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Journal of Steroid Biochemistry and Molecular Biology 212 (2021) 105939

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

# 7-Ketocholesterol: Effects on viral infections and hypothetical contribution in COVID-19

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ARTICLE INFO

*Keywords:*
Adjuvant therapies
COVID-19
7-Ketocholesterol
Oxysterols
SARS-CoV-2
Viral diseases

ABSTRACT

7-Ketocholesterol, which is one of the earliest cholesterol oxidization products identified, is essentially formed by the auto-oxidation of cholesterol. In the body, 7-ketocholesterol is both provided by food and produced endogenously. This pro-oxidant and pro-inflammatory molecule, which can activate apoptosis and autophagy at high concentrations, is an abundant component of oxidized Low Density Lipoproteins. 7-Ketocholesterol appears to significantly contribute to the development of age-related diseases (cardiovascular diseases, age-related macular degeneration, and Alzheimer's disease), chronic inflammatory bowel diseases and to certain cancers. Recent studies have also shown that 7-ketocholesterol has anti-viral activities, including on SARS-CoV-2, which are, however, lower than those of oxysterols resulting from the oxidation of cholesterol on the side chain. Furthermore, 7-ketocholesterol is increased in the serum of moderately and severely affected COVID-19 patients. In the case of COVID-19, it can be assumed that the antiviral activity of 7-ketocholesterol could be counter-balanced by its toxic effects, including pro-oxidant, pro-inflammatory and pro-coagulant activities that might promote the induction of cell death in alveolar cells. It is therefore suggested that this oxysterol might be involved in the pathophysiology of COVID-19 by contributing to the acute respiratory distress syndrome and promoting a deleterious, even fatal outcome. Thus, 7-ketocholesterol could possibly constitute a lipid biomarker of COVID-19 outcome and counteracting its toxic effects with adjuvant therapies might have beneficial effects in COVID-19 patients.

## 1. Origin and metabolism of 7-ketocholesterol

Cholesterol ((3β)-cholest-5-en-3-ol; $C_{27}H_{46}O$; molecular weight: 386.65 g/moL) is a lipid which is both provided by the diet and formed endogenously, except in the brain where the cholesterol present is only produced by astrocytes [1,2]. After a meal, cholesterol transiting through enterocytes is taken up by the enteric capillaries as chylomicrons which will transport it to the liver, where it will be distributed to

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https://doi.org/10.1016/j.jsbmb.2021.105939
Received 29 April 2021; Received in revised form 4 June 2021; Accepted 7 June 2021
Available online 9 June 2021

the peripheral organs via low density lipoproteins (LDLs); its return to the liver is then ensured by the high density lipoproteins (HDL) [3]. In all the cells of the body, acetyl-CoA is the first element involved in the biosynthesis of cholesterol. Endogenous cholesterol synthesis involves several enzymes which, after conversion of acetyl-CoA into mevalonate by the enzyme HMG-CA reductase, will then be converted to squalene, which is subsequently metabolised to lanosterol. From lanosterol, which contains thirty carbon atoms (C30), the biosynthesis of cholesterol (C27) takes two routes: the Bloch pathway and the Kandutsch-Russell pathway. The Bloch pathway produces cholesterol precursors ranging from 14-demethyl-14-dehydrolanosterol (ff-MAS; C29) to desmosterol (C27) and includes zymosterol (C27); the Kandutsch-Russell pathway generates products spanning from 24-25-dihydrolanosterol (C30) to 7-dehydrocholesterol (C27) and includes zymostenol (C27) and lathosterol (C27) [4]. These two pathways can generate cholesterol via the enzymes 24-dehydrocholesterol reductase (DHCR24) and 7-dhydrocholesterol reductase (DHCR7): the enzyme DHCR24 generates cholesterol from desmosterol while the enzyme DHCR7 generates cholesterol from 7-dehydrocholesterol [5,6]. Whether endogenous or exogenous in nature, in a pro-oxidant environment cellular, cholesterol can then give oxidized derivatives in positions 4, 5, 6 and 7 [7–9]. Most often radical attacks by reactive oxygen or nitrogen species (ROS or RNS) take place on carbon 7, because of the weak link between carbon and hydrogen at this position [9,10]. This local oxidation at carbon 7 then generates a peroxyl radical (ROO$^{\bullet}$) which, by reacting with hydrogen, forms cholesterol hydroperoxide (7α- or 7β−OOHC) [11]. As the hydroperoxide bond is very unstable, 7-ketocholesterol (7KC), also named 7-oxo--cholesterol [12,13], is formed in the majority of cases, and 7β-hydroxycholesterol and 7α-hydroxycholesterol in smaller quantities [11] (Fig. 1). Under certain conditions, it has also been shown that 7KC can be formed via the enzyme sterol 7-hydroxylase (CYP7A1) from 7-dehydrocholesterol (7-DHC) present in large quantities in the plasma of patients with Smith Lemli Opitz (SLO) syndrome [4]. In addition, 11β-hydroxysteroid-dehydrogenase type 2 (11β-HSD2), which converts cortisol to cortisone, is also responsible for the conversion of 7β-hydroxycholesterol to 7KC [14].

