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(54) **Title:** PANNEXIN-1 INHIBITORS FOR THE TREATMENT OF SARS-COV-2 INFECTED COVID-19 PATIENTS WITH OR WITHOUT AN ASSOCIATED ACUTE RESPIRATORY SYNDROME

(57) **Abstract:** The present invention relates to the use of probenecid and other pannexin-1 blockers for the treatment of novel coronavirus infection with or without an associated acute respiratory syndrome. Probenecid may be administered either alone or in combination with drugs interfering with the viral replication like, but not limited to, RNA-dependent RNA polymerase inhibitors or with the drugs interfering with the lung hyperinflammation like, but not limited to, IL-6 pathway inhibitors. In particular, the present invention includes the use of probenecid in combination with quercetin, a flavonoid described to reduce lung hyperinflammation, IL-6 production and bronchoconstriction. Further, the present relates to a pharmaceutical composition comprising a combination of an amount of a Pannexin-1 inhibitor with an additional polyphenol in an amount effective to treat a coronavirus infection in a human subject in combination with a pharmaceutically acceptable carrier, additive or excipient wherein the polyphenol includes Epigallocatechin gallate (EGCG).

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"PANNEXIN-1 INHIBITORS FOR THE TREATMENT OF SARS-CoV-2 INFECTED COVID-19 PATIENTS WITH OR WITHOUT AN ASSOCIATED ACUTE RESPIRATORY SYNDROME"

FIELD OF THE INVENTION

The present invention relates to the use of agents that inhibits the target pannexin-1 for the treatment of SARS-CoV-2 infection. More particularly, the invention relates to a use of probenecid and other pannexin-1 blockers in the treatment of infectious disease caused by the novel coronavirus-2 (SARS-CoV-2) in Coronavirus disease (COVID-19) infected human subjects with or without an associated acute respiratory syndrome. The invention further describes methods, compositions and pharmaceutical compositions useful in inhibiting pannexin-1, and in treating infected human subjects suffering from SARS-CoV-2 causing COVID-19 disease.

BACKGROUND OF THE INVENTION

The eruption of the novel coronavirus disease, COVID-19, caused by the new coronavirus 2019-nCoV is now a pandemic threat to global public health and is officially designated as severe acute respiratory syndrome-related coronavirus SARS-CoV-2.

Coronaviruses are large, enveloped, plus-stranded RNA viruses. They cause the common cold in all age groups accounting for approximately 15% of all colds. Coronaviruses have been implicated in the etiology of gastrointestinal disease in infants. They also cause economically important diseases in animals (e.g. avian infectious bronchitis and porcine transmissible gastroenteritis). Coronaviruses get their name because in electron micrographs the envelope glycoproteins appear to form a halo or corona around the periphery of the virion. The coronaviruses are also interesting because they are the only plus-strand RNA viruses with a helical nucleocapsid. Coronaviruses are a major cause of common colds in the winter months. The virus is found throughout the world. Antibodies begin to appear in childhood, and are found in more than 90% of adults. The frequency of coronavirus respiratory infections is highly variable from year to year. The highest incidence occurs in years when rhinovirus colds are lowest. Coronavirus colds tend to occur

in defined outbreaks.

Coronaviruses have the largest genomes of all RNA viruses and replicate by a unique mechanism which results in a high frequency of recombination. Virions mature by budding at intracellular membranes, and infection with some coronaviruses induces cell fusion.

All the viruses, SARS-COV-1 as well as MERS-CoV share a similar protein-cutting enzyme, called the "main protease" in coronavirus and the '3C protease' in enterovirus, that is essential for viral replication. (Zhang et al., *J. Med. Chem.* Feb, 2020). Further, it is disclosed that main protease of coronaviruses and 3C protease of enterovirus share a similar active-site architecture and a unique requirement for glutamine in the P1 position of the substrate. Because of their unique specificity and essential role in viral polyprotein processing, these proteases are suitable targets for development of anti-viral drugs. Interestingly, flavonoids like quercetin have been shown to inhibit MERS-CoV 3C-like protease (Jo et al., *Chem. Biol. Drug. Des.* (2019): vol. 00, pages 1-8).

