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REVIEW

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Coronavirus disease 2019 (COVID-19), human erythrocytes and the PKC-alpha/-beta inhibitor chelerythrine –possible therapeutic implication

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ABSTRACT

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes COVID-19. Until now, diverse drugs have been used for the treatment of COVID-19. These drugs are associated with severe side effects, e.g. induction of erythrocyte death, named eryptosis. This massively affects the oxygen (O_2) supply of the organism. Therefore, three elementary aspects should be considered simultaneously: (1) a potential drug should directly attack the virus, (2) eliminate virus-infected host cells and (3) preserve erythrocyte survival and functionality. It is known that PKC-α inhibition enhances the vitality of human erythrocytes, while it dose-dependently activates the apoptosis machinery in nucleated cells. Thus, the use of chelerythrine as a specific PKC-alpha and -beta (PKC-α/-β) inhibitor should be a promising approach to treat people infected with SARS-CoV-2.

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Introduction

The error-prone nature of RNA polymerases, a pre-requisite for fast genomes evolution of RNA viruses

Evolutionary more highly evolved species with their corresponding large genome size require sophisticated energy- and time-consuming mechanisms to produce precise and viable genome copies. This needs DNA polymerases to operate with the highest degree of proofreading to keep error frequencies and mutation rates very low. However, there are exceptions, i.e. suppression or bypass of proofreading mechanisms in some phases of B-cell development to generate the greatest variability of immunoglobulin molecules [1,2] (for reviews see: [3,4]). This is an important aspect of the immune response to maintain the genetic health of mammalian populations.

RNA replicating viruses are naturally equipped with an error-prone RNA polymerase, allowing them a "turn-on-mutagenesis" status and thus, the highest mutation rates and variability, rapid adaptability and fast genomes evolution [5]. Thus, the RNA viruses are always in attack mode in terms of variability and pathogenicity, and their host must first develop a suitable immune response. The single-stranded RNA virus SARS-CoV-2 with its ~30 kb genome and remarkable capability to transmit between humans, caused COVID-19 [6], affected public life worldwide in an unprecedented way and has so far cost a million people their lives. The respiratory disease COVID-19 has a devastating effect on the health of elderly and people with preexisting conditions, e.g. immunocompromised patients. Currently, several substances are used for COVID-19 treatment, such as glucocorticoids [7] and anti-malaria agents, e.g. chloroquine [8]. The latter usually induce eryptosis. Chloroquine exacerbates virus-associated anemia by enhancing virus replication [9]. Glucocorticoids limit the production of pro-inflammatory cytokines, but as immunosuppressants they also impair the protective function of the T cells, which are urgently needed to combat COVID-19. Furthermore, glucocorticoids inhibit the activity of macrophages and antibody-producing B cells. Thus, a sustained increase in plasma viral load is pre-programmed [10]. In this context, glucocorticoids´ ability to limit the

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overproduction of inflammatory cytokines triggered by SARS-CoV-2 should not be overestimated. Hence, the use of substances for COVID-19 treatment that inhibit the protective effect of immune cells and impair the functionality of erythrocytes or even induce their death is counterproductive. The immediate consequence is a sustained increase in plasma viral load and suboptimal oxygen (O_2) supply of the organism, respectively.

Immuno-modulatory and anti-inflammatory function of human erythrocytes

Human erythrocytes play a vital role in immune and defense mechanisms. They attach to bacteria, transport them to liver and spleen for final elimination [11,12]. Human erythrocytes function as a sink for sphingosine-1-phosphate (S1P) [13] and release on demand this molecule into the plasma through a finely regulated mechanism. S1P negatively regulates lymphocytes circulation [14], maintains endothelial cell barrier integrity [15] and exerts a protective effect on kidney (for reviews see: [16,17]). In addition to this, kidney vascular development is positively regulated by interplay between S1P, erythroblasts and vascular endothelium [18]. Each single human erythrocyte contains approximately 270 millions hemoglobin molecules and thus 1.1 billion heme groups. Heme inhibitory binding to pro-inflammatory cytokine IL-36α (in a ratio of 2:1) negatively regulates IL-36 dependent IL-6 and IL-8 expression [19]. Heme-regulated proteins are involved in several physiological processes, e.g. protein synthesis [20]. Physical interaction of human erythrocytes with a sub-group of dendritic cells (slanDCs) prevents their maturation. The maturation inhibitory effect of erythrocytes ends when slanDCs leave the blood and reach their target tissue, where they mature and acquire an IL-12 and TNF-α producing capacity to fight off pathogens [21]. Human erythrocytes with their unique chemokine receptor are capable to bind an array of inflammatory chemokines [22] including IL-8 [23], and thus avoiding the excessive inflammatory response. Interestingly, 20% of SARS-CoV-2 infected patients develop acute respiratory distress syndrome which is associated with abundance release of chemokines and pro-inflammatory cytokines (e.g. IL-8) culminating in multi-organ failure (for review see: [24]). These data show that human erythrocytes have not only respiratory but also immuno-modulatory and anti-inflammatory functions. Thus, the application of such substances which improve the vitality of erythrocytes and simultaneously eliminate pathogens or pathogen-infected host cells is the best choice and of utmost importance for treating COVID-19.

