV-2 and modify the inflammatory signatures in COVID-19 [60,61]. The use of nutraceuticals for modulating the miRNAs responsible for cytokine storm has also been considered to manage COVID-19 [62]. Though there exists a number of proposed therapeutic strategies for COVID-19, further exprimental validation and clinical trials remain to be established. Though the above-mentioned drugs are somewhat prescribed to manage COVID-19, their specificity and efficacy are not concurrent and they are known to be associated with some adverse effects (Table 1). Therefore, identifying the highly specific drug that targets the ultimate cellular source of cytokine storm might be a valuable strategy to tackle COVID-19. Considering the facts, implementation of a drug that has the ability to specifically deplete the activated macrophages could be of potential therapeutic aid in COVID-19.

4. Therapeutic significance of liposomal encapsulated clodronate in effective depletion of activated macrophages in various experimental conditions and diseases

Clodronate has been proven to be a highly specific macrophage and microglia depleting drug [75-78]. Clodronate acts on the mineral surfaces of the bone and suppresses the osteoclast-mediated bone resorption [77]. Clodronate can enter into the macrophages through endocytosis and disrupt the mitochondrial production of adenosine triphosphate (ATP) leading to their apoptotic cell death [78-81]. Mönkkönen et al. demonstrated that upon the intake of clodronate by cells, intracellular accumulation of adenosine 5′ (b,g-dichloromethylene) triphosphate (AppCCl2p) inhibits adenine nucleotide translocator (ANT) and alters mitochondrial membrane potential, thereby initiating apoptotic signalling cascade [78,81]. Clodronate has widely been used in the treatment of osteoporosis, osteoarthritis, myeloma and hypercalcaemia [75,82]. In addition to its anti-bone resorptive

Table 1
List of widely proposed or used drugs against COVID-19 with their advantages and disadvantages.

No	Drugs	Advantages	Disadvantages	Reference
1	Cyclosporine	Immunosuppressive drug, decreases the disease severity in the majority of patients with systemic juvenile idiopathic arthritis (sJIA) associated MAS	Neurotoxic and nephrotoxic effects, Kidney failure and hypertension	[63]
2	Emapalumab	Human monoclonal antibody raised against interferon-γ, used in hemophagocytic lymphohistiocytosis and MAS	Cause pyrexia, constipation and hypertension	[64]
3	Anakinra	An IL-1 receptor antagonist, commonly used against arthritis, used in combination with TCZ and corticosteroids to suppress the pro-inflammatory factors and MAS	Induces edema at the injection site and transaminase activity	[65]
4	Baricitinib along with remdesivir	A JAK-1/2 inhibitor, reduces serum levels of TNF-α, IL-1β, and IL-6 and plays a significant role in quick recovery from COVID-19	Associated with upper respiratory tract infections, increased LDL, cholesterol, nausea and thrombocytosis	[66]
5	Chloroquine and derivatives	Significantly decreases the production of pro-inflammatory factors and reduces disease severity in COVID-19 patients	Gastrointestinal discomfort, Ocular toxicity and cardiotoxicity	[67]
6	Colchicine	Proposed to inhibit inflammasome signalling in COVID-19	Gastrointestinal defects and Pulmonary embolism	[68]
7	Etoposide	Potential candidate drug against MAS as it induces apoptosis in activated T cells and malignant tumor cells	Alopecia and gastrointestinal toxicity	[69]
8	Eculizumab	Immunosuppressant and improves survival and reduces hypoxia in randomized clinical trials	Meningococcal infection	[70]
9	Glycyrrhizic derivatives	Inhibit replication of SARS resulting in reduced pulmonary inflammation and microvascular permeability	Cardiac dysfunction edema, and hypertension	[71]
10	Plasma exchange therapy	Improve survival rate and beneficial in treating cytokine storm associated secondary MAS	Allergic reactions, alveolar damage and possible transmission of HIV and HBV	[72]
11	Rituximab	Anti-CD20 rituximab therapy has been proposed to attenuate MAS in COVID-19	Induces hyper immune reactions	[73]
12	Tocilizumab	Proposed for treatment against COVID-19 as it reduces the circulating levels of CRP	Headache, hypertension and abnormal liver function	[74]

