Pengembangan Produk Kandidat Biosimilar Dan Biobetter Tocilizumab Untuk Penghambatan Interaksi Il6r - Il6 Dengan Pendekatan Bioinformatika Dan Analisis In Vitro = Development Of Tocilizumab Biosimilar and Biobetter Candidate Products for Inhibiting IL6R - IL6 Interactions Using a Bioinformatics And In Vitro Analysis Approach

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Abstrak

Latar Belakang
Biosimilar merupakan produk bioterapeutik yang memiliki kemiripan/kesetaraan mutu, dengan originator, sedangkan biobetter merupakan versi produk biologis lain yang dimodifikasi. Tocilizumab (TCZ) merupakan rekombinan antibodi monoklonal manusia yang dapat menghambat interaksi IL6R dengan IL6 saat inflamasi kronis seperti pada penyakit Rhematoid Arthritis (RA). Saat ini, paten TCZ telah habis, oleh karena itu penelitian ini akan dikembangkan kandidat biosimilar dan biobetter TCZ.
Metode
Penelitian ini merupakan studi in silico dan in vitro untuk pengembangan kandidat biosimilar dan biobetter TCZ dengan mendesain gen kandidat biosimilar TCZ yang dapat terekspresi di sel mamalia dan dilakukan pengujian hasil transfeksi dengan immunofluorescence, pemeriksaan ELISA, SDS-PAGE dan SPR.
Hasil
Hasil penelitian didapatkan struktur 3D kandidat biosimilar TCZ nilai Ramachandran Plot 97.16%, score molecular docking biosimilar TCZ light chain dengan IL6R sebesar -16.0 kcal mol-1 dan lebih besar dari nilai kontrol yaitu -12.5 kcal mol-1. Didapatkan nilai RMSF antara IL6R dengan kandidat biosimilar TCZ rerata <2. Hasil transfeksi kandidatbiosimilar TCZ yang dinilai menggunakan ratio of fluorescence intensity memiliki nilai intensitas diatas 2 sedangkan kontrol memiliki nilai 0. Terdapat dua pita pada 50kDa dan 25 kDa sesuai dengan ukuran protein TCZ reference. Koofisien afinitas IL6R dengan kandidat biosimilar TCZ 9.44e-8 dan menyerupai dengan koofisien afinitas IL6R dengan TCZ Actemra® yaitu sebesar 2.64e-8. Dilakukan modifikasi asam amino pada TCZ light chain paten 1 untuk membuat kandidat biobetter yaitu Ala43, Tyr87 dan Gly41. Hasil validasi 3D kandidat biobetter TCZ dengan hasil 96.70% pada Ramachandran Plot, energi bebas TCZ light chain biobetter sebesar -18.7 kcal mol-1.
Kesimpulan
Produk kandidat biosimilar TCZ memiliki ukuran molekul protein yang sesuai dengan originator TCZ (Actemra®). Selain itu produk biosimilar ini terbukti dapat berikatan spesifik dengan IL6R alfa dengan koofisien afinitas menyerupai Actemra® secara in vitro. Desain in silico kandidat biobetter TCZ dengan modifikasi asam amino Ala43, Tyr87 dan Gly41 menunjukkan afinitas ikatan IL6R alfa lebih kuat dibandingkan Actemra® sehingga diharapkan dapat meningkatkan potensi dalam mengatasi badai sitokin.
.....Background
A biosimilar is a biotherapeutic product that closely matches the quality of the original product, while a biobetter is a modified version of another biological product. Tocilizumab (TCZ) is a synthetic antibody derived from human cells that can block the binding of IL6R to IL6, hence reducing chronic inflammation in conditions like rheumatoid arthritis (RA). At now, the patent for TCZ has lapsed, thus this study aims to

create biosimilar candidates and improved versions of TCZ, known as TCZ biobetter candidates.
Method
This research involves both in silico and in vitro methods to produce biosimilar and biobetter TCZ candidates. The aim is to build a biosimilar candidate TCZ gene that can be expressed in mammalian cells. The transfection results will be tested using immunofluorescence, ELISA, SDS-PAGE, and SPR investigations.
Results
The research results showed that the TCZ biosimilar candidate possessed a Ramachandran Plot value of 97.16% for its 3D structure. Additionally, the molecular docking score of the TCZ light chain biosimilar with IL6R was -16.0 kcal mol-1, which was higher than the control value of - 12.5 kcal mol-1. The analysis showed that the average Root Mean Square Fluctuation (RMSF) value between IL6R and TCZ of the biosimilar candidate was less than 2. The TCZ transfection results of the biosimilar candidate were evaluated by measuring the ratio of fluorescence intensity. The biosimilar candidate had an intensity value more than 2, while the control had a value of 0. Two bands were observed at 50 kilodaltons (kDa) and 25 kDa, corresponding to the size of the reference TCZ protein. The IL6R signal affinity of the biosimilar candidate TCZ is 9.44e-8, which is comparable to the IL6R signal affinity of Actemra® TCZ, which is 2.64e-8. The TCZ light chain patent 1 underwent amino acid changes to generate improved biobetter candidates, specifically Ala43, Tyr87, and Gly41. The 3D validation results of TCZ biobetter show a yield of 96.70% on the Ramachandran Plot. Additionally, the free energy of TCZ light chain biobetter is -18.7 kcal mol-1.
Conclusion
The TCZ biosimilar candidate product has a protein molecular size that is identical to the TCZ (Actemra®). Furthermore, this biosimilar product has demonstrated the ability to selectively attach to IL6R alfa with a signal affinity comparable to Actemra® in laboratory tests. The computational design of the biobetter TCZ candidate, incorporating alterations to the amino acids Ala43, Tyr87, and Gly41, demonstrates an enhanced affinity for IL6R alfa compared to Actemra®. This raises the expectation that it may enhance its efficacy in mitigating cytokine storms.