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Original article

Cytolytic nanoparticles attenuate HIV-1 infectivity

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Background: We investigated whether cytolytic melittin peptides could inhibit HIV-1 infectivity when carried in a nanoparticle construct that might be used as a topical vaginal virucide. Free melittin and melittin-loaded nanoparticles were prepared and compared for cytotoxicity and their ability to inhibit infectivity by CXCR4 and CCR5 tropic HIV-1 strains.

Methods: TZM-bl reporter cells expressing luciferase under the control of the HIV-1 promoter were incubated with HIV-1 NLHX (CXCR4) or HIV-1 NLYU2 (CCR5) viral strains and different doses of soluble CD4 (positive control) or free melittin to determine infectivity and viability. Melittin-loaded nanoparticles were formulated and different doses tested against VK2 vaginal epithelial cells to determine cell viability. Based on VK2 viability, melittin nanoparticles were tested for prevention of CXCR4 and CCR5 tropic HIV-1 infectivity and viability of TZM-bl reporter cells. Low-speed centrifugation was used to compare the ability of blank non-melittin nanoparticles and melittin nanoparticles to capture CCR5 tropic HIV-1.

Results: As expected, the soluble CD4 positive control inhibited CXCR4 (50% inhibitory concentration [IC_{50}] 3.7 μg/ml) and CCR5 (IC_{50} 0.03 μg/ml) tropic HIV-1 infectivity. Free melittin doses <2 μM were not cytotoxic and were highly effective in reducing HIV-1 infectivity for both CXCR4 and CCR5 strains in TZM-bl reporter cells, while VK2 vaginal cell viability was adversely affected at all free melittin doses tested. However, VK2 cell viability was not affected at any dose of melittin-loaded nanoparticles. Melittin nanoparticles safely and significantly decreased CXCR4 (IC_{50} 2.4 μM and IC_{90} 6.9 μM) and CCR5 (IC_{50} 3.6 μM and IC_{90} 11.4 μM) strain infectivity of TZM-bl reporter cells. Furthermore, melittin nanoparticles captured more HIV-1 than blank nanoparticles.

Conclusions: These data illustrate the first proof-of-concept for therapeutic and safe nanoparticle-mediated inhibition of HIV-1 infectivity. Future investigations appear warranted to explore the antiviral prophylactic potential of melittin nanoparticles to capture, disrupt and prevent initial infection with HIV-1 or potentially other enveloped viruses.

Introduction

Despite recent therapeutic advances in the treatment of chronic infection with HIV-1, there is still a pressing need to develop topical vaginal HIV-1 virucidal agents that prevent the initial infection. The principal aim of the present work was to establish proof-of-concept for a potential anti-HIV-1 prophylactic agent based on our previous work with simple, safe and fully biodegradable/biocompatible cytolytic peptide-loaded nanoemulsions that might disable the HIV-1 virus before it infects normal cells [1,2]. The active pharmacological ingredient, melittin, is a 26-amino-acid, amphipathic, membranolytic peptide, which is a component of bee venom. Melittin is quickly and stably integrated into membrane lipid bilayers and is metabolized by natural proteolytic processes *in vivo*. Beyond its direct pore-forming cytolytic activity, the melittin protein can indirectly facilitate cell apoptosis [3,4] and necrosis [5,6].

Free melittin has been shown to peel off the lipid envelope of murine leukaemia virions [7]. Additionally, the uptake of melittin by HIV-1-infected cells leads to decreased HIV-1 gene expression and replication [8]. We have shown previously that circulating synthetic melittin-loaded perfluorocarbon nanocarriers can inhibit melanoma growth *in vivo* [2]. These nanosized constructs also elicit cancer suppression without any of the usual side effects of chemotherapy or radiation therapy. Herein, we present the first proof-of-concept data for melittin nanoemulsion-mediated inhibition of HIV-1 infectivity. Based on this finding, we propose that melittin-loaded nanoparticles are well-suited for use as topical vaginal HIV-1 virucidal agents.