capability, clodronate also exhibits anti-inflammatory and analgesic properties [83,84]. Studies have shown that clodronate reduces the release of inflammatory cytokines such as TNF-α, IL-1b and IL-6 [85]. Clodronate-mediated depletion of macrophages appear to be effective in reducing the levels of proinflammatory cytokines [86,87]. However, use of free clodronate molecules have some practical disadvantages due to their less permeability and short half-life. Alternatively, liposome-encapsulated clodronate has been identified to yield better results in targeting phagocytic macrophages. Initially, the conventional preparation of liposomes involve dissolving the phospholipid molecules into an appropriate organic solvent such as chloroform and methanol followed by evaporation of the organic solvent at 60 °C for 15 min to obtain thin lipid bilayer films. Further, incubation of a salt form of clodronate containing aqueous solution with lipid bilayer films at 60 °C for 15 min and simultaneous sonication can yield the encapsulation of clodronate in the liposomes. The non-encapsulated clodronate can be removed by centrifugation or filtration. In various in vitro and in vivo experiments, the effect of liposomal encapsulated clodronate has been tested against macrophages [18,88] (Fig. 2). It has been well established that liposomes act as an efficient biological cargo of drugs and bioactive molecules. The liposome encapsulated drugs have several advantages including biocompatibility, self-assembly, and the ability to carry ample molecules. Given the wide range of physicochemical and biophysical properties of liposomes, they can be modified to acquire the desired characteristics [89]. Liposomal encapsulation protects the drug from degradation by the gastric acid, ensures the bioavailability of the effectors in the bloodstream and transportation to various organs, whereas ingestion of unbound small molecules can easily be destroyed by gastric acid and their half-life appears to be very minimal in the circulation [89]. Moreover, the systemic circulation of the free molecules may represent some nonspecific and undesirable effects. However, liposome encapsulation of drugs has many practical advantages as it can minimize the dilution of drugs in the circulation with longer half-life. Eventually, liposomal encapsulated drugs can specifically be directed towards or attracted by designated pathogenic cells and organs [90] (Fig. 2).

Earlier, Mönkkönen and Heath have reported that liposome-encapsulated clodronate is about 350-fold more effective in deactivating the macrophages than free clodronate molecules. While free clodronate appears to act on extracellular level, macrophages engulfs liposome-encapsulated clodronate thereby, committing to prominent phagocytic depletion [91] (Fig. 3). Moreover, Van Rooijen and Sanders described the macrophage 'suicide' technique, from which it has become apparent that liposome-encapsulated clodronate treatment specifically eleminates the phagocytic macrophages, while non-phagocytic cells are not vulnerable to liposome-encapsulated clodronate [92]. It has been reported that intravenous administration of 20 mg of clodronate encapsulated with unilamellar liposomes significantly reduced macrophages and diminished inflammation in an experimental rat model of arthritis, while treatment of free clodronate had no effect in targeting macrophages [93,94]. The radioactive labelling-based animal experiments by Buiting have already demonstrated the in vivo distribution of clodronate in various organs including the liver and spleen [95]. Besides, the whole animal in vivo experiments described that the use of clodronate-liposome solution leads to efficient depletion of macrophages in the bone marrow, spleen, liver, lungs, brain, gut, peritoneal cavity, lymph nodes and in circulation thereby, suggesting liposome-encapsulated clodronate can spread to various organs in the body [96] (Figs. 2, 3, 4).