Overall, 7KC can be detected in the plasma at LDL level or bound to albumin as well as in the plasma membrane of the cells of different

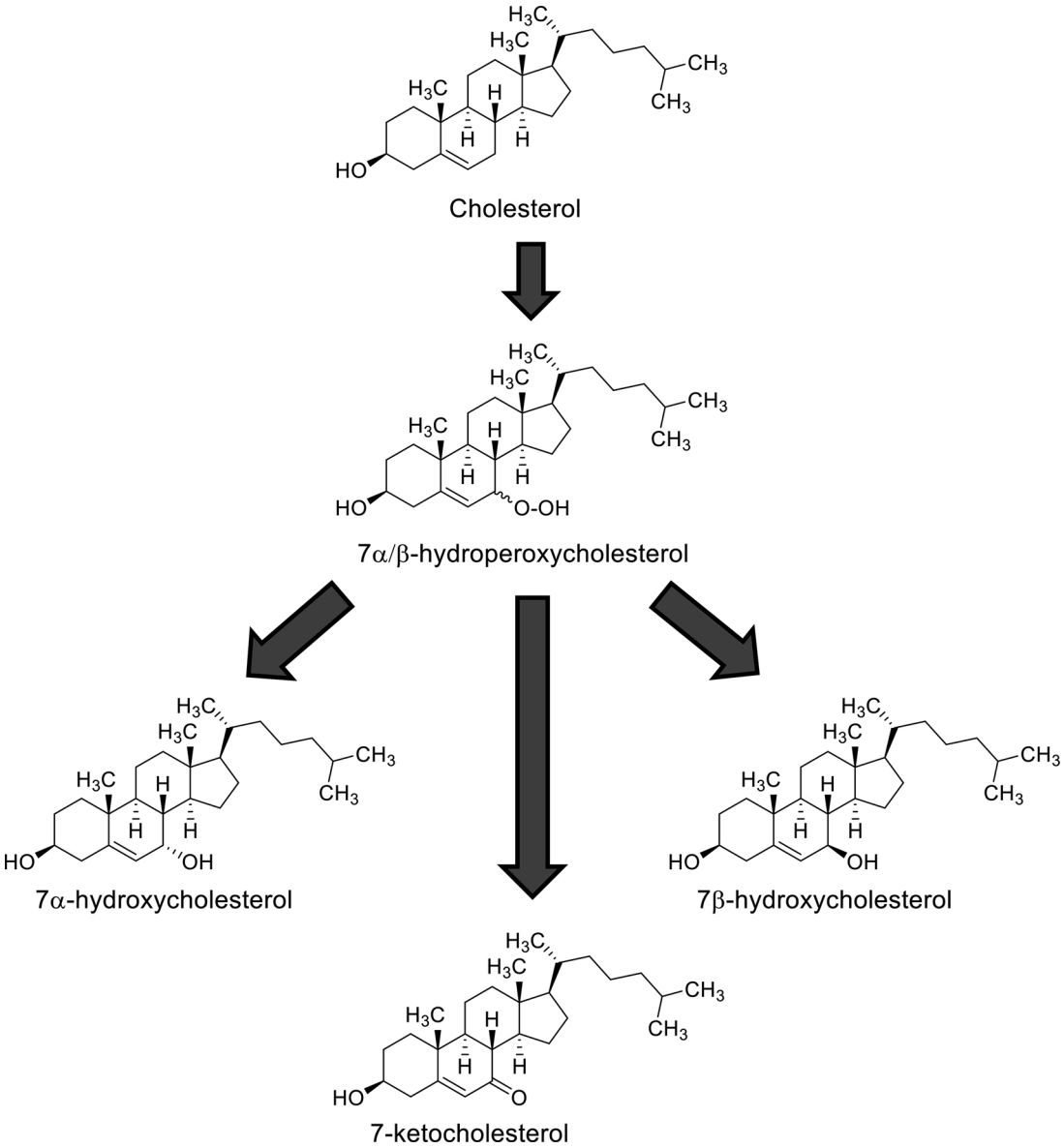


**Fig. 1. Biosynthesis of 7-ketocholesterol.** The biosynthesis and the metabolism of 7-ketocholesterol (7KC) are described in the detail by Wang et al. [23], Brahmi et al. [72], Vejux et al. [14] and Griffiths et al. [24]. For detailed information on the biosynthesis and degradation of 7KC and 7β-hydroxycholesterol, please see the review from Nury T et al. [112].

tissues [15]. It is also well established that 7KC can react with different molecules (sulfate, fatty acids) via the hydroxyl group (OH) localized on C3 in the ring A of the sterane nucleus, which leads to inhibition of its toxicity [16,17]. The enzyme sulfotransferase 2B1b (SULT 2B1b) is involved in the sulfonation of 7KC [18]. As for the esterification of 7KC, a combined action of cytosolic phospholipase A2 alpha (CPLA2 α) and sterol-O-acyltransferase (SOAT1) has been reported [19]. In addition, Acyl-coenzyme A transferase (ACAT, also abbreviated as SOATs) which converts cholesterol into cholesterol ester can use oxysterols as substrates: oxysterols are also substrates for SOAT1 and SOAT2 [20]. The lecithin cholesterol acyl-transferase (LCAT) also esterifies oxysterols in the plasma [15]. At the moment, it has been described in retinal pigment epithelium that 7KC can be metabolised by the enzyme 27-hydroxylase cytochrome P450 27A1 (CYP27A1) to form two metabolites, 3β, 27-dihydroxy-5-cholesten-7-one (7KC-27OHC) and 3β-hydroxy-5-cholesten-7-one-26-oic acid (7KC-27COOH), to reduce the toxicity of 7KC [21,22]. It is therefore important to know the biological activities of 7KC, and of its metabolites which are still not well known [23,24], as well as their related signalling pathways, in order to identify therapeutic targets and develop drugs acting on them to treat diseases associated with high levels of 7KC in tissues and biological fluids.

## 2. Pathologies associated with 7-ketocholesterol

Large amounts of oxysterols formed by auto-oxidation, particularly 7KC, have been initially detected in oxidized LDL (LDLox) and in atheromatous plaques. Since there is a positive correlation between the content of oxysterol-rich LDLox and induction of cell death, it has been suggested that 7KC may play a