NANOTECHNOLOGY; A NOVEL TOOL TO COMBAT COVID-19

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ABSTRACT

Coronavirus disease 2019 (SARS-CoV-2) is a pandemic effecting people worldwide. Number of cases are increasing with the passage of time. SARS-CoV-2 is causative agent of COVID-19 and belong to MRSA and SARS-CoV-1 family. Already approved antiviral drugs (hydroxychloroquine and chloroquine, Remdesivir and immunomodulators like LPV/r plus interferon beta) are considered for their antiviral activity against COVID-19 infection. Extensive research and in-vivo trials are required to validate their efficacy. These drugs also have some side effects. Vaccines are also being produced. Vaccine production is a time-consuming process and required hundreds of trials to be approved for treatment of disease. Nanobiotechnology is an emerging field of medicine. Nanoparticles are target specific, deliver drug safely, evade immune response and have antibacterial and antiviral properties. For treatment of COVID-19, different nanoparticles are being studied and indicated antiviral activity in-vitro. Iron oxide nanoparticles, sliver nanoparticles, decoy nanoparticles and glycyrrhizic acid nanoparticles are some nanoparticles being tested for their antiviral activity against COVID-19 infection. These nanoparticles deliver drug or vaccine to a specific site and improve the treatment outcomes. However, more projects and research should be channeled to validate the use of nanoparticles for COVID-19 treatment.

Key Words: COVID-19, pandemic, Vaccines, Nanotechnology, Nanoparticles

I. INTRODUCTION

Coronavirus disease 2019 (SARS-CoV-2) is a pandemic effecting people worldwide (Cucinotta and Vanelli, 2020). Number of cases are increasing with the passage of time. Case detection rate of COVID-19 is provided by numerous websites for tracking in real time. Figure 1.1. indicates the burden of COVID-19 all around the world; higher in USA following India, Mexico, Brazil, Russian Federation, United Kingdom, Italy, Iran, Egypt and South Africa as described by University of Washington.

Figure 1.1. COVID-19 deaths worldwide (2020 to 2021)
Coronavirus has ability to infect human as well as different animals (Velavan and Meyer, 2020). It has been named as Coronavirus (Latin: Corona-crown) on the bases of morphology as it is spherical and possess surface projections that look like crown. Coronavirus family is divided into subfamilies; alpha (α), beta (β), gamma (γ) and delta (δ). Mammals (Bats) are source of transmission of alpha and beta coronaviruses while other two originate from birds and pigs. Among these subfamilies, beta coronavirus is responsible for high fatality rates in human. Current pandemic is caused by B lineage of beta-coronavirus (SARS-CoV-2) having great similarity with SARS-CoV (Zhou et al., 2020). It was observed that there is 96% similarity between bat coronavirus and coronavirus isolated from COVID-19 patients. This similarity predicts the route and transmission of COVID-19 to human.

Major clinical presentation of COVID-19 is pneumonia. Gastrointestinal symptoms are also reported in younger patients (J. F.-W. Chan et al., 2020). Mean incubation period for coronavirus is five days with 3 days of incubation (Q. Li et al., 2020). Fever, nasal congestion, cough and fatigue are common symptoms that appears within first week of infection. In severe infection, dyspnoea and pneumonia are common observations in case of COVID-19. Pneumonia appears in 2nd to 3rd week of infection and cause drop in oxygen saturation. Changes in lung morphology can be observed in X-Ray findings. Ground glass abnormalities, alveolar exudates, patchy consolidation and interlobular involvement are also seen as the infection proceeds. Lymphopenia becomes more common and inflammatory markers elevates as immune response (Velavan and Meyer, 2020).

II. TRADITIONAL THERAPEUTIC OPTIONS FOR COVID-19

2.1. Drugs

Clinical trials are being conducted on several drugs. WHO has announced a global trial named SOLIDARITY, in which thousands of COVID-19 patients will be registered. These patients were registered from different countries all around the world. This global trial will evaluate the four drugs that WHO considered promising therapies for COVID-19 infection. These drugs include malaria drugs hydroxychloroquine and chloroquine, an HIV drug combination (LPV/r), an Antiviral drug (remdesivir), and an immunomodulator (LPV/r plus interferon beta) (Kupferschmidt and Cohen, 2020).