Activation of protein kinase C-alpha (PKC-α) in respiratory diseases, its role in nuclear export of viral genome and its inhibition by chelerythrine

Respiratory diseases of viral and bacterial origin are associated with PKC-α activation [25,26]. Viral life cycle follows a certain pattern. The negative-strand influenza A RNA virus exclusively persues a nuclear replication and PKC-α promotes the export of ribonucleoproteins (RNPs) complexes from the nucleus of infected cells [27]. Subsequently, RNPs are packaged into budding progeny virions at the cell membrane. The following remarkable review illustrates the principle of virus assembly and budding for an influenza A virus [28]. It is to note that maturation of progeny virions and the intracellular sites at which they bud is determined by the accumulation of their glycoproteins. This principle was demonstrated four decades ago with coronavirus MHV-A59 [29]. Latest studies suggest that positive-strand SARS-CoV-2 RNA virus also follows a nuclear replication strategy [30]. The suppression of PKC-α mediated nuclear export of viral RNPs will be one of the potential targets for chelerythrine, thereby disrupting the formation of the emerging SARS-CoV-2 virions. The following publications describe more details about the pathogenic mechanisms of SARS-CoV-2 [31,32]. The bioactive molecule chelerythrine, a natural product of plant origin is a specific PKC-α/-β inhibitor [33–36], which can exist in

its charged monomeric iminium or neutral alkanolamine forms, enhances the vitality and biological functions of human erythrocytes and prevents stress-induced eryptosis [37]. Several studies underpin the versatility of chelerythrine's mode of action in terms of apoptosis induction [38–40], *in vivo* tumor growth delay [35], kidney [41] and lung protection. The inhibitory effect of chelerythrine on Gram-positive bacteria [42], and its ability to bind to DNA (five nucleotides per chelerythrine) [43–47] and RNA [48] (for reviews see: [49,50]) further underscores the potential of chelerythrine for the use against many diseases including COVID-19.

Inhibitory effect of chelerythrine on virus-mediated PKC-α/-β activation and RNA polymerase phosphorylation

Viral RNA sensing retinoic acid-inducible gene I (RIG-I) as an integral part of host pattern recognition receptors is able to detect both single and double-stranded RNAs in cytosol and initiate the primary line of defense by interferon (IFN)-mediated anti-viral response [25,51,52]. The following work shows some aspects of the SARS-Cov-2 mode of action to undermine IFN-signaling pathways [32]. Both conventional PKC-α and PKC-β are negative regulators of RIG-I. Chelerythrine could reverse PKC-α/-β-mediated RIG-I inhibition and thus initiate the IFN-mediated immune response and, if necessary, depending on the concentration used, eliminate the virus-infected (nucleated) host cells by apoptosis. Several enzymes engaged in DNA and RNA metabolism, e.g. DNA-dependent polymerases and RNA-dependent polymerases, are phospho-activated proteins. It exists a direct correlation between phosphorylation level of hepatitis C RNA polymerase and its RNA replication [53]. In this concept, chelerythrine-mediated inhibition of a putative kinase X activity should decrease RNA polymerase phosphorylation of SARS-CoV-2, severely impair RNA replication and simultaneously enhance RIG-I-dependent INF-mediated anti-viral immune responses. The reciprocal relationship between kinases and phosphatases activities suggests that the use of the kinase inhibitor chelerythrine automatically promotes phosphatase(s) activities. In addition, the protective effect of chelerythrine on human erythrocytes, especially in case of respiratory diseases, is another central point to consider chelerythrine as a potential drug for COVID-19 treatment.

Human erythrocytes vs. SARS-CoV-2

Intact human erythrocytes promote proliferation of activated $CD8^+$ cells [54] whereas they inhibit $CD4^+$ proliferation [55]; they actively adsorb infectious HIV-1 virions [56] for review see: [57], hence reducing HIV infection of $CD4^+$ T-cells. The interaction between virus and the receptive cell is a carbohydrate-dependent mechanism. Human erythrocyte gangliosides [58] as well as sialoglycoprotein glycophorin A (GPA), act as receptors for numerous viruses [59–63] resulting in reduction of both viral infectivity and pathogen load in the circulation [60,64]. In other words, the larger the erythrocyte-bound fraction of a virus, the greater its blood clearance and elimination rate. Each single human erythrocyte with 1×10^6 GPA molecules is capable to bind 1.5×10^5 virions with maximum uptake attaining within 1–2 hours [65,66]. According to the same principle, intact human erythrocytes can capture not only SARS-CoV-2 but also the active particles of this virus and deliver them to macrophages resident in the spleen and liver for final elimination. The elimination of erythrocytes-bound pathogens is a common mechanism. Complement receptor 1-dependent attachment of erythrocytes to bacteria and viruses leads also to phagocytosis and elimination of erythrocyte-bound bacteria [67] (for review see: [68]) and viruses [69,70]. Furthermore, the complexity and high efficiency of the circulatory clearance of bacteria and the involvement of innate (liver, our firewall) and

adaptive (spleen) immunity requires the contribution of additional cells such as platelets [71,72] for preview see: [73].