crucial role in atherosclerosis and cardiovascular diseases [25,26]. Moreover, analogies between atherosclerosis and the development of age-related macular degeneration (AMD) (significant presence of 7KC in lipid deposits called drüsens located between the Bruch membrane and the monolayer of retinal epithelial cells) also indicate involvement of 7KC in the pathophysiology of this disease [27,28]. A potential involvement of 7KC in the pathophysiology of cataract, which affects one person in five from the age of 65, and more than 60 % of people aged 85 and over, is also suspected [29]. Thus, the exposure of membranes isolated from transparent human lenses to 2, 2′-azobis(2-amidinopropane) hydrochloride, a free radical generator, promotes the production of 7KC (74 %) as the main cholesterol oxidation product [30]. In addition, cataract lenses contain quantifiable amounts of 7KC (4.2 +/- 0.32 mmol / mol of cholesterol), whereas clear lenses from cataract-free subjects do not contain detectable amounts [30]. Moreover, the presence of high levels of 7KC in the brain lesions of Alzheimer's disease patients suggests an involvement of 7KC in this prevalent neurodegenerative disease [31,32]. 7KC also appears to be involved in chronic inflammatory bowel diseases [33] as well as in some rare diseases of lipid metabolism, such as X-linked adrenoleukodystrophy (X-ALD) [11,34]. High levels of 7KC have also been described in non-infectious but severe inflammatory lung diseases such as silicosis [35,36]. In addition, air pollution which affects the respiratory system by promoting oxidative stress, increases the production of 7KC and promotes atherosclerosis by activating CD-36 positive macrophages [37]. Currently, several ozone-oxidized cholesterol products have been identified and can be considered as a new class of oxysterols [10,38]. The common denominators of 7KC-associated diseases are high levels of oxidative stress and inflammation which can in turn amplify the formation of 7KC, as well as organelle dysfunction (of mitochondria, peroxisomes, lysosomes, endoplasmic reticulum) [39] and subsequently contribute to the amplification of this stress [40]. Furthermore, organelle oxidative stress, mainly at the mitochondrial and peroxisomal level, could favour the formation of 7KC, which is itself strongly pro-oxidative and pro-inflammatory [41–43].