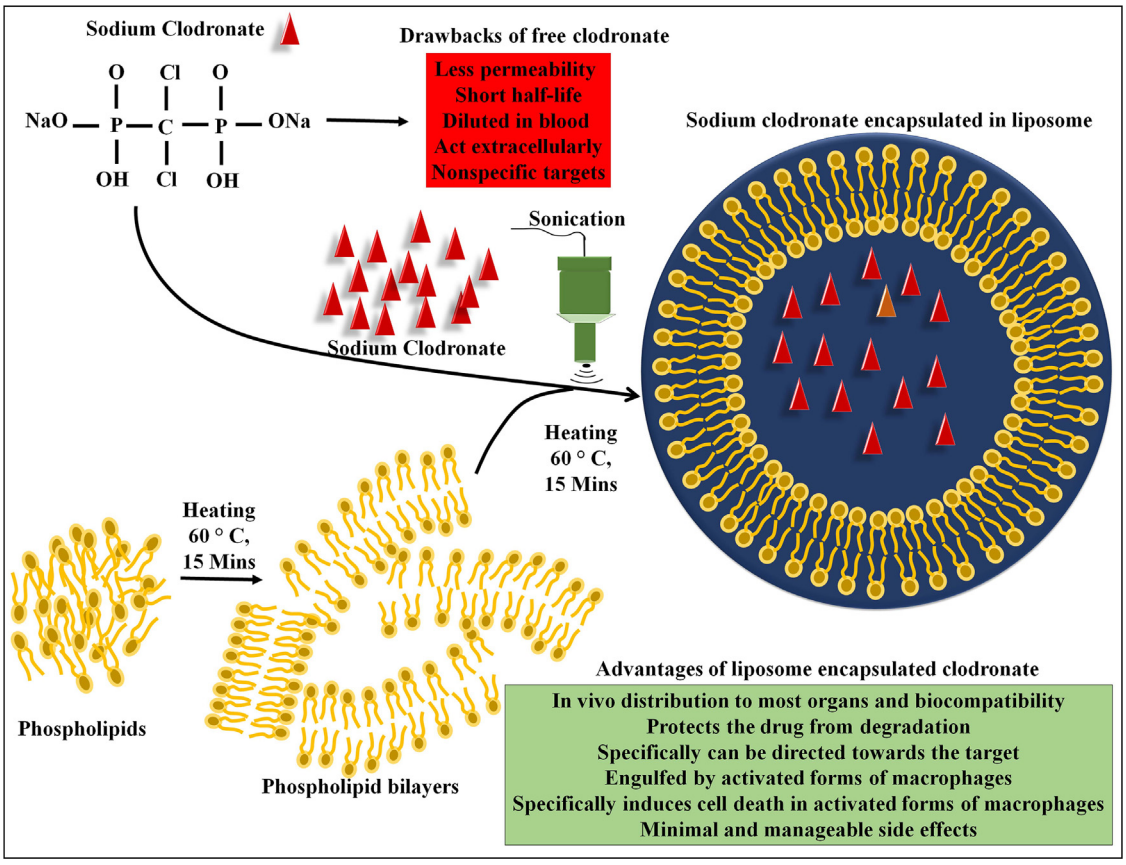

Fig. 2. Schematic representation of steps involved in the preparation of liposomal clodronate. The figure provides an overview of experimental method for the generation of liposomal clodronate that includes the preparation of lipid bilayer films from phospholipids followed by incorporation of sodium chlodronates in liposomes with help of sonication treatment.