Inhibitory effect of chelerythrine on mTORC2-dependent, mTORC1-mediated protein synthesis

Host cell protein synthesis does not necessarily have to be accompanied by its proliferation [74]. This principle can be used by a wide variety of intracellular parasites to proliferate with high velocity, e.g. malaria, or to be multiplied, e.g. viruses. Intense multiplication of SARS-CoV-2 within a single host cell requires the activation of both mammalian targets of rapamycin complexes 1 (mTORC1) and 2 (mTORC2) and the associated protein synthesis machinery. mTORC2 acts as an upstream kinase of serum and glucocorticoid-inducible kinase-1 (SGK-1) and protein kinase B (PKB also called Akt) which in turn activate mTORC1. It is to note that mTORC2 kinase activity is PKC-α-dependent [75]. Phosphorylation-dependent Akt activation is regulated by the following kinases: (a) PKC-α/mTORC2 and (b) phosphoinositide 3-kinase (PI3K)/3 -phosphoinositide -dependent kinase-1 (PDK1). Phosphorylation of BAD (a BH3-only pro-apoptotic protein) by Akt or protein kinase A (PKA) leads to BAD inactivation and promotion of cell survival (for review see: [76]). By activating PKC-α-mTORC2 and PI3K-PDK1 signaling pathways, SARS-Cov-2 could induce the mTORC1-dependent protein synthesis and Akt-dependent pro-survival machinery, thus accelerating its multiplication. The majority of these processes could be inhibited by chelerythrine (Figure 1).

Conclusions

The remarkable properties of chelerythrine: (a) fast diffusion across the cell membrane [77], (b) asymmetric distribution between cytosol and membrane particulate fractions [78], (c) preferential inhibition of both conventional PKC-α and -β (d) causing a pro-apoptotic effect in nucleated cells and thus creation of a hostile environment for intracellular parasites including viruses, (e) creating a pro-survival effect in enucleated human erythrocytes and thus enhancing the anti-viral and -bacterial activities of these cells, (f) its curative effect against viral and bacterial infection-associated anemia [79], and (g) its capability to interact with divers DNA and RNA confirmations,

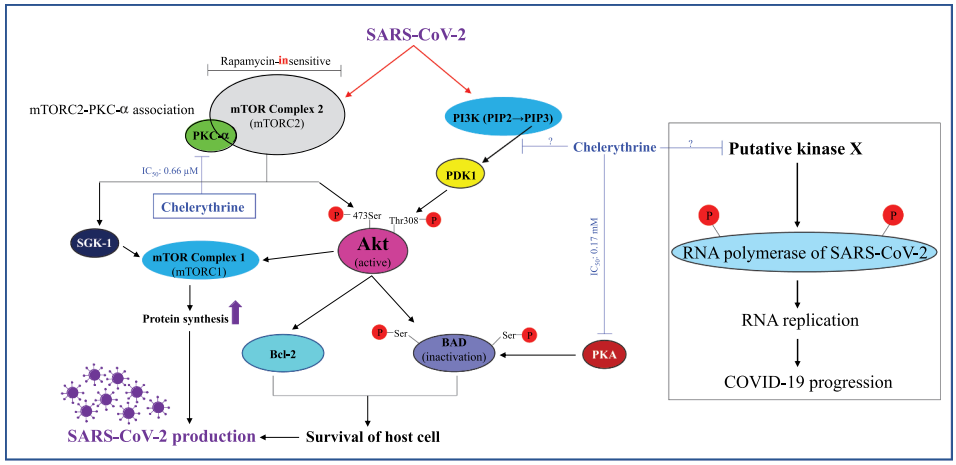

Figure 1. Proposed mechanism of SARS-CoV-2 action and its inhibition by chelerythrine. mTORC2-dependent, mTORC1-mediated protein synthesis as well as mTORC2/PI3K-PDK1-dependent Akt activation and the resulting promotion of cell survival is the basic prerequisite for the synthesis of viral proteins and replication of its genome by the host cell biosynthetic machinery . Therefore, the inhibition of various enzymes of the host cell involved in virus production is an adequate means to stop these processes. Chelerythrine as a specific inhibitor of the protein kinases C alpha and beta (PKC-α/-β) can play an elementary role to accomplish this task. Furthermore, chelerythrine could directly inhibit the upstream kinase of the RNA polymerase of SARS-CoV-2, thus causing its inactivation.

allows the legitimate hypothesis that chelerythrine is predestined for COVID-19 treatment.

Disclosure statement

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Authors contributions

MG designed the project and wrote the manuscript. All figures were made by MG and MK. All authors read, discussed, improved and approved the final version of the manuscript.

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