Beside age-related diseases, increases in 7KC have also been described in the context of viral infections. For example, in patients with type 2 diabetes who are co-infected with herpes virus type 8 (HHV8), significant increases in plasma levels of 7KC have been observed possibly amplifying diabetic complications [44]. It is hypothesized that the HHV8-infection may contribute to ROS overproduction which would trigger lipid peroxidation and cholesterol autoxidation, leading to 7KC formation [44]. Similarly, elevated plasma levels of 7KC have also been measured in patients infected with influenza A virus [45]. In patients infected by the SARS-CoV-2 coronavirus with severe forms of COVID-19 (COrona VIrus Disease - 2019), a potentially fatal acute respiratory distress syndrome due to a bilateral pneumonia, elevated plasma levels of 7KC were observed whereas 27-hydroxycholesterol (also known for its strong anti-viral activity) [46–49] was simultaneously significantly decreased compared to the control group, reaching a marked 50 % reduction in severe COVID-19 cases [49]. SARS-CoV-2 is an enveloped RNA virus; its genome encodes for fifteen genes including a surface protein, the Spike protein, which allows it to enter and to infect the target cells at the level of vital organs [50]. Thus, this Spike protein binds to the angiotensin-converting enzyme 2 (ACE-2) receptor expressed in almost all tissues and abundant in the lungs, kidneys, brain stem, adipose tissue, heart, vasculature, stomach, liver, nasal and oral mucosa [51]. The innate immune cells (neutrophils, monocytes) and adaptive immune cells (T cells) are involved in the response to COVID-19 infection [52,53]. In a retrospective study realized on 175 patients, it was noted that the highest co-morbidity has been observed in COVID-19 patients with cardiovascular diseases [54] in whom it is well known that the level of oxysterols formed by auto-oxidation, including 7KC, is already high [11]. An increased prevalence of fungal and *Pseudomonas aeruginosa* colonization has also been observed in patients with severe forms of COVID-19, suggesting that this association may be the result of a failure in the regulation of immune defenses against pathogens other than SARS-CoV-2, in the case of co-infection [55]. From a clinical point of view, atherosclerosis, AMD, cataract, and Alzheimer's disease are age-related diseases [42,56]. At the beginning of the pandemic, COVID-19 could be considered as an emerging age-related disease. This concept is no longer relevant as it is well established that children, adolescents and young adults can also be infected [57,58]. However, severe forms of COVID-19 most often affect people over 65 years of age due to a combination of several parameters, including nutritional aspects but especially age-related immunological and metabolic alterations [59,60]. About the cardiovascular diseases, it can be assumed that the presence of high concentrations of 7KC in the elderly could be a risk factor that may promote co-morbidity in the case of infection with the SARS-CoV-2. A possible involvement of the 7KC in the pathophysiology and in the fatal or non-fatal outcome of severe forms of COVID-19, would strengthen the interest for this oxysterol in the context of viral diseases.

## 3. Anti-viral activities of 7-ketocholesterol

Currently, there is evidence that several oxysterols have antiviral effects against both enveloped and non-enveloped viruses. An in-depth review of the literature on this topic was carried out in 2016 by Lembo et al. [61]. In addition, several studies have shown the ability of certain cholesterol and oxysterol metabolites to regulate both intrinsic/innate immunity and adaptive immunity, each of which may be involved in bacterial and viral infections [62]. Among the oxysterols with strong anti-viral properties are mainly those derived from cholesterol oxidation on the aliphatic side chain such as 25-hydroxycholesterol and 27-hydroxycholesterol, which could act both by inhibiting viral replication and by activating the immune response [63,46,64,65]. 7α, 25-hydroxycholesterol generated by cholesterol by the sequential action of cholesterol 25 hydroxylase (CH25H) and 7-alpha-hydroxylase (CYP7B1) also has anti-viral activities, and acts as chemo-attractant for cells expressing the cell surface receptor GPR183 present on dendritic and B cells, as well as type 3 innate lymphoid cells (ILC3) [66,67]. Less potent antiviral activities have been reported with 7β-hydroxycholesterol and 7KC [61]. However, 7KC shows anti-viral activities