Methods

Cell lines and viral strains

TZM-bl reporter cells were maintained and cultured in DMEM (Life Technologies, Grand Island, NY, USA) with 10% fetal bovine serum (Sigma-Aldrich, St Louis, MO, USA), 2 mM L-glutamine (Life Technologies), 1 mM sodium pyruvate (Life Technologies), penicillin (1,000 U/ml) and streptomycin (1,000 μg/ml; Life Technologies). TZM-bl cells are HeLa cells engineered to express the HIV-1 receptor CD4 and coreceptor CCR5. HeLa cells endogenously express the CXCR4 coreceptor [9]. They also express luciferase under the control of an HIV-1 promoter allowing for quantification of HIV-1 infectivity. HIV-1 NLHX (CXCR4 tropic) or HIV-1 NLYU2 (CCR5 tropic) viral strains were made by transfecting 293T cells, using TransIT (Mirus Bio LLC, Madison, WI, USA), with HIV-1 NLHX or HIV-1 NLYU2 plasmids [10]. After 3 days, the supernatants were collected and filtered with a 0.45 μM filter. Viruses were then dialysed in Spectrum Float-A-Lyzer columns in 4 l of cold phosphate-buffered saline (PBS) for approximately 6–7 h and then overnight in a fresh 4 l of cold PBS. P24 antigen concentration was measured by ELISA assay [11].

Nanoparticle preparation

All nanoparticle formulation components were purchased from Avanti Polar Lipids (Alabaster, AL, USA). A lipid film containing 99.4 mol% lecithin and 0.6 mol% *N*-(4′-[4′′-maleimidophenyl]butyroyl)-poly(ethylene glycol)2000-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (MPB-PEG2000-DSPE) was prepared by rotary evaporation using an R-210 Rotavapor (BUCHI Labortechnik AG, Flawil, Switzerland). This lipid film representing the 2% surfactant portion was emulsified by sonication in the presence of 20% perfluorocarbon (perfluoro-octyl-bromide; PFOB), 1.85% glycerin and 76.15% water. The emulsion was then formulated into nanoparticles using a 110 S Microfluidizer (Microfluidics Corp., Newton, MA, USA) at 20,000 psi. Subsequently, 1 mg cysteine was added to quench the MPB group and incubated for another 2 h at room temperature followed by three rounds of dialysis for 2 h against 2 l of PBS to remove free cysteine. The size and zeta potential of completed 2 mol% MPB-PEG2000-DSPE PFOB nanoparticles were determined using a ZetaPlus Zeta Potential analyser (Brookhaven Instruments Corp. Holtsville, NY, USA).

Melittin (GenScript Corp., Piscataway, NJ, USA) loaded nanoparticles were produced by incubating nanoparticles with a solution of 0.91 mM melittin in Nanopure water (Thermo Fisher Scientific, Waltham, MA, USA) at 4°C for 72 h with shaking as previously reported [2]. Nanoparticles were isolated by low-speed centrifugation for 20 min at 1,000×*g* to 'softly' pellet the particles. Particle supernatants were analysed for unbound melittin with a fluorescent plate reader (Varian Cary Eclipse, Walnut Creek, CA, USA) at excitation 280 nm and melittin tryptophan 19 emission at approximately 355 nm [1]. Single maximum emission peak sizes corresponding to the amount of free melittin present were then compared with a standard 0.91 mM melittin emission peak and used to calculate supernatant and corresponding nanoparticle pellet concentrations of melittin. The nanoparticles were then washed 5× with PBS using the same centrifugation protocol in order to remove any remaining free melittin. 'Blank' nanoparticles were created using the same method but devoid of melittin.

Vaginal epithelium cytotoxicity assay

Immortalized vaginal epithelial cells (VK2/E6E7, CRL-2616; ATCC, Manassas, VA, USA) were propagated according to ATCC protocol. For cytotoxicity studies, 7,500 cells were added to each well of a 96-well plate and allowed to attach for 24 h. Free melittin, blank nanoparticles or melittin nanoparticles were then added and incubated with the cells for 12 h at 37°C with shaking at 500 rpm. Vaginal epithelial cells were washed once with media and incubated with the MTT reagent (Invitrogen, Carlsbad, CA, USA) for 4 h. The coloured product was solubilized in DMSO and absorbance at 570 nm was measured with a plate reader.