2.1.1. Hydroxychloroquine and Chloroquine

Hydroxychloroquine and chloroquine are well studied anti-malarial drugs. These drugs are safe to use and have mild side effects. However, these drugs should be administrated in prescribed safe dose as there is minor difference between therapeutic and toxic doses. Self-treatment is not recommended for both drugs (Delang and Neyts, 2020). Chloroquine is known for its antiviral activity against other coronaviruses in cell culture (De Wilde et al., 2014; Keyaerts et al., 2004). Clinical trial conducted in many hospitals of China demonstrated the efficacy of chloroquine phosphate against COVID-19. It was observed that chloroquine phosphate inhibits exacerbation of pneumonia, promote infection clearance from body, improve X-Ray findings and shortens disease course (Gao et al., 2020). Hydroxychloroquine indicates therapeutic potential against coronavirus when taken with azithromycin (Gautret et al., 2020). However, detailed study is required to access the efficacy of these drugs as patients registered in the said study were on different stages of COVID-19 (Delang and Neyts, 2020).

2.1.2. Lopinavir–ritonavir

HIV protease inhibitors have ability to inhibit cytochrome P450 in HIV. Lopinavir and ritonavir are two well studied HIV protease inhibitors. Efficacy of HIV protease inhibitors in case of coronavirus is not yet known as Coronavirus has different protease family as compared to HIV protease family (G. Li and De Clercq, 2020). In case of HIV protease, HIV protease inhibitors fit properly and serve as a drug for HIV. However, different protease morphology in coronavirus makes action of HIV protease inhibitor ambiguous. It was reported that LPV/r serves as an effective antiviral agent for SARS-CoV-1 when checked in cell culture (F. Chen et al., 2004). LPV/r also reduce the MERS-CoV load in marmosets (J. F. Chan et al., 2013).

A study conducted by Lim and coworkers indicated no beneficial effect of LPV/r on coronavirus (Lim et al., 2020). In another research, no significant difference in clinical presentation and mortality rates were observed in patients taking LPV/r and control group (Cao et al., 2020).
2.1.3. Remdesivir and Favipiravir
Remdesivir is under development drug against Ebola virus and inhibits viral RNA replication (Warren et al., 2014). It is prodrug and activate in the cell in the form of nucleoside triphosphate. Nucleoside triphosphate is an alternate substrate for RNA dependent RNA polymerase. Nucleoside triphosphate incorporates into viral RNA chain and cause chain termination. However, Remdesivir showed less efficacy in clinical trials conducted in Congo (Mulangu et al., 2019). Remdesivir is an antiviral drug used against different RNA viruses that includes coronavirus also. Antiviral activity of Remdesivir have been proved against coronaviruses in cell culture (Sheahan et al., 2017). It reduces the viral load and helps in pathogen clearance (Sheahan et al., 2020). Antiviral activity of Remdesivir against COVID-19 was also accessed (Wang et al., 2020). Patients on day 11 of infection were treated with Remdesivir and on the 12th day, patients’ condition was improved with improved oxygen saturation in lungs (Holshue et al., 2020). However, positive results of Remdesivir on only one patient cannot be considered sufficient for accessing its efficacy. More clinical studies are being conducted on COVID-19 patients worldwide.

Favipiravir is also an antiviral drug being considered to treat COVID-19 infection. It was initially approved against influenza virus. Favipiravir converts into Favipiravir-RTP when enters in the cells (Naesens et al., 2013). Favipiravir -RTP is a ribofuranosyl 5'-triphosphate metabolite which have ability to inhibit several RNA viruses (Delang et al., 2018). However, mode by which Favipiravir clears infection from body is not known. Antiviral activity of Favipiravir has also been proved in cell culture (Wang et al., 2020). A research conducted in China indicated rapid clearance of SARS-CoV-2 in patients taking oral Favipiravir as compared to patients in control group. X-Ray finding in group taking Favipiravir were also improved as compared to control group (Cai et al., 2020).

2.2. Immunomodulators
Cytokine storm is detected in COVID-19 patients as the infection progress (Hoijyo et al., 2020). This condition arises the need of anti-cytokines therapies (Buckley et al., 2020). However, inhibition of selective cytokines may pose a risk like reactivation of infection and increased risk of bacterial infections.

2.2.1. IL-6 inhibitors
Tocilizumab is an interleukin-6 inhibitor that was used for rheumatoid arthritis treatment. A trial was conducted in which COVID-19 patients were treated with tocilizumab. Body temperature of patients taking tocilizumab remained normal during infection. Oxygen saturation was also improved in 75% patients (Fu et al., 2020). The only shortcoming of this research was absence of control group. Use of tocilizumab is approved in China as an antiviral agent to cure COVID-19 infection. However, detailed and extensive studies are needed to fully demonstrate the efficacy of tocilizumab against COVID-19 infection.