(inhibition of viral replication, viral inactivation) on three enveloped viruses (human papillomavirus-16 (HPV16), human rotavirus (HRoV), human rhinovirus (HRhV)) [46]. In addition, in cultures of green monkey kidney cells (Vero cells) and differentiated human nerve cells (hNP1 cells), 7KC reduces the viral titer of the Zika virus, which is an emerging African virus characterized by significant neurotropism, without affecting cell viability: 7KC reduces the number and infectivity of viral particles released into the culture medium [68]. In Vero-E6/TMPRSS2 cells inoculated with SARS-CoV-2, weak anti-viral activities were demonstrated with 7KC, 22(*R*)-hydroxycholesterol and 22(*S*)-hydroxycholesterol; the latter are weak inhibitors of viral replication, in contrast to 27-hydroxycholesterol and 24(*S*)-hydroxycholesterol [69]. These observations led to the development of semi-synthetic oxysterols with anti-SARS-CoV-2 activity when administered orally to mice which are named ***Oxy210*** (3S,8S,9S,10R,13S,14S,17S)-17-((*R*)-2-hydroxy-4-(pyridin-3-yl)butan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol) and ***Oxy232*** ((3S,5S,8R,9S,10S,13S,14S,17S)-17-((*R*)-3-hydroxy-1-(pyridin-3-yl)pentan-3-yl)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-3-ol) [69]. These different results suggest that 7KC has generally weak anti-viral activities; however, this oxysterol could nevertheless contribute to the immune response due to its pro-inflammatory properties including stimulation of IL-8 and IL-1β production [70,71].

## 4. Potential role of 7-ketocholesterol in the pathophysiology of COVID-19

Although 7KC has weak anti-viral activities, it has strong cytotoxic activities. Indeed, there are several data that favour the pro-oxidative and pro-inflammatory activities of 7KC that may be associated with a type of death defined as oxiapoptophagy (OXIdative stress + APOPTOsis + autoPHAGY), due to the ability of 7KC (at high concentration) to induce oxidative stress and an apoptotic mode of cell death associated with autophagy criteria [14,72,73]. In several cells from different types and from different species, i) oxidative stress is characterized by ROS overproduction including at the mitochondrial level, protein carbonylation, lipid peroxidation and decrease and/or enhancement of anti-oxidant enzymes (catalase, superoxide dismutases (SODs), glutathione peroxidase (GPx), ii) apoptosis is associated by an activation of the mitochondrial pathway leading to a loss of transmembrane mitochondrial potential (ΔΨm), an externalization of phosphatidylserine, an increase of plasma membrane rigidity associated with an increased cell permeability contributing to an altered packing of the lipid bilayer, a cytoplasmic release of cytochrome c, a caspase cascade activation (caspases-3,-7,-8 and -9), poly-ADP ribose (PARP) fragmentation and an internucleosomal DNA fragmentation associated with condensation and/or fragmentation of the nuclei and iii) autophagy is characterized by the presence the presence of autophagosomes and autophagolysosomes revealed by transmission electron microscopy as well as activation of LC3-I into LC3-II [73,74]. In addition, 7KC-induced oxiapoptophagy is associated with a production of inflammatory cytokines (IL-1β, IL-8) [72,14]. Noteworthy, 7KC, together with 7β-hydroxycholesterol (7β−OHC), was significantly increased in the plasma collected from COVID-19 patients compared to age matched healthy controls [49]. A progressive positive trend was found together with the severity of the disease: the highest 7KC and 7β−OHC concentrations were observed in severe COVID-19 patients. In these patients, it was also observed a progressive reduction of the plasma concentrations of lanosterol, lathosterol, and desmosterol, markers of cholesterol synthesis and of the 27-hydroxycholesterol, which has antiviral and immunomodulatory activities against SARS-CoV-2 [49,69]. The rise of 7KC and 7β−OHC was related to the increased oxidative stress caused by respiratory distress [49].