Epigallocatechin gallate (EGCG) has been reported to exert antiviral properties against various harmful viruses in preclinical and clinical setups. These various sets of data support investigating the therapeutic effect of EGCG against COVID-19. For instance, EGCG has been shown to prevent Zika virus entry onto Vero cells (Carneiro et al., 2016 *Virology* 496: 215-218), likely by binding to a the envelop protein E4 (Sharma et al., 2017 *Int. J. Biol. Macromol.* 104: 1046-1054). In addition, EGCG dose-dependently inhibited Zika virus replication in Vero cells (IC_{50}=0.02 μM) and inhibited the viral protease ZIKV-NS2B-NS3 (EC_{50}=0.73 μM) that is critical for Zika virus replication (Zou *et al.*, 2020 *Biochem. Pharmacol.* 113962). At the molecular level, EGCG has been shown to interact with the catalytic triad (His51, Asp75, Ser135) of the active site (Yadav *et al.*, 2020 *J. Biomol. Struct. Dyn.* 1-13). In vitro, EGCG decreased H1N1 replication by increasing interferon IFN- λ2 secretion in human lung epithelial BEAS-2B cells (Zhu *et. al.*, 2020 *J. Thorac. Dis.* 12: 989-997, 2020. In vivo, EGCG decreased cytokine storm, lung tissue damage and protected respiratory function in a mouse model of H9N2 swine flu virus. EGCG (10 mg/kg, gavage, 5 days) reduced immune infiltration and IL-1β and TNF-α levels in bronchoalveolar fluid. In addition, increased mice survival rate (Xu *et al.*, 2017 *Int. Immunopharmacol.* 52: 24-33).

The effect of EGCG has been extensively studied on hepatitis-C virus. In vitro, EGCG has been demonstrated to block HCV entry in human liver cells Huh-7 and to prevent cell-to-cell transmission (Calland *et al.*, 2012 *Hepatology* 55: 720-729). This effect may be due, at least in part, to ECGC binding onto the viral protein NS5B (Roh and Jo 2011 *Talanta* 85: 2639-2642) and/or repressing CD81 expression (Mekky *et al.*, 2019 *Arch. Virol.* 164: 1587-1595), preventing the virus from attaching to the cell membrane. Most importantly, these preclinical data translated into clinical results. In a phase 3 open-label clinical trial (www.clinicaltrials.gov NCT03186313), EGCG 400 mg per day during 12 weeks, potentialized the effect of the standard of care drug sofosbuvir 400 mg + daclatasvir 60 mg as shown on the viral load decline (Shiha *et. al.*, 2019 *Sci. Rep.* 9: 13593). EGCG effect was ascribed to EGCG inhibition of virus entry into the hepatocytes. It must be noted that the EGCG daily dose of 400 mg used in this trial represents 50% of the maximum EGCG daily dose recommended by EMA and FDA.

EGCG also demonstrated an antiviral effect against dengue (Ismail *et. al.*, 2017 Interdiscip Sci 9: 499-511 – Raekiansyah *et. al.*, 2011 *Arch. Virol.* 163: 1649-1655), HBV (Lai et al., 2018 *BMC. Complement. Altern. Med.* 18: 248), Japanese encephalitis (Wang *et. al.*, 2018 *Virus. Res.* 253: 140-146) and chikungunya viruses (Lu *et. al.*, 2017 *Biochem. Biophys. Res. Commun.* 491: 595-602 – Weber *et. al.*, 2015 *Antiviral. Res.* 113: 1-3).