2.2.2. Granulocyte-macrophage colony-stimulating factor
Granulocyte-macrophage colony-stimulating factor (GM-CSF) provides defense against different viruses and boost immune system. GM-CSF is an inflammatory cytokine and myelopoietic growth factor (Mehta et al., 2021). A recombinant GM-CSF (sargramostim) has been recommended and approved by FDA against COVID-19 (Lang et al., 2020). A pilot study named SARPAC is also being done in Belgium to access the beneficiary effects of leukine in COVID-19 patients on respirator (Delang and Neyts, 2020).

Following table indicates the Side effects of Antiviral Drugs and Immunomodulators use to treat COVID-19 infection.

Table 1.1. Side effects of Antiviral Drugs and Immunomodulators use to treat COVID-19 infection

<table>
<thead>
<tr>
<th>Serial Number</th>
<th>Drugs</th>
<th>Side Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hydroxychloroquine</td>
<td>Adverse effects involving the hair, nails or skin</td>
<td>(Sharma et al., 2020)</td>
</tr>
<tr>
<td>2</td>
<td>Chloroquine</td>
<td>Blurred vision, pruritus, paresthesia, insomnia</td>
<td>(Martins et al., 2015)</td>
</tr>
</tbody>
</table>
3. Vaccines

Vaccination is a reliable preventive and treatment option in case of viral diseases. As COVID-19 emerged as a pandemic, vaccination preparation is a global concern. Clinical trials are being conducted for vaccination preparation. To date, three vaccines are recommended by Center of Disease Control (CDC). These vaccines are Pfizer-BioNTech, Moderna and Johnson & Johnson’s Janssen. These vaccines are safe and effective and reduce the possibility of severe disease. Among these vaccines, Pfizer-BioNTech and Moderna are mRNA in nature and Johnson & Johnson’s Janssen is viral vector. Pfizer-BioNTech and Moderna are two shots while Johnson & Johnson’s Janssen is one shot. People 12 years and older can be vaccinated by Pfizer-BioNTech, while People 18 years and older can be vaccinated by Moderna and Johnson & Johnson’s Janssen.

### 2.3. Nanoparticles-base Treatment

Nanobiotechnology is an emerging field of medicine. Nanoparticles are being used for diagnosis and treatment of several diseases. Nanoparticles are known for their low toxicity, precise size, charges and capabilities for modification. Unique properties of nanoparticles make them able to overcome barriers while passing through different administration routes in the body. Nanoparticles are designed with extensive efforts to use in an NP-based intranasal delivery system. Nanoparticles are being used to deliver drugs, vaccines, peptides, antibodies and siRNA in mucosal administration (Alshweiat et al., 2019). Nanoparticles ensure safe delivery of drug or vaccine by avoiding enzyme degradation, extending release time, ensuring adjuvant co-delivery, increasing conjugated material level, offering target specific delivery and boosting immune system (Zhao et al., 2014).

Mucosal surface is the main site for infection initiation. Internasal delivery of drugs ensures non-invasive, simple, inexpensive and simple administration. Internasal route is most convenient route for drug delivery in case of respiratory diseases including coronavirus (Costantino et al., 2007). Numerous studies were conducted to evaluate the properties of nanoparticles used for pulmonary and nasal administration. It was observed that nanoparticles should be smaller than 100-200nm, have slight positive charge with sufficient hydrophobicity (Marasini and Kaminskas, 2019).

There are different types of nanoparticles that are used for internasal administration. These nanoparticles are divided into three major categories like organic nanoparticles, inorganic nanoparticles and self-assembling protein (virus like) nanoparticles (Itani et al., 2020). Following characteristics of nanoparticles make them useful for diagnostic and treatment purposes.

#### Drug Delivery

Peptide inhibitors restrict virus entry into cell. Peptide inhibitors are used in high dose and have several side effects. Nanoparticles serve as nanocarriers and ensures target specific delivery of drugs. Nanocarriers deliver low doses of drugs with minimal side effects (Xu et al., 2018). It was observed that anticancer drugs with drug delivery system possessed high bioavailability. Sunitinib and Erlotinib are two anticancer drugs are validated to reduce viral load in targeted cells in case of COVID-19. Both drugs conjugated with nanoparticles are predictable to impart positive outcomes in patients suffering with COVID-19 infection (Itani et al., 2020).

