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Literature Review

Potential of Antisense Oligonucleotides (ASO) Through Inhalation Based on Gold Nanoparticle (AuNP) Delivery System in Inhibiting SARS-CoV-2 Replication And Transcription

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ABSTRACT

Introduction: Coronavirus disease 2019 (COVID-19) is an emerging infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). This virus infects the respiratory, digestive, and nervous systems with a rapid transmission and a fairly high mortality rate. However, there has been no specific therapy to treat COVID-19. Previous studies have shown that antisense oligonucleotide (ASO) has good efficacy in DNA and RNA viral infections. This literature review aims to investigate the potential of inhaled ASO based on gold nanoparticles (AuNp) delivery system in inhibiting the replication and transcription of SARS-CoV-2. **Method**: Literature searching using several databases, such as Google Scholar, Science Direct, ResearchGate, and NCBI. Inclusion and exclusion criteria are used to eliminate the journals that does not match the criteria, thus 28 journals are obtained. **Results**: The results show that ASO has the potential to inhibit the replication and transcription of the SARS-CoV-2 virus through different mechanisms by binding to the target RNA and modulating the viral protein synthesis. One form of ASO modification that is often used is LNA GapmeR. LNA GapmeR stimulates viral RNA cleavage and can be administered by inhalation with nebulized ASO solution. AuNP as an ASO delivery system through inhalation can reduce toxicity and increase ASO concentrations in reaching target cells. **Conclusion**: Therefore, ASO therapy with AuNP through inhalation needs to be considered for COVID-19 treatment. Further clinical study about the ideal delivery system and optimal dosage of ASO based AuNP via inhalation for COVID-19 are needed to investigate soon.

Keywords: Antisense oligonucleotide, gene editing therapy, gold nanoparticles, inhalation, SARS-CoV-2 replication and transcription

ABSTRAK

Latar belakang*: Coronavirus Disease* 2019 (COVID-19) merupakan penyakit jenis baru yang disebabkan oleh virus *Severe Acute Respiratory Syndrome Coronavirus 2* (SARS-CoV-2). Virus ini menyerang sistem pernafasan, pencernaan, dan persarafan dengan proses penularan yang cepat serta angka kematian yang cukup tinggi. Namun, hingga saat ini belum ada terapi yang spesifik untuk menangani COVID-19. Penelitian sebelumnya diketahui bahwa *Antisense Oligonucleotide* (ASO) diketahui memiliki efikasi yang baik pada penyakit infeksi virus RNA. *Literature review* ini ditujukan untuk mengetahui potensi *Antisense Oligonucleotides* (ASO) berbasis inhalasi nanopartikel emas dalam menghambat replikasi dan transkripsi SARS-CoV-2. **Metode**: Pencarian literatur menggunakan mesin pencari berupa *Google Scholar*, *Science Direct*, *ResearchGate*, dan *NCBI*. Kriteria inklusi dan eksklusi digunakan untuk mengeliminasi jurnal yang tidak berkaitan sehingga didapatkan 28 jurnal pada penulisan ini. **Hasil**: Hasil telaah pustaka menunjukkan bahwa ASO memiliki potensi dalam menghambat replikasi dan transkripsi virus SARS-CoV-2 melalui dua mekanisme yang berbeda dengan pengikatan terhadap RNA target dan memodulasi sintesis protein virus tersebut. Salah satu bentuk modifikasi ASO yang sering digunakan ialah LNA GapmeR. LNA GapmeR merangsang pembelahan RNA virus dan dapat diberikan secara inhalasi dengan nebulisasi larutan ASO. Penggunaan nanopartikel emas sebagai sistem penghantar ASO melalui inhalasi dapat menurunkan toksisitas dan meningkatkan konsentrasi ASO dalam mencapai sel target. **Simpulan**: Oleh karena itu,

terapi ASO dengan sistem penghantar nanopartikel emas (AuNP) melalui inhalasi perlu menjadi pertimbangan untuk terapi COVID-19. Penelitian klinis lebih lanjut diperlukan untuk mengetahui sistem penghantaran yang ideal dan dosis optimal ASO dengan penghantaran AuNP melalui inhalasi untuk COVID-19.