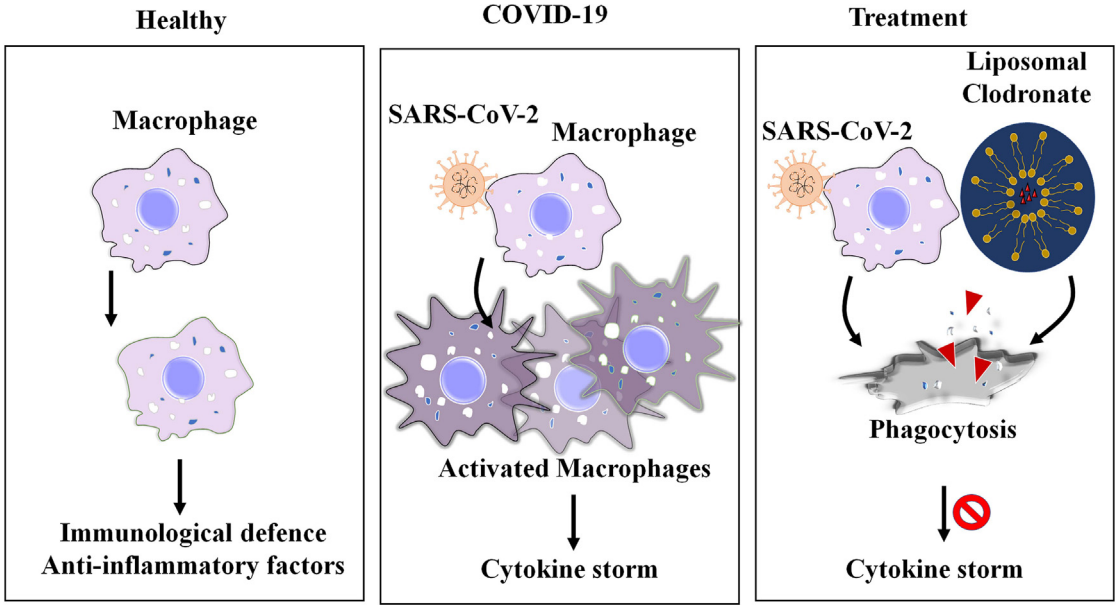

Fig. 3. Graphical illustration for the clodronate liposomes mediated depletion of activated macrophages in COVID-19. The figure depicts the role of macrophages in healthy condition and SARS-CoV-2 mediated macrophage activation leading to cytokine storm, and depletion of activated macrophage by clodronate liposomes as a proposed treatment option to mitigate cytokine storm in COVID-19.

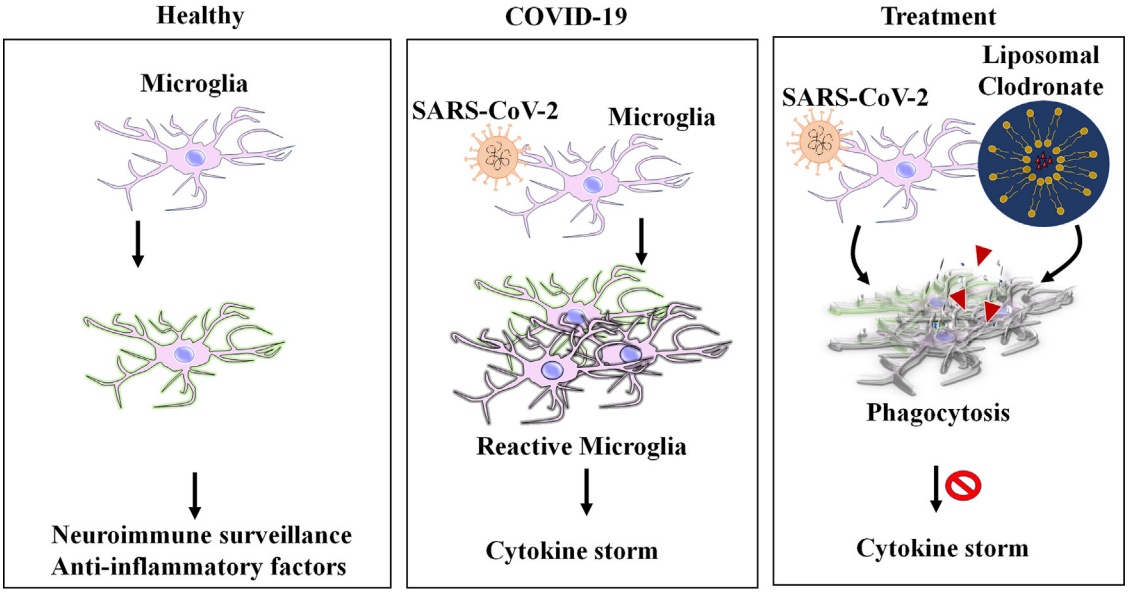

Fig. 4. Graphical illustration for the clodronate liposomes mediated depletion of activated microglia in COVID-19. The figure depicts the function of the brain resident microglia in healthy condition, SARS-CoV-2 mediated activation of macrophages responsible for cytokine storm, and depletion of activated microglia by clodronate liposomes as a possible treatment option to eradicate cytokine storm in the brain of subjects with COVID-19.

Notably, pulmonary surfactant is a mixture of phospholipids and proteins secreted by the alveolar cells in the lungs. The pulmonary surfactants have high biocompatibility with liposomes that are used for encapsulation of drugs. Thus, formulated liposomes can efficiently be attracted and observed by the lungs via pulmonary surfactants. Ample reports indicated that liposomal inhalants represent emerging carriers in pulmonary drug delivery strategy and play an important role in treating various lung disorders including acute respiratory distress syndrome (ARDS). Elder et al. reported that intratracheal inhalation of liposomes containing clodronate efficiently depletes alveolar macrophages in experimental rats [97]. Recent data suggests the therapeutic effectiveness of liposome-encapsulated clodronate through intratracheal instillation against pulmonary fibrosis by inhibiting the macrophages in the lungs of LyzM-Cre/ Methyl-CpG–binding domain 2 lox/flox (Mbd2-CKO) mice [98].