HIV-1 infectivity assay

TZM-bl cells were serum starved for 24 h. They were then plated at a density of 10,000 cells/well in 96-well plates and allowed to attach for 24 h. A quantity of 50 ng of HIV-1 NLHX (CXCR4) or HIV-1 NLYU2 (CCR5) as measured by p24 ELISA assay was incubated with the agent of interest for 1 h with soluble CD4 (sCD4; ProScI Inc., Poway, CA, USA) or nanoparticles or for 2 h (free melittin) at 37°C. If the agent of interest was a nanoparticle, it was removed from the mixture following the incubation by centrifugation for 20 min at 14,000 rpm. The treated virus was then used to infect TZM-bl reporter cells for 2 days at 37°C. Following the infection, culture media was removed and the cells were lysed with PBS containing 0.2% Triton-X100. Luciferase activity in relative light units was then determined as a measurement of HIV-1 infectivity using an Opticomp luminometer (MGM Instruments, Hamden, CT, USA). Luciferase activity in the absence of the agent of interest was normalized to 100% and values reported as percentage infectivity relative to no agent. Viability of TZM-bl cells exposed under the same conditions was measured using the Cell Titer-Blue cell viability assay (Promega, Madison, WI, USA) according to the manufacturer's directions.

HIV-1 capture assay

HIV-1 NLYU2 (CCR5) strain supernatants produced by transfection of 293T cells with TransIT (Mirus Bio LLC) were harvested 72 h post-transfection, filtered and assayed for p24 antigen content by ELISA. Fresh virus was resuspended in culture media and aliquoted. Viral p24 values were normalized so that equal amounts of p24 were added to each assay confirming that the same amount of virus was present in each sample. A quantity of 50 ng of the HIV-1 NLYU2 viral strain in 90 μl was incubated with 10 μl of blank or melittin nanoparticles at an equivalent melittin concentration of 10 μM for 1 h at 37°C. In order to separate nanoparticle-bound virus from free virus particles, this mixture was centrifuged at 1,000×*g* for 10 min. This speed is sufficient to pellet the very dense (approximately 1.9 g/ml) perfluorocarbon nanoparticles [12] and bound virus but not free virus particles, which require ultracentrifugation forces of approximately 100,000×*g* given their small 100 nm diameter and low density of 1.16–1.18 g/ml [13]. To determine the amount of captured virus, the level of the viral protein p24 in the supernatant and pellet was compared. The total amount of p24 in the supernatant and pellet combined was normalized to 100%, and the percentage of p24 in the pellet was reported for each type of nanoparticle.

Statistics

Assuming a random normal distribution, a two-tailed Student's *t*-test assuming equal variance was used to calculate *P*-values for α=0.05.

