Apolipoprotein A-I as a platform for modulating and prolonging the activity of cytokines fused with it.

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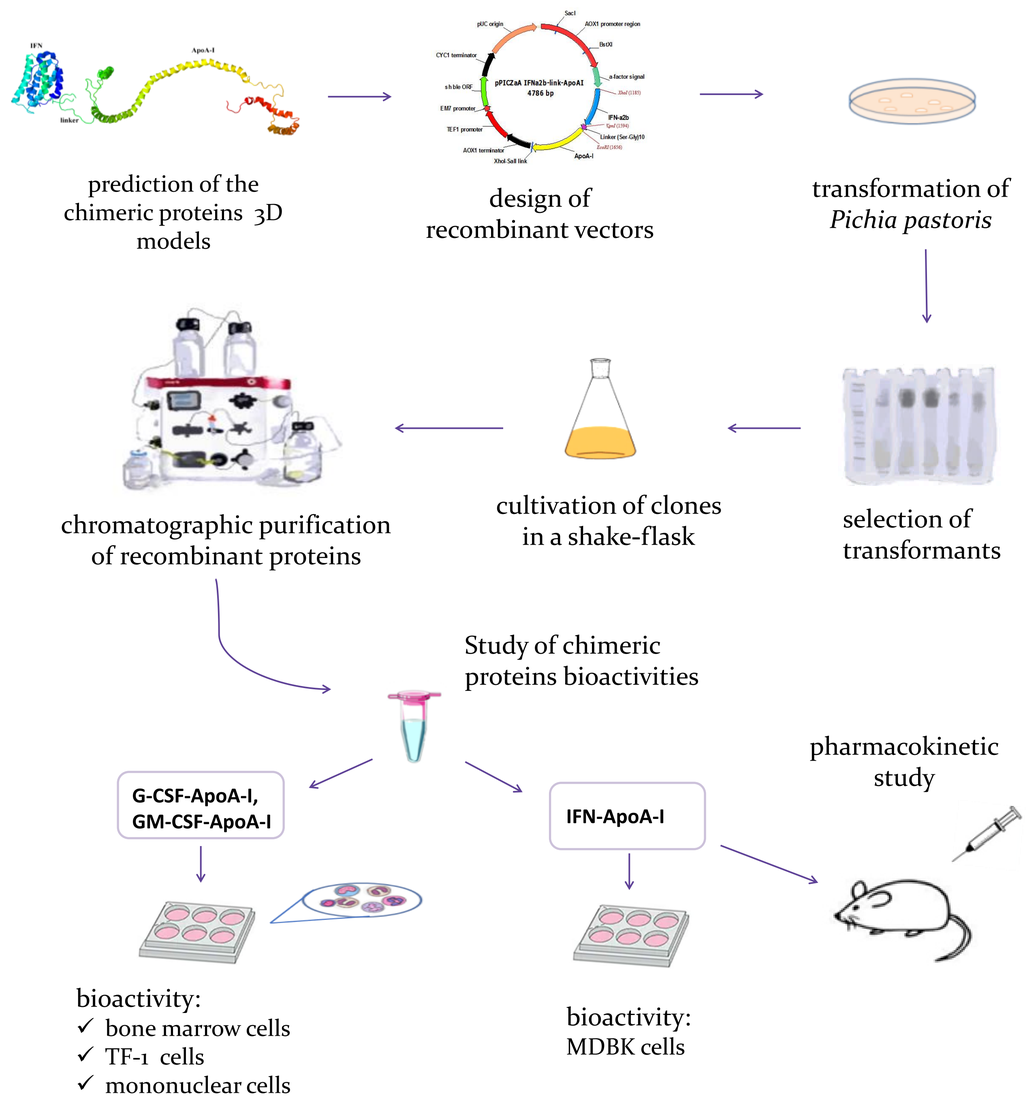
Abstract

Currently, over 200 recombinant proteins have been approved by the FDA for treatment of a wide range of diseases. However, the clinical use of most protein drugs is limited by their toxicity, short half-life, and low solubility and bioavailability. In this regard, the development metabolizable agents of targeted delivery and prolongation of the life of therapeutic proteins is currently of relevance. Recently, plasma lipoproteins and their protein components, apolipoproteins, have been studied as a new nanoplatform for the transport of various therapeutic molecules. Apolipoprotein A-I (apoA-I) has a long half-life in the body, is not immunogenic and binds to receptors of many cell types. In our studies, genetically engineered constructs were created in which apoA-I was fused with clinically used cytokines – granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF) and interferon-α2b (IFN).