Pneumonia infection leads to a massive inflammatory reaction, called 'cytokines storm', that is well documented in the context of viral lung infection (Short *et. al.*, *Lancet Infect. Dis.* (2014): vol. 14(1), pages 57-69), including coronavirus infection due to SARS-CoV and MERS-CoV (De Wit *et al.*, *Nature* (2016): vol. 14, pages 523-534). It is characterized by the activation of an inflammasome cascade involving NLRP3/pannexin-1 ATP channel/P2X7 ATP receptor/IL- 1β and the release of other pro-inflammatory chemokines like IL-6 and TNF-α. Cytokine storm has also been recently identified in COVID-19 infected patients (Mehta *et al.*, *Lancet* (2020): vol. 395(10229), pages 1033-1034).

Panx-1 channels appears to be at the crossroad of upstream receptor like vanilloid TRPV and downstream ATP receptors like P2Y and P2X, all described as key actors of lung inflammation. Therefore, blocking ATP release through Panx-1 will offer the advantage of

an upstream MoA capable of altering the course of pulmonary hyperinflammation, neurogenic bronchoconstriction and cough reflex.

The pandemic Covid-19 disease has become a serious threat to the global public health due to unavailability of drugs or vaccines to treat this infectious disease. Hence, novel drugs and new approaches to develop them are urgently needed. Both *de novo* drug discovery and drug repurposing have been used in the search for an effective antiviral drug. Unlike the lengthy and costly process of de novo drug discovery, drug repurposing can reduce the time, cost and risk associated with drug innovation. Though probenecid has been used for other clinical indications, till date it has not been repurposed for the treatment of Covid-19 infectious disease with or without an associated acute respiratory syndrome either alone or in combination with other drugs and flavonoids like quercetin.

SUMMARY OF THE INVENTION

The present invention discloses the therapeutic use of probenecid and other pannexin-1 blockers for the treatment of novel coronavirus infection with or without an associated acute respiratory syndrome. The probenecid can be administered either alone or in combination with drugs interfering with the viral replication like, but not limited to, RNA-dependent RNA polymerase inhibitors, with drug interfering with the virus cell entry or with drugs interfering the lung hyperinflammation like, but not limited to, IL-6 pathway inhibitors. In particular, the present invention includes the use of probenecid in combination with EGCG, a green tea flavonoid described to interfere with the virus cell entry or quercetin, a flavonoid described, like EGCG, to reduce lung hyperinflammation, IL-6 production, lung tissue damage and bronchoconstriction. The probenecid either alone or with combination of other drugs can be administered by oral or parenteral or aerosol or intranasal routes. The said formulation may optionally contain other conventional pharmaceutical aids such as pharmaceutically acceptable carrier, additive or excipients such as stabilizers, preservatives, or buffering agents.

DESCRIPTION OF THE INVENTION

The present invention relates to the use of probenecid and other pannexin-1 blockers for the treatment of novel coronavirus infection with or without an associated acute respiratory syndrome. Probenecid may be administered by oral, parenteral, aerosol or intranasal route. Probenecid may be administered either alone or in combination with drugs interfering with the viral replication like, but not limited to, RNA-dependent RNA polymerase inhibitors or with the drugs interfering with the lung hyperinflammation like, but not limited to, IL-6 pathway inhibitors. In particular, the present invention includes the use of probenecid in combination with quercetin, a flavonoid described to reduce lung hyperinflammation, IL-6 production and bronchoconstriction.

Probenecid is an OATP-3 solute transporter antagonist (Hagos *et al.*, *Xenobiotica* (2017): vol. 47(4), pages 346-353) and a pannexin-1 ATP channel blocker (Silverman *et al.*, *Am J Physiol Cell Physiol* (2008): vol.295(3), pages C761-767). Probenecid blocks the replication of the influenza virus strain H1N1 in infected mice during in vitro and in vivo studies (Perwitasari *et al.*, *Antimicrob Agents Chemother* (2013): vol. 57(1), pages 475-483). The solute transporter OAT-3, a member of SLC22 family of transporters, is necessary to the replication of the influenza virus in lung epithelial cells. Probenecid is a known OAT-3 antagonist in the kidney and constitute its mechanism of action for a gout treatment. Blocking OAT-3 in lung epithelial cells may results in a reduction of viral titer and related respiratory syndrome.

