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# The antimicrobial peptide Dermaseptin S4 inhibits HIV-1 infectivity in vitro

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**Abstract**

Most of HIV-1 infections are acquired through sexual contact. In the absence of a preventive vaccine, the development of topical microbicides that can block infection at the mucosal tissues is needed. Dermaseptin S4 (DS4) is an antimicrobial peptide derived from amphibian skin, which displays a broad spectrum of activity against bacteria, yeast, filamentous fungi, and herpes simplex virus type 1. We show here that DS4 inhibits cell-free and cell-associated HIV-1 infection of P4-CCR5 indicator cells and human primary T lymphocytes. The peptide is effective against R5 and X4 primary isolates and laboratory-adapted strains of HIV-1. Its activity is directed against HIV-1 particles by disrupting the virion integrity. Increasing the number of DS4-positive charges reduced cytotoxicity without affecting the antiviral activity. The modified DS4 inhibited HIV-1 capture by dendritic cells and subsequent transmission to $CD4^+$ T cells, as well as HIV-1 binding on HEC-1 endometrial cells and transcytosis through a tight epithelial monolayer.


*Keywords:* Dermaseptin S4; Microbicide; Herpes simplex virus type 1

## Introduction

The predominant mode of human immunodeficiency virus type 1 (HIV-1) transmission worldwide is via heterosexual contact, with a higher rate of transmission to females than to males (UNAIDS, 2002). For sexual transmission to occur, infectious HIV-1 must cross the mucosal epithelium. Epithelial cells in genital and gastrointestinal tracts do not express CD4, but HIV-1 can bind to the cell membrane using a galactosyl-ceramide (Bomsel and Alfsen, 2003). HIV-1 could cross the pluristratified squamous epithelium of the vagina, exocervix or anus by physical breaches, or after capture or infection of dendritic cells (DCs). The transfer of HIV-1 across the monostratified epithelium of the endocervix, rectum, or gastrointestinal tract can occur by transcytosis, binding on Langerhans cells or infection of the intraepithelial lymphocytes (Bomsel, 1997; Bomsel and Alfsen, 2003; Pope and Haase, 2003; Stone, 2002). After crossing the epithelial barrier, HIV-1 can infect CCR5-expressing DCs, macrophages and T lymphocytes in the submucosa (Geijtenbeek et al., 2000; Greenhead et al., 2000; Spira et al., 1996), then spread to circulating $CD4^+$ T cells.

Since most new infections occur in developing countries, new methods of HIV prevention that can be controlled by women are urgently needed. These methods include the use of topical microbicides able to prevent HIV-1 entry through mucosa. Topical HIV-1 inhibitors may target the incoming virus at several steps of molecular events that drive viral entry (Eckert and Kim, 2001; Wyatt et al., 1998). They may also target specifically submucosal cells to prevent infection (Hu et al., 2004; O'Hara and Olson, 2002; Pierson and Doms, 2003; Reimann et al., 2002; Shattock and Moore, 2003).

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Inhibitors that target the envelope glycoprotein (Env) inhibit the binding of gp120 to CD4 or co-receptor (Bewley, 2001; Chang et al., 2003; Moulard et al., 2000; Poignard et al., 2001) or the fusion stage (Kilby et al., 1998). HIV-1 membrane is fragile and prone to disruption by many chemical agents. However, viral membrane is derived from the host cells from which it buds. Thus, the cytotoxicity of drugs that target viral membrane has to be evaluated on epithelial cells (Van Damme et al., 2002). Sexually transmitted diseases (STDs) have a marked effect on both viral shedding in the genital tract and the risk of acquiring HIV-1 infection, since they increase inflammation and create lesions in the vaginal or rectal tissues (Kovacs et al., 2001). Hence, an ideal microbicide should protect not only against HIV-1 but also against genital herpes and common bacterial infections.

Dermaseptin S4 (DS4) is a 28-residue antimicrobial peptide isolated from frog skin (Mor et al., 1991, 1994). This linear cationic peptide adopts an amphipathic α-helical conformation upon association with lipid bilayers, leading to membrane permeabilization and microbe death. Selective membrane recognition is related to the lipid composition of the target membrane and its electrical potential (Gaidukov et al., 2003; Kustanovich et al., 2002). DS4 displays a broad spectrum of activity affecting Gram-negative and Gram-positive bacteria, yeast, filamentous fungi, *Plasmodium falciparum*, and the enveloped herpes simplex virus type 1 (HSV-1) (Belaid et al., 2002; Efron et al., 2002; Mor et al., 1994). Increasing the positive charge and reducing the hydrophobicity of the native peptide have been shown to correlate with selective antimicrobial activity and reduced toxicity in mammalian cells (Kustanovich et al., 2002).