Kata Kunci: Antisense oligonukleotida, inhalasi, nanopartikel emas, replikasi dan transkripsi SARS-CoV-2, terapi pengeditan gen
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INTRODUCTION

Coronavirus Disease 2019 (COVID-19) is an infectious disease caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). SARS-CoV-2 belongs to the type of positive single-chain RNA virus -coronavirus group, which is in the same family as SARS-CoV and MERS-CoV.[1] This virus infects the respiratory, digestive, and nervous systems with a fast transmission process and a relatively high mortality rate.[2] Based on data from the World Health Organization (WHO), until 15th February 2021, global COVID-19 cases reached 412,351,279 cases with 5,821,004 deaths, while in Indonesia, it had reached 1,147,010, and death with 31,393 cases.[3,4]

Until now, there is no specific therapy to treat COVID-19. The management of COVID-19 patients is focused on symptomatic and supportive therapy such as antipyretics for fever, oxygenation, and fluid management.[5] Meanwhile, repurposed drug combinations are still the main therapy used in COVID-19 patients who have severe symptoms and are hospitalized. WHO published the Solidarity Clinical Trial program for the treatment of COVID-19 involving various countries to determine specific and effective therapies for COVID-19 patients, it was found that remdesivir, hydroxychloroquine, lopinavir, and interferon had little or no effect on patients with COVID-19 based on indicators of mortality, ventilator use, and duration of hospitalisation.[6]

In the last decades research on targeted therapies with biomolecular approaches such as SHERLOCK-based CRISPR-Cas12/13, DETECTR, CARVER and PAC-MAN, ASO, antisense peptide nucleic acids, ribozymes, aptamers, and RNAi silencing therapy have been developed.[7] One of the potential gene therapies for treating COVID-19 is Antisense Oligonucleotide (ASO). ASO has several advantages over other gene therapies, such as easy to design, affordable production costs, and has many optimizations for chemical modifications in various diseases.[8] ASO is a gene-editing-based molecular biology therapy targeting mRNA, small RNA, or long non-coding RNA.[9] ASO chemically synthesizes 15-50 nucleotides in length designed to bind to target RNA via complementary base pairs and modulate RNA function.[10] Previous study reported that ASO is known to have good efficacy in viral infectious diseases, especially in RNA viruses such as Ebola, influenza, hepatitis C, Japanese encephalitis, and SARS-CoV.[7]

In COVID-19 therapy, inhalation delivery is required to reduce toxicity and increase ASO concentrations reaching the infected lung epithelium. In addition, inhalation administration can promote higher bioavailability, thus having a longer half-life than oral and intravenous administration.[11] ASO can be administered by inhalation via a

nanoparticle delivery system. Nanoparticles has great potential to assist the distribution of drugs with various properties, such as small sizes, making it easier to deliver drugs to places are difficult to reach.[12] Inhalation through nanoparticles as drug delivery agents is known to have longer retention in the lungs and better penetration of mucous mucus.[13] Among other nanoparticles, gold nanoparticles (AuNP) has good biocompatibility in size, shape, and stability and is easy to synthesize.[14] In addition, AuNPs are known to have good intrinsic antiviral activity. Previous study reported that AuNP is effective against various types of viruses, namely HIV-1, H1N1, H3N2, H5N1, hand foot and mouth disease (HFMD), and dengue virus.[15]

Based on the high number of COVID-19 cases and the absence of specific COVID-19 therapy, this literature review aim to determine the potential of gold nanoparticle-based Antisense Oligonucleotides (ASO) in inhibiting the replication and transcription of SARS-CoV-2. This literature review is expected to contribute to the development of knowledge.