Probenecid decrease OAT-3 expression and decreases H1N1 replication during in vitro studies in human A549 lung epithelial cells and during in vivo studies in infected mice (Perwitasari *et al.*, 2013). Probenecid administered to H1N1 infected mice following a prophylactic or a therapeutic regimen resulted in a decrease of the lung viral titer and an increase of the survival curve of the animals. Antiviral effects were observed at doses of 10 and 25 mg/kg intraperitoneally, of which corresponding Human Equivalent Dose falls within the therapeutic range. This explains the effect of probenecid on viral replication.

Analogs of the probenecid derivative N-(3-chlorophenyl)-4-(N,N-diethylsulfamoyl)-benzamide (NCDB) exert an antiviral effect on several strains of enterovirus during *in vitro* studies (Sethy *et al.*, *Future Med. Chem.* (2018): vol.10(11), pages 1333-1347). Sethy *et al.*,

developed analogs of the probenecid derivative NCDB and assessed their antiviral properties on two enterovirus strains, D68 and A71 during in vitro studies. The best compounds inhibited the enterovirus replication in human cells with an EC_{50} of approximately 2 μM. It is disclosed that the NCDB analogs binds to a pocket inside the viral capsid protein VP1. This binding site is identical to the binding site of pleconaril, an antiviral developed to treat respiratory infections caused due to enterovirus and rhinovirus.

There are many evidences existing which show the role of ATP receptors and ATP channels/receptors in obstructive airway diseases which leads to airway inflammation and hyperresponsiveness. It has been observed that ATP concentrations are higher in bronchoalveolar fluids of smokers versus non-smokers, in smokers with chronic obstructive pulmonary disease (COPD) versus smokers without COPD, irrespective of the smoking status, and patients suffering from lung fibrosis versus healthy subjects (Lommatzsch *et al.*, *Am. J. Resp. Crit. Care Md.* (2010): vol. 181, pages 928-934 and Riteau *et al.*, *Am. J. Resp. Crit. Care Med.* (2010): vol. 182, pages 774-783). Further, in lung epithelium, ATP has been shown to trigger mast cells degranulation, bronchoconstriction and mucus production during in vitro and in vivo studies. (Kim *et al.*, *Biochem. Biophys. Res. Comm.* (2018): vol 503, pages 657-664 and Li *et al.*, *Exp. Cell. Res.* (2018): vol. 366, pages 1-15). ATP is a well-known pro-inflammatory molecules in the lung epithelium in asthma, allergen reactivity, cough and smoking-related COPD and its release through Panx-1 channels is now evident (Lommatzsch *et al.*, *Am. J. Resp. Crit. Care Md.* (2010): vol. 181, pages 928-934). Also, it is observed that pro-inflammatory vanilloid receptors TRPV-1 and TRPV-4 triggers ATP release in epithelial airway though Panx-1 channels in human COPD lung tissue. Probenecid inhibits ATP release from human bronchoepithelial cells which are exposed to cigarette smoke (Baxter *et al.*, *Thorax* (2014): vol. 69, pages 1080-1089).

Probenecid reduces lung inflammation in mice infected with Pseudomonas aeruginosa (Wonnenberg *et al.*, *Int. J. Med. Microbiol.* (2014): vol. 304, pages 725-729). It is observed that probenecid when administered through oral route at the dose of 80 mg/kg for two days significantly decreased the lung inflammation and the concentration of pro- inflammatory chemokines IL-1β and TNF-α in the bronchoalveolar fluid of mice infected with Pseudomonas aeruginosa. The therapeutic dose of probenecid falls within the Human

Equivalent Dose range used in clinic.

