**Human Hemoglobin derived peptide prevents HIV-1 infection and protects cells from HIV-1 induced inflammation**

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**Introduction:**

The heterosexual transmission of HIV, facilitated by inflammation and related epithelial barrier perturbation, is the leading cause of virus spread worldwide. Hence, preventing HIV infection at this portal of entry is pivotal in combating AIDS epidemic. Various anti-HIV molecules/microbicides have been developed to curb new HIV-1 infections, however, none of them has been approved by FDA till date for reasons owing to cytotoxicity, efficacy and others. In the absence of a vaccine, it is therefore essential to identify/design new molecules form natural sources, such as antimicrobial peptides from host molecules to prevent the spread of new HIV infections. This work is an effort in this direction to design an anti-HIV peptide from human hemoglobin.

**Aim:**

To design and characterize anti-HIV peptide/s from hemoglobin and evaluate their anti-HIV potential

**Objectives:**

1) *In silico* design of peptide/s against CD4 binding domain of gp120 of HIV-1  
2) To evaluate anti-HIV activity and safety of designed peptide/s

**Experiments:**

a) Binding of peptide with gp120 by SPR  
b) Anti-HIV activity of peptide by p24 antigen assay  
c) Specificity of binding of peptide to gp120 on cells by Flow cytometry  
d) MTT and TER assay  
e) Immunofluorescence  
f) CD Spectroscopy  
g) HIV-1 Pseudotyping

**Results**

**gp120 binding assay (SPR)**

- HbAHP-25 binds to gp120 in a dose-dependent manner  
- HbAHP-25 inhibits CD4 from binding to gp120 of HIV-1 Bal & HIV-1

**Competitive ELISA**

- HbAHP-25 inhibits early steps of entry/fusion into cells  
- HbAHP-25 inhibits HIV when it gets a chance to interact directly with HIV-1 gp120

**P24 antigen assay**

- HbAHP-25 inhibits HIV-1 in a dose dependent manner, with an IC50 of 35.7µM for all 4 strains of HIV-1

**Time of addition Assay**

- HbAHP-25 inhibits early steps of entry/fusion into cells  
- HbAHP-25 inhibits HIV when it gets a chance to interact directly with HIV-1 gp120

**Flow Cytometry**

- HbAHP-25 binds specifically to (a) HL2/3 cells expressing gp120 on the surface and not to gp120 negative cells i.e (b) HeLa and (c) TZMbl cells

**HIV-1 VSV assay**

- HbAHP-25 specifically targets HIV-1 but not HIV-1 VSV virus  
- HbAHP-25 retains its structure at different pH

**CD Spectroscopy**

- HbAHP-25 maintains its anti-HIV potential in presence of seminal plasma (SP) and vaginal fluid (VF)

**Immunofluorescence**

- HbAHP-25 does not alter tight junction formation; it did not affect the TER values

**Conclusion**

- HbAHP-25 prevents cells from HIV-1 entry/fusion.
- HbAHP-25 blocks CD4 from binding to gp120, and doesn’t have any adverse effect on cell viability and monolayer integrity.
- Further characterization may lead to development of a potential anti-HIV therapeutic agent/molecule.

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