METHOD

Search keywords, such as "antisense oligonucleotides (ASO)", "gold nanoparticles (AuNP)", "inhalation", "SARS-CoV-2 replication and transcription", and "gene therapy here" were used as a keyword for literature searching. The literature search was conducted using search engines in several database such as Google Scholar, Science Direct, ResearchGate, and NCBI. The inclusion criteria used in the literature are evidence-based medicine research journals at levels 1, 2, and 3 and experimental research both in vitro, in vivo, ex vivo, and clinical research with publication provisions within the last ten years. The exclusion criteria are related to the studies other than Indonesian and English.

Evaluation of inclusion and exclusion criteria was carried out by assessing the title and abstract as a first step, and then the full text was reviewed if there was a correlation of keywords with each other in the journal so that it could support writing a description or analysis in this literature review. From the results of the literature search using inclusion and exclusion criteria, 28 journals were used in this work.

DISCUSSION

Pathogenesis and Potential Targeted Therapy Against SARS-CoV-2

The immune response to COVID-19 causes an increase in inflammatory cytokines and chemokines produced by protein synthesis from the SARS-CoV-2 genome. After inhalation through aerosol droplets containing the virus, SARS-CoV-2 binds to the ACE2 receptor of the nasal epithelium.[16] SARS-CoV-2 infection occurs via an endocytosis process in which the viral S1 subunit of the spike protein (S) binds to the human angiotensin-converting enzyme-2 (ACE2) mammalian cell receptor.[17] If containment of the virus remains unsuccessful, the virus spreads to other organs in the body that have ACE2 receptors, including the heart, liver, kidneys, and intestines. This triggers the activation of the interferon-type innate immune response and an increase in neutrophils; pro-inflammatory cytokines such as TNF, IL-6, IL-1β, and IL-17 were observed, which ultimately lead to inflammation.[18]

SARS-CoV-2 has a unique replication strategy, as it has a nonsegmental positive-sense RNA genome containing a 5' cap structure along with a 3' poly-A tail, allowing it to be read by the polyprotein

replicase for translation.[8] Two-thirds of the genome is composed of nonstructural proteins (NSP) genes. The most important structural proteins of Coronavirus are spike protein (S), membrane protein (M), envelope protein (E), and nucleocapsid protein (N) (Figure 1). The other proteins are generated as two polyproteins (ORF1a and ORF1ab), which are prepared into 12 nonstructural proteins (NSPs) by three viral proteases.[19] All of these NSPs have essential roles in replication and transcription, making each a potential drug target.

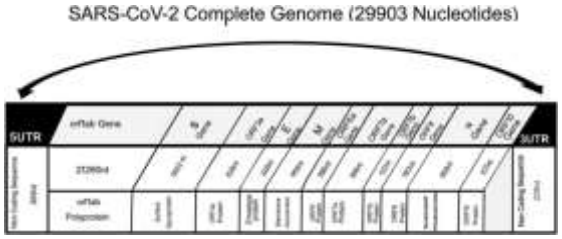

Figure 1. Structure of the SARS-CoV-2 genome. [19]

The therapy essentially targets eight major steps in the coronavirus replication cycle, including drugs viral endocytosis, fusion, helicase, protease, replication, and translation. Nucleic acid-based therapies, especially RNA therapy including RNAi (RNA interference), siRNA (small interfering RNA), RNA aptamers, ribozymes, and ASO will both target and neutralize important components of mRNA molecules that synthesize viral proteins such as E (envelope), M (membrane), or N (nucleocapsid), or SARS helicase.[16]

Oligonucleotide-based therapeutic strategies can target the virus itself by inhibiting its spike protein (S) or interfering its replicative protein via protein-nucleic acid interactions — as in aptamers — or by directly targeting its large genome via siRNA or ASO. — mediation of gene silencing.[8] As an alternative, gene silencing (or activation) approaches can be used to reduce the effects of inflammation in the lungs and other organs, leading to death in severe COVID-19 cases.[20]