As an associated advantage, Probenecid reduces lung airways hyperinflammation and increases survival in mice infected with the influenza virus strain H1N1 and H3N2 (Rosli *et al.*, *Br. J. Pharmacol.* (2019): vol. 176, 3834-3844). It is disclosed that probenecid when given by intranasal route (40 mg/kg every 2 days over 9 days) inhibited the activation of the NLRP3 inflammasome shown by a significant reduction of immune cells infiltration and a lower concentration of the proinflammatory chemokines IL-1b, IL-6 and TNFa in the bronchoalveolar fluid of mice infected with either H3N2 or H1N1 flu virus. This reduction of the lung hyperinflammation was accompanied by a dramatic increase of the mice survival. This therapeutic effect was equivalent in magnitude to the one obtained with AZ11645373, antagonist of the ATP receptor P2X7. This subset of data indicates that probenecid acted by blocking pannexin-1 ATP channels and inhibiting ATP release through, preventing ATP from acting on its downstream receptor P2X7, therefore interfering with the development of the hyperinflammation.

In the present invention, probenecid and other pannexin-1 blockers are formulated in several dosage forms and used for the treatment of novel coronavirus infection with or without an associated acute respiratory syndrome. In particular, the present invention includes the use of probenecid in combination with quercetin, a flavonoid described to reduce lung hyperinflammation, IL-6 production and bronchoconstriction (Micek *et al.*, *Molecules* (2016): vol. 21(5), pages E623).

In an embodiment, Probenecid is administered either alone or in combination with drugs interfering with the viral replication like, but not limited to, RNA-dependent RNA polymerase inhibitors or with drugs interfering the lung hyperinflammation like, but not limited to, IL-6 pathway inhibitors.

In another embodiment, present invention includes the use of probenecid in combination with quercetin, a flavonoid described to reduce lung hyperinflammation, IL-6 production and bronchoconstriction.

In another embodiment, present invention includes the use of probenecid in combination with Epigallocatechin gallate (EGCG). Also known as epigallocatechin-3-gallate, EGCG

is the ester of epigallocatechin and gallic acid, and is a type of catechin. EGCG the most abundant catechin in tea is a polyphenol under basic research for its potential to affect human health and disease. EGCG has been reported to exert antiviral properties against various harmful viruses in preclinical and clinical setups. These various sets of data support investigating the therapeutic effect of EGCG against COVID-19.

EGCG, at doses corresponding to Human Equivalent Doses relevant to human use, reduced lung hyperinflammation, cytokines storm and secretion of IL-6 in the bronchoalveolar fluid and lung tissue in a mouse model paraquat intoxication (Shen *et al.*, 2017 *Life. Sci.* 170: 25- 32). Moreover, EGCG, also at doses corresponding to Human Equivalent Doses relevant to its use in humans, decreased the production of pro-inflammatory cytokines TNF-α, IL-1β and IL-6 in the bronchoalveolar fluid of a mouse model of lung inflammation and injury triggered by the bacterial toxin LPS. The reduction of cytokines production was associated to a reduction of lung tissue damage (Wang *et al.*, 2019 *Braz. J. Med. Biol. Res.* 52(7): e8092). Interestingly, quercetin, another polyphenolic flavonoid, has been described to also reduced TNF-α and IL- 6 secretion induced by LPS in the bronchoalveolar fluid in a rat model of lung inflammation and to prevent the development of the associated tissue damage (Huang *et al.*, 2015 *Arch. Med. Sci.* 11(2): 427-432). Furthermore, in an *invitro* model of viral lung infection, quercetin inhibited the secretion of IL-6 triggered by the TLR-7 agonist imiquimod in A459 human lung cells (Gauliard *et al.*, 2008 *J. Med. Food.* 11(2): 82-84). These results further support the use of EGCG and drugs interfering with the IL-6 pathway in combination with probenecid as a treatment for coronavirus infection.