Keywords: apolipoprotein A-I; bioactivity modulation; fusion protein; granulocyte colony-stimulating factor; granulocyte-macrophage colony-stimulating factor; half-life prolongation; interferon a-2b; *Pichia pastoris*

Study design



Methods

1. Construction of *P. pastoris* strains, producing recombinant proteins: G-CSF-ApoA-I, GM-CSF-ApoA-I, IFN-ApoA-I.
2. Isolation and purification of recombinant proteins.
3. Evaluation of the biological activity of chimeric proteins *in vitro* :
   - On rat and human bone marrow cells (BMCs) (G-CSF-ApoA-I, GM-CSF-ApoA-I)
   - human erythroleukemia cell line TF-1, human mononuclear cells (GM-CSF-ApoA-I)
   - cytopathic effect on MDBK (Madin-Darby bovine kidney) cells (IFN-ApoA-I)
4. Study of the pharmacokinetic properties of the chimeric protein IFN-ApoA-I on CD-1 mice male.

Results

1. P. pastoris strains, providing the secretion of chimeric recombinant proteins, have been created.
2. The chimeric proteins were obtained and purified to a purity of 90-95%.
3. The biological activities of the cytokines fused with ApoA-I are completely preserved.
   - **G-CSF-ApoA-I, GM-CSF-ApoA-I**: stimulation of granulopoiesis on human bone marrow cells (BMCs) comparable to G-CSF (Fig.1) and GM-CSF
   - **IFN-ApoA-I**: high antiviral activity (1.6×10^8 IU/mg)
4. ApoA-I fused with IFN prolongs the effect of IFN in mice by 1.8 times after a single subcutaneous injection of CD-1 mice male (Fig.3).

ApoA-I modulation of cytokine activity

ApoA-I in chimeric colony-stimulating factors modulates the activity of G-CSF and GM-CSF fused with it:
- ✓ reduce apoptosis (Fig.2),
- ✓ increase the viability of bone marrow cells, including blast cells of the granulocytic series (Fig.1, 2),
- ✓ increase the total number of bone marrow cells

G-CSF-ApoA-I:
- ✓ increases the number of monocytes by 1.4 times,
- ✓ reduce the number of abnormally segmented neutrophils versus non-chimeric cytokines (Fig.1).

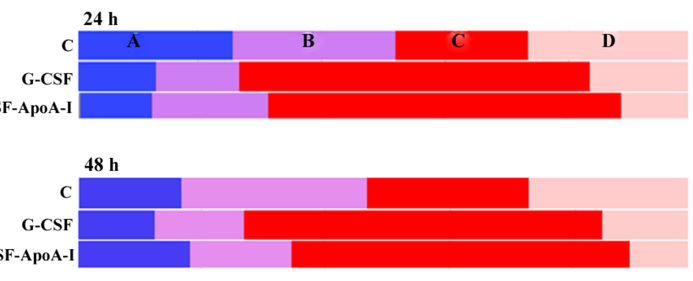


Fig. 1. Distribution granulocyte series of human BMCs, incubated for 24 and 48 hours in the presence of G-CSF and G-CSF-ApoA-I.
Group A - blasts (myeloblast, promyelocyte, myelocyte), B - young - metamyelocyte, stab neutrophil, C - mature - segmented neutrophils, D - abnormal phenotype - stab and 2-segment neutrophils with a less mature nucleus.

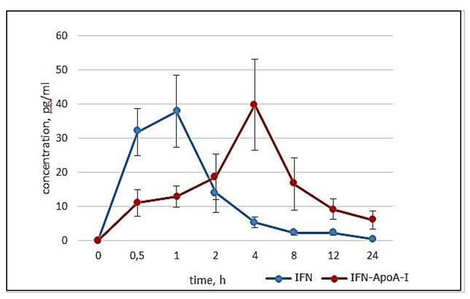


Fig. 3. Pharmacokinetic of IFN and IFN-ApoA-I in CD-1 mice male. Numerical data are presented as mean ± standard deviation (n = 5).

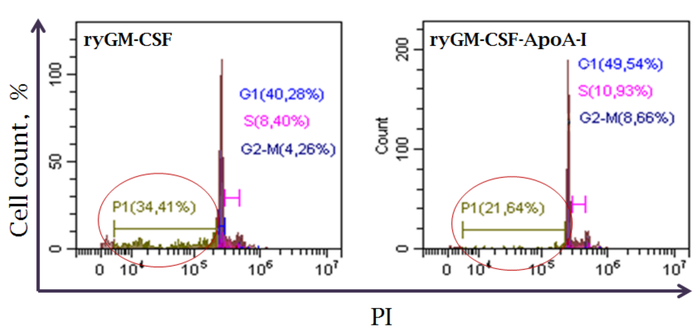


Fig. 2. Flow cytometric analysis of cell cycle of all BMCs treated by ryGM-CSF and ryGM-CSF-ApoA-I .
SubG1- 34.41% in case of ryGM-CSF vs. 21.64% - ryGM-CSF-ApoA-I.

Conclusion

Cytokines fused with ApoA-I retain their original activity. ApoA-I also modulates the activity of G-CSF and GM-CSF , resulting in chimeric cytokines also exhibiting new properties. ApoA-I fused to IFN demonstrates an extended half-life in mice, demonstrating the promise of ApoA-I technology for extending the life of fused cytokines. ApoA-I can be used as a platform to create new therapeutic biomolecules.

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