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Malva sylvestris derivatives as inhibitors of HIV-1 BaL infection

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Abstract

The emphasis of the present study is to evaluate a natural product and the potential microbicide activity using a dual chamber infection method. *Malva sylvestris* extracts and fractions were screened for anti-HIV activity by measuring the virus-antibody neutralization. Plant extracts with strong antiviral activity working in nanomolar or picomolar range can be used to enhance the activity of synthetic compounds and work as anti-HIV agents. The aqueous fraction (AF) of *M. sylvestris* demonstrated antiviral activity in a model with epithelial and blood cell lines. The AF showed an effective antiviral potential on the TZM-bl cells with reduction scores higher than 60% of infectivity. Quantification of p24 in the supernatant of the co-culture model demonstrated a reduction in the number of viral particles after AF treatment ($p < 0.05$). Cytokines were quantified and all signaling inflammatory markers; IL1-alpha, IL-beta, IL-6, IL-8 and GM-CSF ($p<0.05$) were modulated by positive control and AF treatments. In particular, IL-6 had lower levels of expression in Malva groups when compared to the Zidovudine positive control group. Natural occurring derivatives of *M. sylvestris* demonstrated to work inhibiting reverse transcriptase enzyme action. *M. sylvestris* contains highly potential anti-HIV-1 BaL components and may be considered a potential source for new formulations in the development of topical microbicides.

Graphical Abstract

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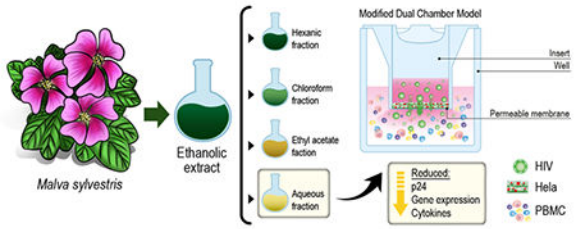

Keywords

Malva sylvestris; HIV infections; inflammation; anti-infective agents

1. Introduction

The currently approved pharmacological therapy changed HIV from a deadly into a chronic infection, but it is still not a curative therapy (Cutler and Justman, 2008). Attention has been given to discovering and developing novel agents, including microbicides that can be applied topically and protect against sexually transmitted infections, especially HIV (Pirrone et al., 2012). The future distribution of microbicides may have an important social impact, reducing health care costs and the risk of infection by HIV (D'Cruz and Uckun, 2004). Many natural compounds have a specific biological activity and low toxicity effects working on a particular target that may act to complement traditional antiviral medications (Yang et al., 2001). *M. sylvestris* is distributed worldwide as a traditional medicine and due to its medical importance; it is a good candidate for drug discovery (Benso et al., 2015; Benso et al., 2016). Therefore, the aim of this study was to investigate the potential anti-HIV activity of the aqueous fraction (AF) of *M. sylvestris* on cells infected by HIV-1BaL using a dual chamber model.

2. Results and Discussion

The pharmacological evaluation for a microbicide candidate may start with cytotoxicity test as the first step to establishing concentrations and determining activity (Pasetto et al., 2015). The aqueous fraction of *M. sylvestris* did not alter cell metabolic viability at different tested concentrations in TZM-bl; HeLa or PBMC (Peripheral blood mononuclear cells) lines (Figure S1). Subsequently, the anti-HIV activity of AF was assessed in TZM-bl cells demonstrating a percentage of reduction that reached the 67%. The positive control Zidovudine (60 μM) was tested at highest and non-toxic concentration that inhibited 80% of the HIV-1BaL infection. The vehicle control (0.1% DMSO, for all compounds tested) did not affect the infectivity of HIV-1 BaL (Figure S2). Plant extracts with strong activity acting in a nanomolar/picomolar range can be used to enhance the activity of synthetic compounds and work as an anti-HIV agent (Singh, Bodiwala 2016).

The first assay, TZM-bl infection model, revealed that the identified fraction presented positive results against HIV-1BaL in very low concentrations. The highest concentration with non-toxic activity of the natural compound resulted in an IC50 of 37 μg/mL. Additionally, the idea was to maintain both the highest and non-toxic suppression values

against the HIV infections. In accordance to that, authors demonstrated similar values for Zidovudine (10 μM; 50 μM) to reduce infectivity and the differentiation of bone marrow cells to granulocyte-monocyte colony forming unit (Chitnis et al., 2002).