Cell entry of coronavirus depends on binding of the viral S-protein to its cellular receptor and on S-protein priming by the host cell proteases. COVID-19 use angiotensin- converting enzyme 2 (ACE2) to bind onto the cell and the serine protease TMPRSS2 to prime its S-protein (Hoffmann *et al.*, 2020 *Cell* 181(2): 271-280). A recent study showed that camostat mesylate, an approved TMPRSS2 inhibitor indicated in pancreatitis, blocks in vitro COVID- 19 cell entry (Hoffmann *et al.*, 2020 *Cell* 181(2): 271-280). Camostat mesylate is currently in a phase 1/2 as a treatment for COVID-19 infection (www.clinicaltrials.gov NCT04321096). Interestingly, EGCG has been demonstrated to decrease TMPRSS2

expression in LNCaP cells (Farooqi *et al.*, 2010 *World J. Oncol.* 1: 242-246). Altogether, these data strongly suggest that EGCG would constitute an interesting therapeutic strategy to treat SARS-CoV2.

The present invention proposes a combination of Probenecid and EGCG. Mechanism of action of Probenecid mainly involves interfering with the cytokines storm that takes place in the lung upon infection and preventing any loss of respiratory function from happening. Probenecid acts at the host level. EGCG binds onto viral proteins and block the virus capacity to interact with the host cells. It blocks virus cell entry and replication. EGCG mainly acts at the virus level. By combining both active compounds, the newly created combination drug acts on the host response and on the virus capacity to interact with the host, both mechanisms of action complementing each other, hence potentializing the therapeutic response.

In another preferred embodiment, probenecid and other pannexin-1 blockers are formulated in several dosage forms and administered for the treatment of novel coronavirus infection with or without an associated acute respiratory syndrome. The probenecid alone or with combination of other drugs can be administered by oral or parenteral or aerosol or intranasal routes. The formulation optionally contains other conventional pharmaceutical aids such as pharmaceutically acceptable carrier, additive or excipients such as stabilizers, preservatives, or buffering agents.

The present invention thus provides use of probenecid and other pannexin-1 blockers for the treatment of coronavirus-2 (SARS-CoV-2) infection alone or as a combo with drugs interfering with the viral replication like, but not limited to, RNA-dependent RNA polymerase inhibitors. Additionally, the composition may include drugs interfering with the lung hyperinflammation like, but not limited to, IL-6 pathway inhibitors. In particular, the present invention includes the use of probenecid in combination with quercetin, a flavonoid described to reduce lung hyperinflammation, IL-6 production and bronchoconstriction.

Probenecid may be administered by oral route, injectable, aerosol, intranasal.

In another embodiment, Probenecid is an already marketed drug with favorable

toxicological profile. Probenecid alone or with combination of other drugs is proposed to be developed as a sterile injectable solution, an aerosol or a solution for intranasal instillation.

In another embodiment, the present invention provides a method of treating novel coronavirus infection with or without an associated acute respiratory syndrome comprising administering an effective amount of at least one Pannexin-1 inhibitor to a human subject; wherein, said coronavirus infection is a severe acute respiratory syndrome-related coronavirus-2 (SARS-CoV-2) causing novel coronavirus disease (COVID-19); said Pannexin-1 inhibitor is Probenecid, pharmaceutical salts and/or alternative salts thereof; and said Pannexin-1 inhibitor is co-administered in combination with an additional polyphenol in an amount effective to treat a coronavirus infection in a human subject or optionally with at least one flavonoid.

In another embodiment, the present invention provides a pharmaceutical composition comprising a combination of an amount of a Pannexin- 1 inhibitor with an additional polyphenol in an amount effective to treat a coronavirus infection in a human subject in combination with a pharmaceutically acceptable carrier, additive or excipient; wherein, said Pannexin-1 inhibitor is Probenecid, pharmaceutical salts and/or alternative salts thereof; and said polyphenol includes Epigallocatechin gallate (EGCG).

In another embodiment, the present invention provides a pharmaceutical composition comprising a combination of an amount of a Pannexin-1 inhibitor with an additional flavonoid in an effective amount to treat a coronavirus infection in a human subject in combination with a pharmaceutically acceptable carrier, additive or excipient; wherein said Pannexin-1 inhibitor is Probenecid, pharmaceutical salts and/or alternative salts thereof; and said flavonoid is quercetin or its pharmaceutical salts and/or alternative salts thereof.