#### Specific siRNA Delivery

Coronaviruses possess ORF1a and ORF1b replicate. In this case, RNA interferences (RNAi) may serve to control infection by silencing coronavirus mRNA in human cells. RNAi incorporates non-coding RNA to RNA-induced silencing complex (RISC). When specific sequences are identified, RNAi separates from RISC and bind with specific sequence. Complementary strands of mRNA are degraded with specific enzymes (Y. Li et al., 2013). Therefore, viral replication and infection can be controlled with siRNAs.
Efficacy of siRNA has been observed in case of Hepatitis C virus (HCV) (Kim et al., 2009). However, target specific delivery is important factor that predict efficacy of treatment. Naked siRNA can be degraded by host enzymes, become instable and may cause toxicity in host body (Tseng et al., 2009). Therefore, specific nanocarriers are required to deliver a fragile molecule (siRNA). MERS-CoV was targeted by siRNA and delivery was enhanced by different nanocarriers (inorganic, lipid and polymeric nanoparticles) (Sohrab et al., 2018).

**Peptide Inhibitors**

Coronaviruses have spikes proteins. These viruses infect the cells through fusion mediated by S protein with host cell (Yuan et al., 2017). Two subunits S1 and S2 of Spike protein bind with Dipeptidyl peptidase 4 (DPP4) receptor (Raj et al., 2013). Therefore, restricting the fusion between host cell and pathogen can be helpful for treating COVID-19 infection.

A peptide inhibitor heptad repeat 1 (HR1) suppress HR1/HR2 mediated fusion between host cell and coronavirus. It was observed that peptide inhibitor delivered with gold nanorods showed good results as compared to inhibitor alone (Huang et al., 2019). These nanocomplexes exhibit biocompatibility and were more stable. Hence, these nanocomplexes can be used for coronaviruses treatment.

**Prevention of Coronaviruses Entry into Cells**

Nanoparticles can also be used to restrict the entry of coronavirus in the cells. Nanoparticles have large surface area and have ability to attach with antigens present on surface of pathogen. Therefore, restrict the entry of virus in host cell. Gold nanoparticles are known for their interaction with viruses (Szunerits et al., 2015).

A recent study indicated that Boronic acid ligands conjugated with carbon quantum dots have ability to bind with S protein of coronavirus and restricts its entry into the host cell (Łoczechin et al., 2019). It also reduces the infection rate of cells by inhibiting the viral replication. These nanoparticles possess diameter of 10nm and have high water solubility. These characteristics make these nanoparticles able to invade the cell (endocytosis) and prevent viral replication by interacting with it.

**Immune System stimulation by Virus-like Nanoparticles**

Viruses are used to activate immune system against a particular pathogen. Vaccine should mimic the original pathogen. So, Virus-like nanoparticles (VLNP) are of great interest in case of COVID-19 infection. Nanoparticles can easily invade lymphatic system and can present more than one antigen as compared to antigen presenting cells (presenting one antigen) (Moon et al., 2012). These characteristics of VLNP make them able to activate immune system and also serve as therapeutic agents in the body. Nanoparticles also have surface energy that cause strong adhesion with the host cells (Tenzer et al., 2013). In this way, they can mimic viruses more accurately and activate immune cells along with antibodies production.

A study represented that VLNP showed increase stability and S-protein retention as compared to antigen. This study also indicated increased delivery in lymphatic system as nanoparticles and S protein showed strong adhesion. VLNP cause IgG synthesis in abundance and as these can easily invade the cells and activate complement (H.-W. Chen et al., 2016).

Another study indicated that MERS-CoV nanoparticle vaccine with Matrix-M1 efficiently blocked the MERS-CoV replication in mice. It also produced high titer of Anti-S antibodies in-vivo (Coleman et al., 2017). These studies indicated that VLNPs have potential to activate immune system and also protect the host cells against specific infection.