In this study, we investigated the antiviral effect of DS4 against HIV-1 in vitro. The ability of DS4 to reduce cell-free or cell-associated HIV-1 transmission was evaluated on different target cells: P4-CCR5 indicator cells, human primary T lymphocytes, and monocyte-derived dendritic cells (DCs). We also investigated the capacity of DS4 to impair HIV-1 attachment to human epithelial cells and DCs, as well as transcytosis through a tight epithelial barrier. The effect of the peptide on the viral particle was explored, and to pre-clinically test DS4 as a potential topical microbicide, we established the levels of efficacy versus toxicity.

## Results

### *Dermaseptin S4 inhibits HIV-1 infection of P4-CCR5 indicator cells*

The antiviral effect of DS4 was first evaluated using a single cycle virus infectivity assay on P4-CCR5 indicator cells (Lorin et al., 2004). These cells express the CD4, CCR5, and CXCR4 HIV-1 receptors and are stably transfected with *LacZ*, inducible by HIV Tat. Therefore, they are susceptible to HIV-1 isolates and express β-galactosidase upon infection. P4-CCR5 cells were infected with HIV-$1_{LAI}$ in presence of increasing concentrations of DS4 (0.35–11.2 μM). Fig. 1 shows that DS4 inhibited HIV-1 infection of reporter target cells in a dose-dependent manner. More than 90% reduction of infection was observed in presence of 3.5 μM DS4 and 50% reduction in presence of 1.5 μM. P4-CCR5 cells viability was determined after 24-h exposure to increasing concentrations of DS4. Fig. 1 shows that cells remained viable in the presence of DS4 concentrations that inhibited 90% of HIV-1 infection. However, DS4 concentrations higher than 5 μM were significantly cytotoxic in this assay. For this reason, we modified DS4 peptide with the aim to reduce its toxicity without affecting its antiviral activity.

### *Biochemical modifications reduce DS4 cytotoxicity*

It has been previously shown that increasing the net-positive charge and reducing the hydrophobicity of DS4 resulted in reduced hemolytic activity and high antibacterial activity (Efron et al., 2002; Feder et al., 2000; Kustanovich et al., 2002). In the present study, DS4 analogs were synthesized by introducing deletions, substitutions, or both in the native 28-aminoacid sequence of DS4 (Table 1). Both antiviral activity and cytotoxicity of these analogs were evaluated using P4-CCR5 target cells. Peptide concentrations causing 50% inhibition of HIV-1 infectivity ($IC_{50}$) and concentrations causing 50% cytotoxicity ($CC_{50}$) after 24 h of exposure were measured. The selectivity index (SI), the ratio $CC_{50}/IC_{50}$, was calculated and results for all analogs are summarized in Table 1. The C-terminal amidation of native DS4 had no significant effect on the peptide toxicity for P4-CCR5 cells or on its anti-HIV-1 activity. Deletions of the C-terminal region dramatically affected the anti-HIV-1 activity: DS4-(1–16)a was 5-fold less active than the native peptide, and DS4-(1–12)a or DS4-(1–9)a were inefficient, even at a concentration of 100 μM. Likewise,

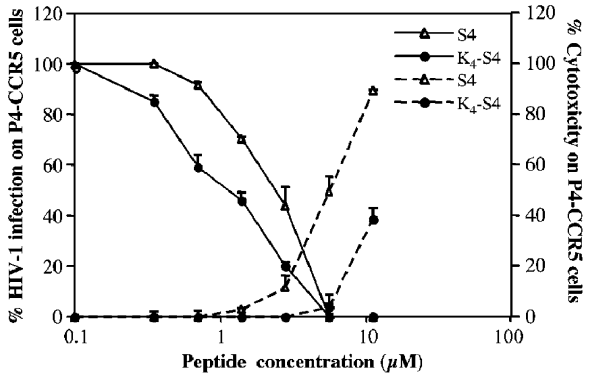


Fig. 1. Effect of dermaseptins S4 and $K_4$-S4 on HIV-$1_{LAI}$ infection and cytotoxicity. P4-CCR5 cells were subjected to a 2-h infection with HIV-$1_{LAI}$ in presence of the indicated concentration of the different forms of dermaseptin S4. β-Galactosidase production was quantified 48 h post-infection (solid lines). Cytotoxicity was quantified by trypan blue exclusion following a 24-h exposure on P4-CCR5 cells (dashed lines).