The composition is in a dosage form selected from a non-limiting group of inhalation dosage form, oral dosage form, intranasal dosage form, or parenteral dosage form. The probenecid and other pannexin-1 blockers are used for the treatment of SARS-CoV-2 infection alone or as a combination with drugs interfering with the viral replication like, but not limited to, RNA-dependent RNA polymerase inhibitors and with the lung

hyperinflammation like, but not limited to, IL-6 pathway inhibitors.

The following non-limiting examples are provided to further exemplify the invention.

EXAMPLES

ANTIVIRAL EFFECT ON MULTIPLE STRAINS OF SARS-COV-2 VIRUS

The pannexin-1 inhibitors probenecid and the green tea catechin EGCG resulted in a signification inhibition of the viral replication of several viral strains. The degree of inhibition depended on the type of strain and concentration. Both the pannexin-1 inhibitors successfully inhibited more than 50% the replication of SARS-CoV-2 UK strain as depicted in **Table 1 and 2**.

Table 1

Probenecid antiviral effect on multiple strains of SARS-CoV-2 virus. The values are presented in % of inhibition of the viral replication

	IHUMI 3	IHUMI 845	IHUMI 2096	IHUMI 2137	UK
1 mM	80.2	0	9.9	48.1	not tested
10 mM	55.3	53.7	0	37.5	59.2

Table 2

EGCG antiviral effect on multiple strains of SARS-CoV-2 virus. The values are presented in % of inhibition of the viral replication

	IHUMI 3	IHUMI 845	IHUMI 2096	IHUMI 2137	UK
10 mM	31.9	93.4	75.2	18.4	49.8
20 mM	42.7	0	70.1	4.5	19.4
30 mM	47.2	0	77.5	0	53.8

Therefore, probenecid and EGCG exhibited strong antiviral properties which signifies for their development as a treatment, curative and preventative for COVID-19.

EXPERIMENTAL STUDIES

Materials and Methods

Test Articles:

EGCG is procured from Taiyo Kagaku Japan, batch 002211 and Probenecid is procured from Teva Generics Society, batch 2307-01219.

Experimental Conditions:

Assay Temperature: 37±1 °C

Test articles concentrations:

EGCG: 10, 20 and 30 µM; and

Probenecid: 1 and 10 µM;

Contact duration: 48 h;

Viral strain: IHUMI 3, 4th passage;

IHUMI 845, 2nd passage ;

IHUMI 2096, 3rd passage ;

IHUMI 2137, 3rd passage ; and

IHUMI 3076 (UK), 3rd passage;

Cell line: VERO C1008 (ATCC CRL-1586).

Experimental Protocol:

At Day-1, VERO C10008 cells are plated in 96-well plates at 5.10^5 cells/ml in 200 µl (1.105 cells/well). 8 wells per experimental condition (viral strain and test article concentration) are prepared.

At Day-2, test articles at corresponding concentrations and controls are added to the wells and pre-incubated at 37°C under 5% CO_2 for 4 hours. 50 µl of the viral suspension (MOI=0.0001, 29-30 Ct.) or negative control (M4 medium) are then added to the corresponding wells. 100 µl is sampled from 1 well corresponding to each experimental condition and constitute the H0 control. Plates are incubated at 37°C under 5% CO_2 for 48 hours. RT-PCR is then performed on 100 µl of each well.

At time H0 and H48, RNA is extracted from corresponding samples using QIAamp 96 Virus QIAcube HT kit (Qiagen). Real-time RT-PCR is carried out specifically targeting the N gene of the SARS-CoV-2 virus. The primers used are forward GACCCCAAAATCAGCGAAAT, reverse TCTGGTTACTGCCAGTTGAATCTG and probe FAM-ACCCCGCATTACGTTTGGTGGACC. The RT-PCR is conducted using the Superscript III Platinium One-Step Quantitative RT-PCR systems with ROX kit (Invitrogen). The final concentration is 320 nM primer and 200 nM probe in a volume of 25 µl containing 2 µl RNA. The equipment used is a LightCycler 480i (Roche Diagnostics).