The role of ASO in inhibiting SARS-CoV-2 replication and transcription

ASO is a gene-editing-based molecular biology therapy that targets mRNA, small RNA, or long non-coding RNA. ASO can act on and modulate RNA through two different mechanisms within the cell; (1) in the cytoplasm, ASO interferes with mRNA function and prevents mRNA translation by ribosomes and blocks protein synthesis; (2) in the nucleus, ASO binds to complementary mRNA strands. Complementary base pairing between ASO and mRNA causes a decrease in endonuclease-mediated transcription as the RNA-DNA hybrid becomes a substrate for RNase H. Transport of mRNA to the cytoplasm is inhibited, thereby preventing protein production (Figure 2).[7]

There are several kinds of chemical modification of ASO such as phosphorothioate (PS), phosphorodiamidate morpholino oligomer (PMO), peptide nucleic acid (PNA), locked nucleic acids (LNAs), and nucleobase modification. ASO was designed to target the genomic expression of 5′-UTR, ORF1a, and ORF1b of the transcriptase replicase complex, and the N genes encoding genomic-associated nucleoproteins.[21]

Each ASO modification has its own mechanism and advantages depending on the intended purpose. Modifications of ASO that are often used in a number of therapeutic applications are phosphorodiamidate morpholino oligomers (PMOs) and locked nucleic acids (LNAs).[10] Modifications of ASO that can be applied as a therapy for SARS-CoV-2 by

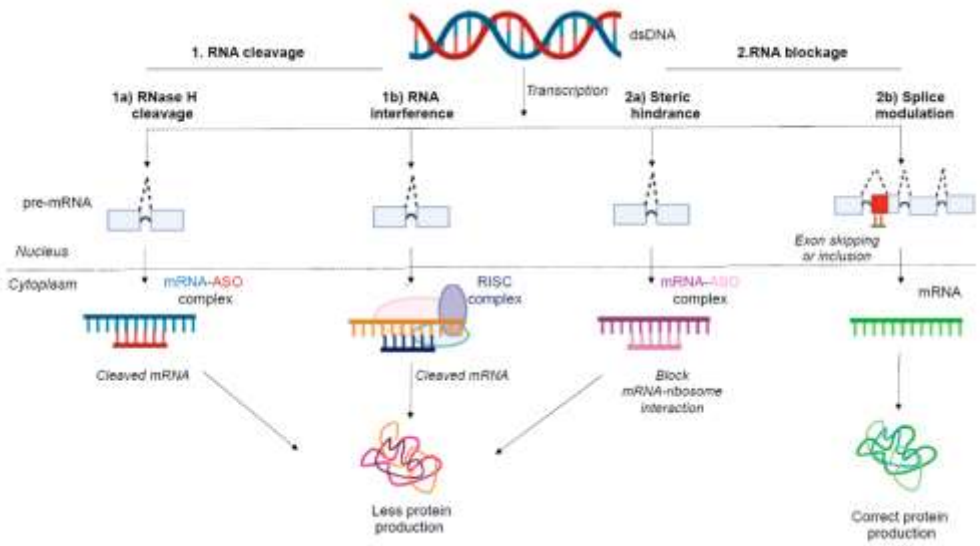

Figure 2. ASO mechanism of action: (1) RNA cleavage and (2) RNA blocking[10]

inhalation are locked nucleic acids (LNAs). The form of the LNA is the GapmeR LNA, an LNA-DNA-LNA-based design containing continuous DNA nucleotides between two LNA nucleotides located at the terminal (Figure 3). It has been shown that GapmeR LNA stimulates the RNase H1-based cleavage mechanism.[10]

Figure 3. Modification of ASO with the form of LNA GapmeR[10]