The HPLC chemical analysis made bioassay-guided fractionation feasible and the peak corresponding to rutin was identified as having the greatest peak area (Benso et al., 2015). Positive ion ESI-mass spectrometry for molecular ions at m/z 611 and 633 confirmed the compound identification from 23.8 mg of the total sample (Benso et al, 2016). Tao et al. reported anti-HIV activity for sodium rutin sulfate, which is a polyanionic compound activity for rutin in the TZM-bl HIV-1 BaL infection model; however, for the modified structure a significant virus inhibition IC50 8.5 μM (5.19 μg/mL) was demonstrated. The authors correlated the activity with the presence of sulfated polysaccharides in the structure, such as dextran sulfate, that may bind mainly to the V3 loop of X4 gp120 rather than that of R5 gp120.

In the supernatant of a dual chamber infection model, it was quantified the HIV-1 p24 antigen. Results after PBMC were treated with the AF and showed a statistically significant reduction of the infection. The positive control AZT and the natural compound tested AF 50 μg/mL were not statistically different (Figure S3). Data from other laboratories have indicated that AZT and derivatives belonging to the nucleoside reverse transcriptase inhibitors NRTI group effectively inhibits the HIV-1 replication based on p24 assays (Turk et al., 2002).

The p24 protein production is reportedly connected to reverse transcriptase (RT) activity, and the assay assumes the relationship between the number of target-infected PBMC and the antigen. Rutin is associated in AF with a secondary molecule that may have a synergic effect and or enhance its antiviral activity. Reports in the literature describe a variety of pharmacological activities for the flavonoid group and the association between the chemical structure and the biological activity (Tao et al, 2007; Asada et al., 2013). Moreover, the biochemical effects are caused by the ability to inhibit a number of enzymes such as reductase, lipoxygenase, cyclooxygenase and different hormones (Soto-Cabrera et al., 2015). Naturally occurring compounds, like flavonoids, have been reported to inhibit the reverse transcriptase enzyme but with differential sensitivity according to the structure and concentration tested (Spedding et al., 1989). Quercetin, rutin and catechin are flavonoids that possess antiviral activity that may be related to the non-glycosidic compounds and hydroxylation at the 3-position permitting the inhibition activity (Shi et al., 2002).

Overall, these flavonoids have been shown to have pharmacological activity and important components in traditional medicine for many years (Rathee et al, 2009). In our study, we reported the cytotoxicity assay data for the AF of *M. sylvestris*, which revealed that, for all cell lines, the AF is shown to be pharmacologically safe, maintaining cell viability. Our data agree with previous findings that screened *M. sylvestris* leaves against four tumor cell lines - L929sA, MCF 7 and MDA-MB 231 and the IC50 was not defined due to a lack of response (Kaileh et al., 2007).

Cytokines modulate monocyte function as well as HIV replication with these cells and they are considered to be major reservoirs of HIV-1, playing an important role in the pathogenesis of AIDS (Badley et al., 2013). Furthermore, to understand the mechanism of the AF in the replication of HIV-1BaL, proinflammatory cytokines were assessed [19]. The AF reduced the expression of all the cytokines studied - IL1-alpha, IL-beta, IL-6, IL-8 and GM-CSF - compared to the vehicle control group. IL-6 had low levels of expression compared to the positive control group AZT (Figure S4).

Here, we proposed the mechanism of action of AF to reduce HIV infection: it prevents RT activity (Figure S5). The promotion of alternative compounds on RT activity may contribute to the disease therapeutics; since the enzyme lacks a proofreading capability, very high mutation rates are found (Esposito et al., 2011; Kharlamova et al., 2009). To reduce the drug-resistance events, a combination therapy is frequently recommended with different targets and mechanisms of actions (Mascola et al., 2002; Yang et al., 2018).

3. Conclusions

We proposed a study focusing on the investigation of a natural product modulating HIV-specific RT activity. Enhancing the understanding of *M. sylvestris* favor to the discovery of a novel anti-HIV-1 components and potential new formulations for topical microbicides.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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