In the case of COVID-19, high serum levels of 7KC were observed: in male/female control subjects of the same age range as COVID-19 patients (70 ± 10 years old), the serum level of 7KC is about 20 μg/L whereas the serum level of 7KC was increased by a factor of 2–3.5 and of 2–5 in moderate and severe COVID-19 patients, respectively [49]. Similarly, while the serum level of 7β−OHC is about 8 μg/L in control subjects, this later was increased by a factor of 1.5–2.5 and of 1.5–5 in moderate and severe COVID-19 patients, respectively [49]. As patients with severe forms of COVID-19 may have the same 7KC and 7β−OHC levels as patients with moderate forms, this suggests that increased serum levels of these oxysterols are not indicative of disease severity. On the other hand, the very high serum levels of 7KC observed in some patients with severe COVID-19 - which may be a consequence of the infection and result from an exacerbated immune response - may contribute to the deterioration of the patients' condition. Thus, it can be assumed that the anti-viral activity of 7KC should be outweighed by its toxic effects, which could contribute to cytokine storm [12,75] and also to the activation of coagulation in the capillaries of the pulmonary alveoli [76], and to the deterioration of alveolar epithelial cells [77]. It is assumed that the organism, with the contribution of 7KC, is overwhelmed by a storm of cytokines that have a deleterious impact on host cells, particularly alveolar epithelial cells, and is therefore unable to inhibit and destroy the SARS-CoV-2. Through its cytotoxic activities associated with oxiapoptophagy, it is hypothesized that 7KC could contribute to the progression and outcome of COVID-19 pathophysiology at different levels: a) by activating the TLR4 receptor which would contribute to increased secretion of pro-inflammatory cytokines (IL-1β, IL-8) [78]; b) by promoting the externalization of phosphatidylserine at the level of endothelial cells of the alveolar capillaries [79] and the eryptosis of red blood cells [80] as well as platelet aggregation [76] which would activate coagulation and the induction of thrombosis c) by altering the viability of the epithelial alveolar cells (oxidative stress, induction of cell death) [77] which would have the consequence of favouring acute respiratory distress syndrome. In addition, elevated plasma levels of 7KC could have systemic effects by altering mitochondrial function [81] with a consequent decrease in ATP production and general exhaustion of the patient. In addition, mitochondrial dysfunction could lead to peroxisomal alterations with subsequent amplification of oxidative stress, deregulation of non-cytokine-mediated inflammation due to the fact that leukotrienes, some of which are also pro-coagulants, are catabolized at the peroxisome level [82–85]. Furthermore, the activation of autophagy by 7KC could also have consequences on the infection. Indeed, autophagy initially considered as anti-viral also seems capable of promoting infection by acting at the level of viral replication and viral cycle [86,87]. Given the toxic characteristics of 7KC, its high level in COVID-19 patients could contribute to cytokine storm, thrombosis and respiratory distress (Fig. 2). To better understand the involvement of 7KC in the pathophysiology of COVID-19, rapid and easy to use analytical tests are a necessity. These could include ELISA tests using anti-7KC mouse monoclonal antibodies [88] or dried blood tests [89] associated with gas chromatography or liquid chromatography coupled with mass spectrometry allowing the simultaneous identification and quantification of several oxysterols and of their metabolites [90,24].

## 5. 7-Ketocholesterol-modifying drugs in COVID-19

Depending on the risk factors presented by SARS-CoV-2 infected patients, the infection may cause an acute respiratory distress syndrome, and multiple organ failure, which can be fatal. To prevent and/or cure COVID-19, the vaccine strategy seems the most promising approach whereas conventional drugs can provide potential alternative treatments as adjuvants [91,92]. These alternative approaches using drugs acting on the viral cycle and/or on the cytopathic effects of SARS-CoV-2 should not be neglected. As cholesterol is essential for the assembly, replication and infectivity of enveloped viruses such as SARS-CoV-2, several cholesterol-modifying drugs could alter the SARS-CoV-2 life cycle [93]. In addition, as cholesterol is also a major component of