Clodronate liposome treatment in a mouse model of colorectal cancer revealed a reduction in the expression of macrophage cell markers and cytokines such as IL-13, TGF-β, IL-10 and CCL-17 in the colon leading to arrest in tumor progression [99]. Besides, an experimental murine model of myeloma treated with clodronate liposomes has also showed a proper depletion of macrophages and considerable redcution of the tumor mass [20]. Satyanarayanan et al. reported that clodronate treatment-induced drastic reduction in peritoneal macrophages in a mouse model of peritonitis [100]. Besides, Kameka et al. demonstrated

that a single dose of clodronate liposomes can be sufficient for the depletion of macrophages in the spleen and lungs of experimental chickens [101]. Moreover, the depletion of macrophages by clodronate-encapsulated liposomes have been reported to be highly efficient in infectious models such as Taenia crassiceps cysticercosis, influenza virus and dengue [102-105]. Roscic-Mrkic et al. demonstrated clodronate liposome-mediated depletion of monocyte/-macrophages in a genetically modified mouse model with measles virus infection [105]. Several studies highlighted the depletion of macrophages in experimental mice by administration of clodronate-liposomes at weekly intervals [106,107]. In the influenza pig model, Kim et al. reported the depletion of alveolar macrophages by liposome-encapsulated clodronate [81]. In addition, Mert et al. demonstrated the anti-inflammatory effect of liposome-encapsulated clodronate against activated macrophages and neutrophils in the carrageenan-induced inflammation model [84]. Histological and immunohistochemical studies in a mouse model of rheumatoid arthritis (RA) indicated that macrophage depletion by clodronate liposomes is beneficial against inflammation [108]. In an immunofluorescence based study by Wang et al. to analyse the effect of clodronate liposome against neuropathic pain in a rat model indicated a transient depletion of microglia in the spinal cord [109]. Moreover, animal experimental studies revealed that treatment of liposamal clodronate leads to depletion of microglia in the brain and retina [110,111]. Besides, liposamal clodronate treatment has been reported to attenuate the zymosan-induced activation of NF-kB, Fos expression and hypothermia [112]. Han et al. demonstrated that the direct injection of liposome-encapsulated clodronate to the striatum of experimental animals result in depletion of microglia [113]. Moreover, liposome-encapsulated clodronate has also been considered as an antiosteolytic therapeutic agent in bone pain coupled with skeletal metastases in patients with breast cancer and multiple myeloma [114,115]. In addition, clodronate has proven to be effective against bone-related diseases like osteoporosis, osteopenia, osteolytic lesions, and hypercalcemia [116]. A clinical trial by Frediani et al. indicated that intramuscular injection of clodronate provides therapeutic relief against osteoarthritis and bone marrow edema [117]. Besides, clodronate treatment appears to be effective against radiation induced bone necrosis in head and neck cancer patients [118]. Taken together, the use of clodronate has been prevalent in the treatment of breast cancer, bone metastasis, multiple myeloma, osteolytic lesions, and osteoporosis. Moreover, clodronate has been suggested to reduce inflammatory cytokines and also act against nitric oxide secretion from macrophages [119,120].

5. Prospective for the use of liposome encapsulated clodronate for the treatment regime against COVID-19 and to manage COVID-19 vaccinations related adverse effects

It has become clearly evident that elevated levels of proinflammatory cytokines have been associated with various pathogenic events, irreversible multiorgan failure and mortality in a significant portion of severely affected COVID-19 patients. Thus, the pathogenic severity of COVID-19 has been directly linked to cytokine storm. Despite the accumulation of reports advocating the use of many anti-inflammatory drugs, COVID-19 has been refractory to the tailored treatment options. Though vaccinations have been implemented to prevent the spread of SARS-CoV-2 infection, improper vaccination drives and the emergence of new viral variants pose a huge challenge to the health care system in many countries. Therefore, establishment of better alternate therapeutics targeting the activated immune cells needs to be considered. Chronically activated macrophages have been very well established potential cellular sources of cytokine storm in COVID-19. Besides, neuropathogenic events and neuroinflammation resulting from SARS-CoV-2 infection have been reported to be associated with activated microglial cells in the brain. Therefore, it can be hypothesized that depletion of activated macrophages and microglial cells by a specific drug could be highly beneficial in eliminating the life-threatening cytokine storm in COVID-19. A plethora of preclinical studies and clinical trials in patients with cancer and bone disease has clearly demonstrated that liposome encapsulated clodronate has the ability to deplete macrophages responsible for cytokine storm. Considering a well-established specificity in depleting activated forms of macrophages, biocompatibility, widespread in vivo distribution, it can be proposed that liposome encapsulated clodronate might be an ideal therapeutic candidate to harness the activated macrophages and microglia in COVID-19. The liposome encapsulated clodronate can be delivered via non-invasive or minimal invasive routes in the forms of oral suspensions, intravenous injections and nasal spray. The use of liposome encapsulated clodronate can be expected to result in ablation of the abnormal cytokine storm and thereby preventing the multiorgan failure in COVID-19 (Figs. 3 and 4).