The advantage of using modified LNA GapmeR is that it can be delivered in vivo to target cells without transfection reagents. This modification is stable in sterile aqueous solutions and can be administered by subcutaneous injection. In respiratory infections, LNA GapmeR can be administered by aerosol inhalation. Such aerosols can be produced by nebulizing ASO solution. The advantages of inhalation delivery would be lower toxicity and higher ASO concentrations reaching the lung epithelium infected by SARS-CoV-2.[21]

Based on the previous study conducted by Berber et al., ASO provides several advantages compared to other molecular biology therapies based on gene editing, namely easy to design, cost-effective to produce, low toxicity and already has many chemical modifications that optimize its use in various infectious diseases. Thus, Optimization of medicinal chemistry will be easier when a pandemic occurs where rapid drug development is urgently needed.[7,8]

The Delivery of ASO by Inhalation

The lungs are the entry site and intracellular formation of many airborne pathogens, including viruses (e.g., influenza, SARS, RSV, and the common cold) and bacteria (e.g., *Streptococcus pneumonia* and *Mycobacterium tuberculosis*). The lungs can be one of the target organs for drug delivery in respiratory tract infections. Lung's large surface area, thin epithelial layer and rich blood supply ensure the rapid absorption of the drug. The metabolic rate in the lungs is much lower than in the gastrointestinal tract and liver. Therefore, most inhaled medications pass metabolism directly through the lungs to target cells and avoid systemic degradation.[22]

Oligonucleotide-based therapies across the lung mucosal epithelium are inhibited by several biological barriers (Figure. 4).[23] These include the physical barrier of the overlying mucus layer and the tight arrangement of epithelial cells combined with the sweeping motion of the apical cilia that removes luminal material from the mucosal surface. In addition, unconjugated and formulated siRNA and ASO are susceptible to the recognition and subsequent destruction by alveolar macrophages that are part of the mononuclear phagocyte system.[24]

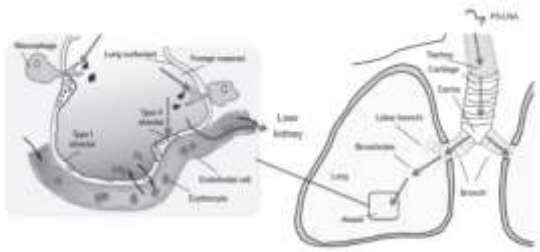

Figure 4. Illustration of intratracheal delivery and macrophage capture of PS-LNA in the lung. PS-LNA accumulates in macrophages and alveolar cells which further facilitates systemic migration to the kidney and liver.[23] LNA, locked nucleic acid; PS, phosphorothioate.

Upon reaching the target cell, the oligonucleotide moves across the cell membrane, escape hydrolytic damage in the endosome, and interact with the target mRNA before translation.[25] Optimization of delivery systems and oligonucleotide design are important considerations to enable the clinical application of nucleic acid-based therapies to the lung. Advances in nanotechnology offer potential solutions to the challenges of oligonucleotide delivery, particularly the requirements for crossing biological barriers and transmembrane intracellular delivery.

The Role of Gold Nanoparticles (AuNP) as a Delivery System for Antisense Oligonucleotides (ASO) through Inhalation

In the delivery of antiviral drugs by inhalation, the nanoparticle size should be in the range of 3 nm - 7 nm.[26] Nano-sized particles have deposition characteristics that are ideal for delivery to the alveolar areas of the lungs, settle widely, and spread more evenly in the lungs. Drug delivery in nano-sized particles offers additional advantages. Nano-sized particles can escape macrophage clearance and penetrate the epithelium into therapeutic target cells in the lung.[22]

Research conducted by James and Howard's 2012 highlighted the importance of combining LNA-ASO or siRNA with delivery reagents such as lipids and polyplexes to increase gene knockdown efficiency in lung tissue.[27] The disadvantage of these lipid-based nanocarriers is that their delivery is limited to the liver and reticuloendothelial system as the sinusoidal capillary epithelium in these tissues provides a sufficiently large space to allow entry of these relatively large nanoparticles.[25]