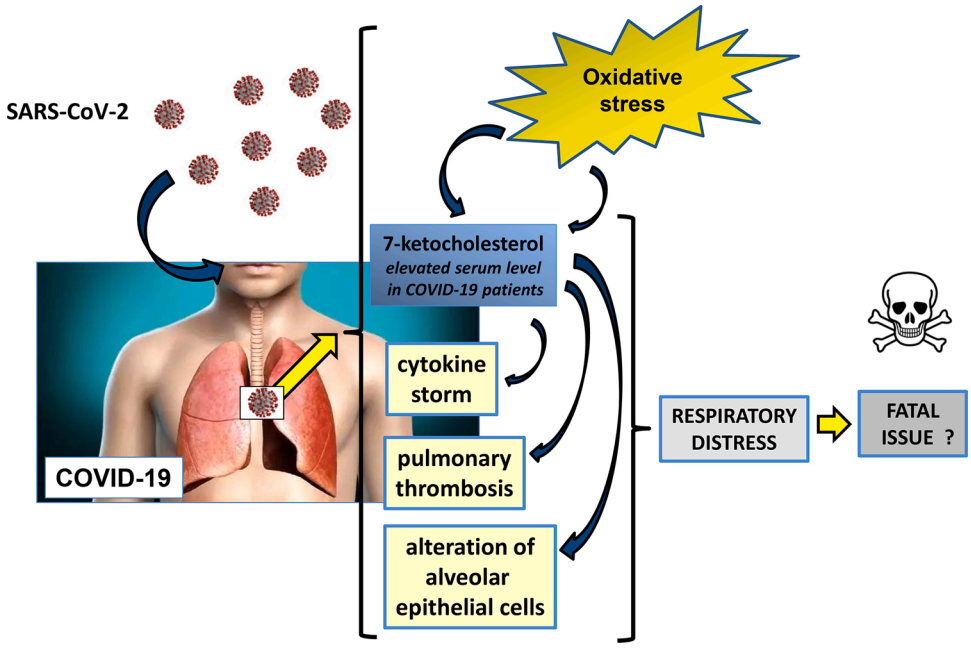


**Fig. 2. Potential contribution of 7-ketocholesterol in the progression and outcome of COVID-19.** Due to high serum levels of 7KC in COVID-19 patients, it is hypothesized that this oxysterol may be involved in the progression and outcome of the disease. The anti-viral effects of 7KC have been described, but these are often weak compared to those of oxysterols such as 24(*S*)-, 25- or 27-hydroxycholesterol [61,69]. On the other hand, 7KC at high concentrations is known for its toxicity (pro-oxidative and pro-inflammatory effects, induction of cell death) [73]. Because of these different activities, 7KC could contribute to the pathophysiology of COVID-19. It is suggested that the measurement of 7KC plasma levels could provide information on the evolution of patients with COVID-19 (use of 7KC as a prognostic biomarker) and that adjuvant therapies mitigating 7KC toxicity could benefit patients and help reduce the number of patients on ventilatory support in emergency departments.

immune cell membranes, excess cholesterol levels in patients with obesity and/or cardiovascular diseases could contribute to dysregulate acquire immunity and promote abnormal inflammatory responses [93]. Moreover, as cholesterol oxidation under the effect of ROS or RNS or through the intermediary of enzymes can lead to the formation of oxysterols [94], and in particular 7KC, which can impact the redox status, inflammation, coagulation, and cell viability, cholesterol-modifying drugs could be of interest to target the side effects of 7KC in the management of SARS-CoV-2 infection [95].

To have a more targeted activity against 7KC, natural and synthetic cytoprotective molecules have been identified and some of them could be used [72,73,85]. Among the natural molecules, fatty acids that can be used as a basis for the design of synthetic analogues, making it possible to neutralise 7KC by esterifying it at the level of the hydroxyl residue on carbon 3 of the A ring of the sterane nucleus, and/or by acting on its signalling pathways, are promising molecules [96,72]. Natural polyphenols [72,73] and derivatives, such as aza- and azo-stilbenes, which are bio-isosteric analogues of resveratrol [97], could be also of interest, as well as tocopherols which have strong anti-oxidant properties and prevent the accumulation of 7KC in the lipid rafts to trigger several signalling pathways [98,99]. In addition, the phospholipid bis(monoacylglycero)phosphate (BMP) is a structural isomer of phosphatidylglycerol that exhibits an unusual sn1:sn1′ stereoconfiguration based on the position of the phosphate moiety of its two glycerol units [100]. BMP prevents the formation of 7KC in murine macrophagic RAW264.7 cells [101]. Recently, it has been suggested that BMP could be useful in the prevention of SARS-CoV-2 infection [102]. It is hypothesized that BMP, which is present in the endosomes and is involved in the intracellular cholesterol trafficking, could act on the SARS-CoV-2 viral cycle and reduce virus production.