The percentage of inhibition of the viral replication is calculated using the $2^{\wedge(-\text{delta delta Ct})}$ method, as follow:

$DCt_{treated}$ = mean [$Ct_{test\ article\ H48}$ – mean Ct_{H0}]

$DCt_{control}$ = mean [$Ct_{untreated\ H48}$ – mean Ct_{H0}]

$DDCt = DCt_{control} - DCt_{treated}$

% inhibition = $100 - ((2^{-DDCt}) \times 100)$

CLAIMS

We claim:

1. A method of treating novel coronavirus infection with or without an associated acute respiratory syndrome comprising administering an effective amount of at least one Pannexin-1 inhibitor to a human subject.

2. The method according to claim 1, wherein said coronavirus infection is a severe acute respiratory syndrome-related coronavirus-2 (SARS-CoV-2) causing novel coronavirus disease (COVID-19).

3. The method according to claim 1, wherein said Pannexin-1 inhibitor is Probenecid, pharmaceutical salts and/or alternative salts thereof.

4. The method according to claim 3, wherein said Probenecid exhibits antiviral effect on multiple strains of SARS-CoV-2 virus wherein percent inhibition of viral replication is 55.3, 53.7, 37.5, and 59.2 for IHUMI 3, IHUMI 845, IHUMI 2137 and UK viral strains respectively at 10 μM concentration of the Probenecid and percent inhibition of viral replication is 80.2, 9.9, and 48.1 for IHUMI 3, IHUMI 2096 and IHUMI 2137 viral strains respectively at 1 μM concentration of the Probenecid.

5. The method according to claim 1, wherein said Pannexin-1 inhibitor is co-administered in combination with an additional polyphenol in an amount effective to treat a coronavirus infection in a human subject or optionally with at least one flavonoid.

6. The method according to claim 5, wherein said polyphenol includes Epigallocatechin gallate (EGCG).

7. The method according to claim 5, wherein said flavonoid is quercetin, pharmaceutical salts and/or alternative salts thereof.

8. A pharmaceutical composition comprising a combination of an amount of a Pannexin- 1 inhibitor with an additional polyphenol in an amount effective to treat a coronavirus infection in a human subject in combination with a pharmaceutically

acceptable carrier, additive or excipient.

9. The composition according to claim 8, wherein said Pannexin-1 inhibitor is Probenecid, pharmaceutical salts and/or alternative salts thereof.

10. The composition according to claim 8, wherein said polyphenol includes Epigallocatechin gallate (EGCG).

11. The composition according to claim 10, wherein said EGCG exhibit antiviral effect on multiple strains of SARS-CoV-2 virus wherein percent inhibition of viral replication is 31.9, 93.4, 75.2, 18.4 and 49.8 for IHUMI 3, IHUMI 845, IHUMI 2096, IHUMI 2137 and UK viral strains respectively at 10 μM concentration of the EGCG and percent inhibition of viral replication is 42.7, 70.1, 4.5 and 19.4 for IHUMI 3, IHUMI 2096, IHUMI 2137 and UK viral strains at 20 μM concentration of the EGCG and 47.2, 77.5, and 53.8 for IHUMI 3, IHUMI 2096, and UK viral strains at 30 μM concentration of the EGCG.

12. The composition according to claim 8, wherein the composition comprises an additional flavonoid in an effective amount to treat a coronavirus infection in a human subject.

13. The composition according to claim 12, wherein said flavonoid is quercetin or its pharmaceutical salts and/or alternative salts thereof.

14. The composition according to claim 8, wherein said composition is in a dosage form selected from a non-limiting group of inhalation dosage form, oral dosage form, intranasal dosage form, or parenteral dosage form.

15. The composition according to claim 8, wherein the composition is used for the treatment of SARS-CoV-2 infection alone or as a combination with drugs interfering with the viral replication like, but not limited to, RNA-dependent RNA polymerase inhibitors and with the lung hyperinflammation like, but not limited to, IL-6 pathway inhibitors.