AuNP, as one of the ASO delivery systems, offers several benefits in

achieving target cell therapy as an antiviral. AuNPs conjugated with drugs create attractive potentials because they are able to bind to a variety of organic and biological molecules, have lower toxicity, and have a more substantial absorption spectrum than other nanoparticles. Drug delivery system with AuNP through inhalation can also increase the concentration of ASO in reaching the lung epithelium infected by SARS-CoV-2.[21]

AuNPs were reported to inhibit H1N1 influenza virus replication by regulating the expression of IFN-β and other IFN-stimulated-genes (ISG), resulting in decreased viral replication. Hyaluronic acid AuNP and IFN complex have been used for the treatment of hepatitis C (HCV) infection. AuNP also can suppress influenza, HSV, and HIV viruses. AuNP with sialic acid function inhibits influenza virus infection by multivalent interaction, relatively better than some synthetic clinical drugs such as zanamivir and oseltamivir which are susceptible to resistance by influenza virus. Therefore, apart from being a therapeutic delivery system, AuNPs also have an important role in antiviral activity.[28]

To this date, there is no in vitro and in vivo study regarding ASO-AuNP in SARS-CoV-2.[29] However, Li et al., used modified ASO, peptide-conjugated PNA (PPNA) in cultured Vero-E6 cells to target 5'UTR in SARS-CoV-2 replication in cultured cells.[29] It was found that sequences were very effective where 5'UTR-3 sequences could reduce titers by up to 75% in the SARS-CoV-2 test. [29] A previous in vivo study by Sekimukai et al. stated his research on Virus-like particles (VLP). VLP was produced with 10 nm CpG oligodeoxynucleotides (ODN) with AuNP and core antigen of hepatitis B. A 50 g conjugate (i.p.) of VLP injected into mice, showing a 200% increase in antibody titers in mice compared to ODN without AuNp. In addition beside increasing antibody titers, the use of ODN and AuNp led to increase in CD4 T cells and cytotoxic T cells with increased secretion of IL-4 and IFN-γ as well as Th1 and Th2 immunostimulating responses.[30]

Many AuNP polymer-based therapeutic delivery systems such as poly(lactide-co-glycolide) (PLGA), poly(β-amino ester) (PBAE), and polyethylenimine (PEI) have been used as ASO delivery system. PBAE and PEI exert a 'proton sponge' effect which reduces endosomal enzyme immobilization and increases ASO concentrations to target cells. PLGA conjugated nanoparticles have been approved by the FDA and are widely used as ASO delivery systems. However, the minimal side effect that can occur with the use of these conductors is toxicity that can be caused by excessive cationic loads so that rational and efficient doses must still be considered in their use.[10]

CONCLUSION

ASO has great potential as a SARS-CoV-2 therapy through a AuNP delivery system inhalation. ASO is able to inhibit the replication and transcription of SARS-CoV-2 through cleavage and blockade of viral RNA. ASO has several advantages, such as being easy to design, cost-effective to manufacture, low toxicity, and already has many chemical modifications that optimize its use in various infectious diseases. The use of AuNP by inhalation can reduce toxicity and increase the concentration of ASO in reaching the infected lung epithelium. In addition, the AuNP delivery system has antiviral capabilities as a distinct advantage that can synergise with ASO to inhibit SARS-CoV-2 virus infection. However, the minimal side effects that can occur with these conductors are caused by excessive

cationic loads, so rational and efficient doses must be considered in their use.

RECOMMENDATIONS

Further research on the specific properties of ASO with the ideal inhalation administration based on AuNP delivery system with the optimal dose for COVID-19 therapy and the regulatory system for drug release through inhalation is urgently needed. The synergy of researchers, health workers, and government is required in a pandemic situation to create implementable steps in research related to COVID-19 to reduce the rate of death due to COVID-19.

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