Among the synthetic molecules, dimethyl-fumarate (DMF), which is marketed under the name Tecfidera (Biogen) for the treatment of multiple sclerosis and psoriasis, exerts anti-inflammatory activities on T and B cells, as well as on dendritic cells via an inhibitory effect on nuclear factor kappa B (NF-κB) [103] and anti-oxidant activities by activating the erythroid 2-related factor 2 (Nrf2) signalling pathway. This induces the expression of several anti-oxidant enzymes (hemeoxigenase 1 (HO-1), catalase (CAT), superoxide dismutases (SODs), glutathione peroxidase (GPx)), the expression of phase II detoxifying enzymes, such as glutathione S-transferase (GST), and enzymes responsible for glutathione (GSH) synthesis, such as glutamine-cysteine ligase (GCL) and glutathione synthetase (GS) [104,105]. However, as the suppression of pyroptosis by DMF is independent of Nrf2, this supports that several signalling pathways can be activated by this molecule [106]. DMF also attenuates *in vitro* oxidative stress and cell death induction triggered by 7KC on 158N oligodendrocytes [107,108]. Noteworthy, it is currently reported that DMF could reduce lung alveolar cells damage in COVID-19 patients [109].

As 7KC accumulates in the lysosome, a strategy based on the use of bacterial enzymes targeting this organelle, defined as medical bioremediation, could also be used to inactivate 7KC and to prevent 7KC-induced cytotoxic activities [110,111].

## 6. Conclusion

Compared to oxysterols derived from oxidation on the alkyl chain of cholesterol, 7KC has weak anti-viral activities. In severe and often fatal forms of COVID-19, it is hypothesized that 7KC could be a predictive biomarker for assessing the severity of the disease and its progression to a fatal outcome. Indeed, while 7KC has slight antiviral activities, its cytotoxic activities can be considered dominant in many diseases associated with high levels of this oxysterol. These include age-related diseases, among which COVID-19 could be included. It can be assumed that decreasing the amount of 7KC by promoting its degradation and inhibiting or mitigating its toxicity could constitute adjuvant therapies that would if not eliminate, at least reduce, mortality and morbidity associated with COVID-19 infection. Due to the severity of the pandemic, it is reasonable to consider all hypotheses and explore all avenues to treat patients with COVID-19, especially those with potentially fatal outcomes. Therefore, given the biological activities of 7KC, adjuvant treatment with drugs that reduce 7KC toxicity could help to reduce the number of patients with severe forms of COVID-19 and thus reduce the number of patients on respiratory support in emergency departments.

## Authors statement

This work was supervised by Gérard Lizard (GL).

Imen Ghzaiel (IG) and GL mainly contributed to the writing and editing of the manuscript; Khouloud Sassi (KS), Amira Zarrouk (AZ),

Thomas Nury (TN), Mohamed Ksila (MK) and John J Mackrill (JJM) also contribute to the writing and the editing.

Valerio Leoni (VL) was consulted as an expert in oxysterols and COVID-19. He did a careful reading of the manuscript.

Balkiss Bouhaouala-Zahar (B B–Z) and Mohammad Samadi (MS) were consulted as experts in the field of antibodies raised against oxysterols and in the field of sterol chemistry, respectively. They did a careful reading of the manuscript.

Sonia Hammami (SH), Mohamed Hammami (MH) and Taoufik Ghrairi (TG) provided helpful criticisms on the manuscript.

## Declaration of Competing Interest

The authors declare no conflict of interest.

## Aknowledgements

The authors acknowledge the European Network for Oxysterols Research (ENOR: http://oxysterols.net) which permits discussion and collaborative research between the members. Khouloud Sassi (MS; Ph.D student), Amira Zarrouk (Ph.D), Thomas Nury (Ph.D), Valerio Leoni (M. D, Ph.D), John J. Mackrill (Ph.D), Mohammad Samadi (Ph.D), Anne Vejux (Ph.D) and Gérard Lizard (Ph.D, D.Sc) are all members of ENOR. The authors acknowledge the University Tunis El Manar for the financial support of Mrs Imen Ghzaiel (M.S, Ph.D student), Mrs Khouloud Sassi (M.S, Ph.D student) and Mr Mohamed Ksila (M.S, Ph.D student) as well as the "Association Bourguignonne pour les Applications des Sciences de l'Information en Médecine" (A.B.A.S.I.M).

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