Results

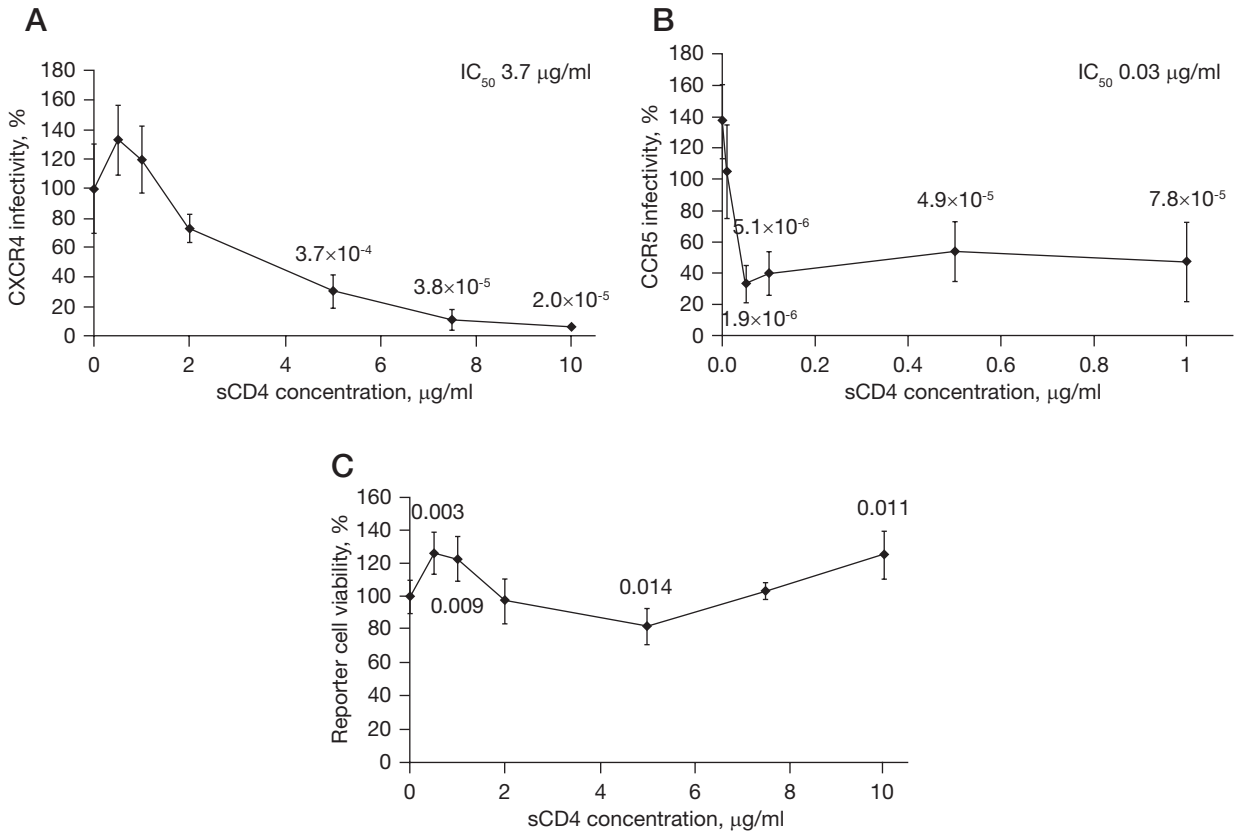
Given the known activity of melittin for disruption of viral lipid envelopes [8], we hypothesized that melittin delivered in both free and nanoparticle formats might inhibit infection by HIV-1. To test this hypothesis, we first assessed CXCR4- and CCR5-dependent HIV-1 infectivity of TZM-bl cells in the presence of an sCD4 molecule designed for HIV-1 neutralization assays. We reasoned that sCD4 could serve as a positive control for our subsequent melittin nanoparticle experiments to assess inhibition of HIV-1 infectivity because both sCD4 and our nanoparticles rely on a neutralization mechanism. As expected, sCD4 inhibits both CXCR4- and CCR5-dependent HIV-1 infectivity (Figure 1A and 1B). A higher concentration of sCD4 was required to inhibit CXCR4 (50% inhibitory concentration [IC_{50}] 3.7 μg/ml) than CCR5 (IC_{50} 0.03 μg/ml). However, inhibition of CCR5 infectivity quickly reached a plateau of approximately 40% at higher sCD4 dosing (Figure 1B), whereas CXCR4 infectivity was almost eliminated at higher sCD4 concentrations (Figure 1A). Moreover, reporter cell viability was not negatively influenced by sCD4 (Figure 1C).

