Desain Antiviral Berbasis Small Interfering RNA dan Efikasinya Secara In Vitro Terhadap Virus Avian Influenza Subtipe H5N1 Asal Indonesia = Antiviral Design and Itâs In Vitro Efficacy based on small interfering RNA against Avian Influenza Viruses Subtype H5N1 circulating in Indonesia

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Abstrak

Latar Belakang: Penyakit avian influenza subtipe H5N1 Asian lineage yang mulai mewabah di kawasan Asia, Afrika dan Eropa sejak tahun 1997 selain menimbulkan kerugian ekonomi yang sangat signifikan juga mengancam aspek kesehatan manusia dimana sejumlah korban meninggal dunia karena infeksi virus yang bersifat zoonosis. Penanganan penyakit dilakukan dengan antiviral yang berbasis neuraminidase inhibitor. Permasalahan timbul sebagai akibat mutasi beberapa strain virus menjadi resisten terhadap antiviral yang ada. Penelitian ini bertujuan untuk melakukan desain antiviral alternatif berbasis siRNA terhadap gen nucleoprotein yang lebih sesuai terhadap virus avian influenza subtipe H5N1 yang bersirkulasi di Indonesia. Metode: Desain siRNA dilakukan secara in silico dengan program siDirect 2.0 berdasarkan 210 sekuen gen nucleoprotein virus H5N1 yang bersirkulasi di Indonesia. Dua kandidat siRNA-NP672 dan siRNA-NP1433 dipilih berdasarkan kajian bioinformatik. Selanjutnya, kedua kandidat siRNA-NP tersebut ditantang secara in vitro pada sel Mabin-Darby canine kidney (MDCK) terhadap virus H5N1 asal Indonesia clade 2.1.3 dan 2.3.2 dengan menggunakan siRNA-NP1496 sebagai pembanding. Paramater yang diamati adalah produksi virus dan ekspresi gen virus. Terakhir, analisa mutasi gen nucleoprotein virus H5N1 dilakukan untuk melihat paparan siRNA-NP secara berulang kali. Hasil: Kandidat siRNA-NP672 memberikan efek penurunan infeksi virus H5N1 yang lebih baik dalam menurunkan tingkat infeksi virus HPAI subtipe H5N1 baik clade 2.1.3 dan 2.3.2 secara in vitro pada sel MDCK yang dicerminkan dengan titer produksi virus dibandingkan dua desain lainnya yaitu siRNA-NP1433 dan siRNA-NP1496. Pemberian siRNA-NP672 juga memberikan efek peredaman yang lebih tinggi dan konsisten terhadap ekspresi gen-gen virus, antara lain nucleoprotein, polymerase acidic, hemagglutinin, neuraminidase, Matrix, dan non-structural. Hasil kajian bioinformatik terhadap struktur sekunder dan tersier RNA gen nucleoprotein menunjukkan bahwa target siRNA-NP672 lebih berinteraksi karena memiliki bagian bebas (loop) yang lebih banyak dibandingkan dua kandidat siRNA-NP lainnya. Selanjutnya, paparan siRNA-NP tidak memicu terjadinya mutasi gen target pada virus H5N1 baik clade 2.1.3 dan clade 2.3.2 setelah 3 kali paparan. Kesimpulan: Desain siRNA-NP672 menunjukkan prospek yang lebih baik dalam menurunkan tingkat infeksi virus avian influenza subtipe H5N1 baik clade 2.1.3 dan clade 2.3.2.

.....Introduction: Avian influenza disease outbreak of subtype H5N1 Asian lineage that has spread in Asia, Africa, and European continental since 1997 caused massive economic drawbacks as well as a zoonotic threat where numerous deaths related to viral infection. The treatment of viral infection has been done with antiviral based on neuraminidase inhibitors. However, mutation of numerous virus strains has been confirmed that may lead to resistance against current antivirals. This study's objective was to design an alternative antiviral based on siRNA targeting nucleoprotein gene that is more suitable for the avian influenza viruses subtype H5N1 circulating in Indonesia. Methods: The siRNA design was accomplished in

silico using the siDirect 2.0 program based on 210 nucleoprotein gene sequences of H5N1 viruses circulating in Indonesia. Two siRNA candidates (siRNA-NP672 and siRNA-NP1433) were chosen based on bioinformatic analyses. Subsequently, these siRNA-NP candidates were challenged in vitro in Mabin-Darby canine kidney cell culture against the Indonesian H5N1 both clade 2.1.3 and clade 2.3.2 using siRNA-NP1496 as a comparison. The parameters analyzed within the study are including virus production and viral gene expression level. Finally, mutation analysis was performed to evaluate the effect of three serial siRNA-NP exposures to the target gene of the H5N1 viruses. Results: The siRNA-NP672 provides a better reduction of the H5N1 viral infection, especially on viral production titer for both clade 2.1.3 and clade 2.3.2 compared to the two other siRNA candidates, including siRNA-NP1433 and siRNA-NP1496. The siRNA NP672 also provides a better and more consistent reduction of viral gene expression levels, including nucleoprotein, polymerase acidic, hemagglutinin, neuraminidase, Matrix, dan non-structural. This finding was confirmed by bioinformatic analyses of the siRNA-NP672 biding site in the secondary and tertiary structure of the nucleoprotein gene which has more free parts (loop) compared to the two other siRNA-NP candidates. Subsequently, three serial exposures of siRNA-NP do not induce any mutation on the target site of the nucleoprotein gene of the H5N1 virus both clade 2.1.3 and 2.3.2. Conclusion: The design of siRNA-NP672 provides a better prospect to reduce the Indonesian avian influenza virus subtype H5N1 infection for both clade 2.1.3 and 2.3.2.