**3.1. Nanoparticles for COVID-19 treatment**

Nanomaterials possess antiviral ability and clinical trials are being conducted on nanoparticles that serve as antiviral agents against coronaviruses including SARS-CoV-2. Nanotechnology provides information about nanomaterials, drug delivery, biocompatible nanomaterial and route of administration (Tang et al., 2021). Following nanoparticles are being considered for use against COVID-19.
3.1. Iron Oxide Nanoparticles for treating COVID-19

Metal oxide nanoparticles are well known for their antimicrobial activity (Abo-Zeid et al., 2018). Metal oxide nanoparticles produce reactive oxygen species (ROS) which are antimicrobial in nature. ROS oxidize biomolecules in microorganisms and cause cell death. Antiviral activities of metal oxide nanoparticles are also been investigated. Antimicrobial and antiviral activities of iron oxide nanoparticles are also well studied (Pessan, 2018). Based on various studies conducted on Dengue virus, Influenza virus and rotavirus, it was observed that these nanoparticles restrict the virus entry into host cell by interacting with surface protein of viruses (Gutierrez et al., 2009; Kumar et al., 2019; Murugan et al., 2017). As iron oxide nanoparticles are FDA approved, these could be a safe and efficient treatment for COVID-19.

Molecular docking of iron oxide nanoparticles with surface protein of SARS-CoV-2 indicated efficient interaction between iron oxide nanoparticles and SARS-CoV-2. Binding of nanoparticles with S1 protein subunit of SARS-CoV-2 may cause virus destruction through ROS as proven by previous studies. Clinical trials are required to prove iron oxide nanoparticles as a treatment option against COVID-19. Minimum dose, administration frequency, safety profile, drug’s compatibility with patients, interaction with co-administrated medicines and side effects of iron oxide nanoparticles should be addressed before conducting clinical trials (Abo-Zeid et al., 2020).

3.1.2. Silver Nanoparticles for treating COVID-19

Silver nanoparticles are known for their antiviral activities as they have ability to bind with surface glycoproteins of virus and restrict entry of viruses into cells (Park et al., 2014). It was observed that Silver nanoparticles also reduced cytokines like CCL5, TNF and IFNs in experimental mice. It was evaluated that silver nanoparticles of diameter 12nm are non-toxic at dose 50µg/ml with maximum anti-viral activity. Silver nanoparticles also have long half-life which make them a good option for treatment in case of respiratory diseases. A previous study indicated the anti-viral activity of silver nanoparticles (dose 4mg/kg body mass) in mice suffering from respiratory syncytial viruses. In these mice, immunomodulatory effects of silver nanoparticles were also evident (List and Content, 2020). These features make silver nanoparticles a good antiviral agent for COVID-19 treatment. Studies are being devised to evaluate the antiviral activity of silver nanoparticles in case of COVID-19. However, clinical studies are required to prove it fully (Sarkar, 2020).

3.1.3. Decoy nanoparticles for treating COVID-19

Decoy nanoparticles are produced by combining cell membrane nanovesicles (expressing ACE2) and monocytes (presenting cytokine receptors). Decoy nanoparticles have ability to compete host cells and absorb viruses as well as inflammatory cytokines. These characteristics make decoy nanoparticles an efficient antiviral agent. A recent study (Rao et al., 2020) proved the antiviral activity of decoy nanoparticles for COVID-19 infection which represent them as promising antiviral agent. However detailed studies are required to use them as an agent to combat COVID-19 infection.

3.1.4. Glycyrrhizic Acid Nanoparticles for treating COVID-19

Glycyrrhizic acid had been extensively used in Chinese medicines. It is known for antibacterial and antiviral activities. In a recent study, it was observed that Glycyrrhizic acid nanoparticles inhibits the coronavirus proliferation in-vitro. In mouse model, glycyrrhizic acid nanoparticles target the site of inflammation and improve treatment outcomes. These nanoparticles reduce organ damage in mouse models of COVID-19. Extensive and detailed studies are needed to evaluate their anti-viral activity against COVID-19 infection.

IV. CONCLUSION

COVID-19 is a pandemic that is affecting all nations worldwide. Novelty and severe complication are two major factors that are making situation worse. Researches are being conducted to understand SARS-CoV-2 genome, diagnosis and treatment. As SARS-CoV-2 belongs to coronavirus family, already approved antiviral drugs against SARS-CoV-1 and MRSA are considered for their antiviral activity against COVID-19 infection. Extensive clinical trials are needed to check their efficacy against COVID-19. These drugs also have some side effects. Vaccines are also being produced. Vaccine production is a time-consuming process and required hundreds of trials to be approved for treatment of disease. Nanobiotechnology is an emerging field of medicine. Nanoparticles are target specific, deliver drug safely, evade immune response and have antibacterial and antiviral properties. For treatment of COVID-19, different nanoparticles are being studied and indicated antiviral activity in-vitro. These nanoparticles
deliver drug or vaccine to a specific site and improve the treatment outcomes. However, more studies should be conducted to validate the use of nanoparticles for COVID-19 treatment.

REFERENCES