Having validated the HIV-1 NLHX (CXCR4) and HIV-1 NLYU2 (CCR5) viral strains as susceptible to neutralization using sCD4, we next assessed susceptibility to neutralization by free melittin. To assess the influence of free melittin on HIV-1 infectivity, TZM-bl cells were incubated with HIV-1 and varying concentrations of free melittin for 2 days. For both CXCR4- and CCR5-dependent viral strains, a concentration of 1 μM free melittin reduced HIV-1 infectivity to 30% (Figure 2A). The same level of reduction was observed for the CXCR4 strain at 2 μM while CCR5 infectivity was further reduced to <10%. Increasing the melittin dose to >6 μM resulted in no infection. However, given melittin's non-specific cytolytic activity, we simultaneously assessed viability of the TZM-bl reporter cells. Cell viability was stable at 100% until the melittin dose exceeded 2 μM at which point it rapidly decreased (Figure 2B). These results demonstrate a narrow therapeutic range for free melittin.

Having determined the neutralization parameters of free melittin, we subsequently formulated and assessed the physicochemical properties of the melittin-loaded nanoparticles. We first formulated the 'blank' non-melittin nanoparticles. Blank nanoparticles lack specific HIV-1 targeting ligands but incorporate cysteine blocked linkage points on the tips of the polyethylene glycol (PEG) moieties. This ensures that future nanoparticle variations are easily adapted for specific targeting applications if necessary. To convert blank nanoparticles to melittin nanoparticles, free melittin was absorbed onto the blank nanoparticles as described in the *Methods* section. As demonstrated, there was a significant increase (*P*=0.003) in the mean ±SD hydrodynamic diameter of the melittin-loaded (324 ±14 nm) versus the blank (306 ±10 nm) nanoparticles (Figure 3A). The final concentration of melittin in the melittin nanoparticle colloid was 127 μM, which corresponds to approximately 5,737 melittin molecules per nanoparticle. Additionally, the mean ±SD electrokinetic or zeta (ζ) potential of blank (-22 ±3 mV) and melittin (14 ± 3 mV) nanoparticles was significantly different ($P=2.4 \times 10^{-15}$; Figure 3B).

The positive ζ - potential of the melittin-loaded nanoparticles is as a consequence of melittin being a cationic peptide [1]. Based on this analysis, we hypothesized that melittin nanoparticles would have an increased electrostatic affinity for HIV-1 over blank nanoparticles given that HIV-1 has a negative ζ potential (-28.2 mV) in culture conditions [14]. Thus, HIV-1 and equivalent numbers of blank or melittin nanoparticles were incubated together followed by low-speed centrifugation to pellet nanoparticles and any captured virions as determined by HIV-1 p24 levels in the pellet. As shown, the mean ±SD amount of HIV-1 captured by the melittin nanoparticles (19 ±1%) exceeded that observed for the blank nanoparticles (16 ±1%; Figure 3C).