While COVID-19 vaccines are safer and more effective in providing boosting immunity against SARS-CoV-2 infection with relatively very rare side effects, a set of recent clinical evidence indicates the activation of inflammatory cascade upon COVID-19 vaccination in a significant number of receivers. However, the degree of side effects varies among different formulations of vaccines generated against SARS-CoV-2. Notably, COVID-19 vaccinations have been known to be associated with thrombotic thrombocytopenia in some cases [121]. Ample reports indicate that COVID-19 vaccinated individuals have considerably increased risk of reinfection to SARS-CoV-2 [122,123]. Notably, in some individuals, COVID-19 vaccination developed destructive macrophage activation and multisystem inflammatory disease similar to the pathogenesis seen in COVID-19 [124,125]. In some cases, the immoral immune responses of COVID-19 vaccinations appear to be associated with chronic activation of various immune cells including macrophages resulting in cytokine storm and unforeseen adverse effects including MAS and ARDS [126]. Considering the facts, liposome encapsulated clodronate might also be a beneficial in depleting activated macrophages in individuals who exhibit hyperinflammation upon COVID-19 vaccination.

Though the use of clodronate can be effective in depleting the pathogenic macrophages, it might be associated with depletion of the physiological macrophages. The unpresented depletion of physiological macrophages might be related with immunological dysfunction in healthy situations. However, recent reports suggest that physiological macrophages can be repopulated over the time or upon the withdrawal of liposome-encapsulated clodronate. Moreover, ample reports suggested the depletion of macrophages by clodronate have resulted in the amelioration of various disease conditions with no side effects or drawbacks of the treatment. To note, oral administration of clodronate appears to be associated with gastrointestinal toxicities, esophagitis and diarrhoea. Chronic administration of liposome-encapsulated clodronate might be associated with hypercalcaemia. The incidence of adverse effects appears to be very low and are not life-threatening as they can be managed by lowering the doses of clodronate. However, future experimental evaluations and pharmacodynamics of liposome encapsulated clodronate in preclinical models of COVID-19 needs to be considered.

6. Conclusion

The macrophage depleting drug, liposome encapsulated clodronate has gained a greater clinical advantage and use in the treatment of cancer-associated bone diseases and osteoporosis. The macrophages and microglia depleting effect of liposomal clodronate has already been well-established in vitro and in vivo preclinical models of malignant disorders and bone diseases. In addition, the reports on preclinical studies suggest the use of clodronate as an efficient therapeutic strategy to mitigate various human diseases that are associated with

activated immune cells and severe inflammation. Therefore, we propose the transient use of liposome encapsulated clodronate as a potential therapeutic strategy to eliminate the pathogenic macrophages and microglia to defuse cytokine storm in COVID-19 (Figs. 3 and 4). The proposed treatment regime of liposome encapsulated clodronate could be higly effective and beneficial in diminishing the cytokine storm responsible for life-threatening pathogenic events in COVID-19. Though the available reports on the use of clodronate for various disease conditions revealed no considerable adverse effects even when utilising a high dose and for a prolonged period of time, predictable and unknown side-effects associated with clodronate treatment may not be completely ignored.

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Author contributions

MK conceived the idea and generated the graphical illustrations. SR and NM performed the literature search and made the initial draft. SR, NM and MK contributed to the revision of article. All authors discussed the content and contributed to the final manuscript.

Ethical approval and informed consent

Not applicable.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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