Figure 1. Effect of soluble CD4 on HIV-1 reporter cell infectivity and viability

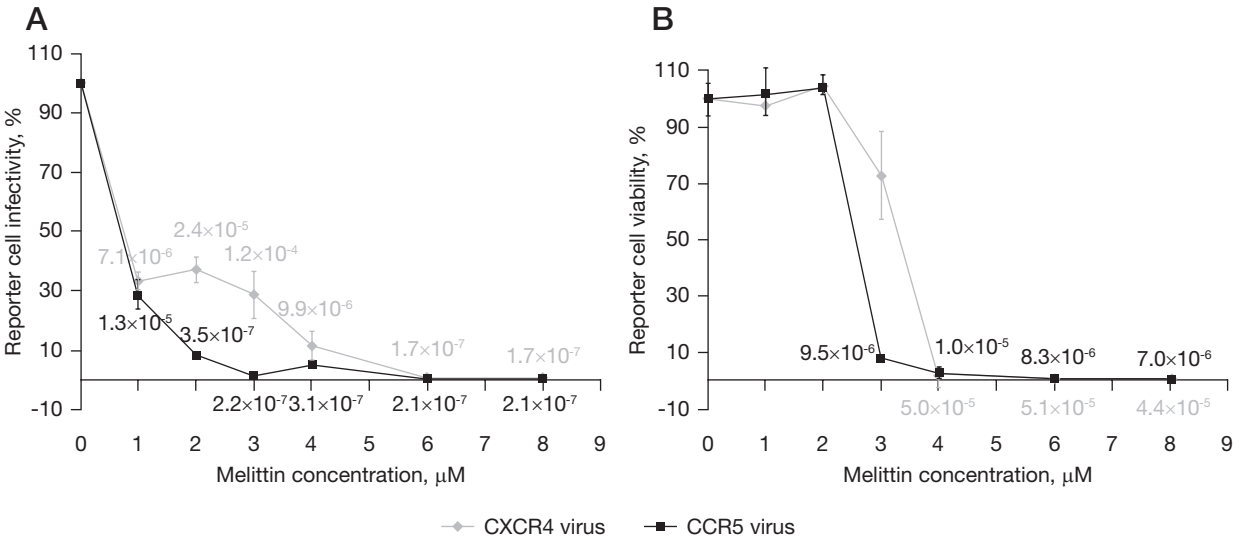


Effect of soluble CD4 (sCD4) on infectivity of TZM-bl reporter cells by **(A)** CXCR4 and **(B)** CCR5 tropic strains of HIV-1. **(C)** Effect of free melittin on TZM-bl reporter cell viability. Data represent the mean ±SD for n=6 determinations. Statistically significant P-values (P<0.05) are listed above or below error bars.

Having demonstrated the effectiveness of free melittin for inhibiting HIV-1 infection, we next sought to harness melittin in nanoparticles for inhibition of viral infectivity. However, in analogy to free melittin, the therapeutic benefit of a melittin nanoparticle-mediated decrease in HIV-1 infectivity could be negated by decreasing cell viability. Thus, we tested selected doses of melittin nanoparticles and equivalent numbers of blank nanoparticles on vaginal keratinocytes. Vaginal keratinocytes were chosen as a cytotoxicity test platform given the therapeutic potential for melittin nanoparticles to serve as an intravaginal gel-based prophylactic against HIV-1 infection. As demonstrated, vaginal cell viability is decreased at all free melittin concentrations tested (Figure 3D). However, with the exception of a slight decrease in viability at the maximal 20 μM dose of blank nanoparticles but not melittin nanoparticles, no decrease in cell viability was observed for an equivalent number of both 'blank' and melittin nanoparticles at any melittin concentration tested (Figure 3D). In fact, cell viability was modestly increased at all doses tested for the melittin nanoparticles. By contrast, a separate non-pegylated version of melittin-loaded nanoparticles that killed tumour cells *in vitro* but proved to be safe and decrease tumour growth *in vivo* [2] demonstrated vaginal cell cytotoxicity when used topically (Additional file 1). This result highlights how simple changes in nanoparticle formulation can greatly influence therapeutic applicability.

Having demonstrated the cytotoxicity profile of melittin nanoparticles *in vitro*, we next determined the IC_{50} and IC_{90} for melittin nanoparticle inhibition of infectivity of CXCR4 and CCR5 viral strains. The IC_{50} and IC_{90} for the CXCR4 strain were 2.4 and 6.9 μM, respectively (Figure 4A). By contrast, the IC_{50} and IC_{90} were higher for the CCR5 strain at 3.6 and 11.4 μM, respectively (Figure 4C). Moreover, blank nanoparticles were also able to modestly reduce infectivity for both viral strains (Figure 4A and 4B) but especially for CXCR4, where we were able to obtain an IC_{50} of 7.4 μM equivalent melittin concentration. Additionally,

Figure 2. Effect of free melittin on HIV-1 reporter cell infectivity and viability



(A) Effect of free melittin on infectivity of TZM-bl reporter cells by CXCR4 and CCR5 tropic strains of HIV-1. **(B)** Effect of free melittin on TZM-bl reporter cell viability. Data represent the mean ±SD for n=3 determinations. Statistically significant P-values (P<0.05) are listed above or below error bars.

there was no adverse effect on reporter cell viability observed for either CXCR4 (Figure 4B) or CCR5 (Figure 4D) strains at any concentration of melittin nanoparticles tested.

Discussion

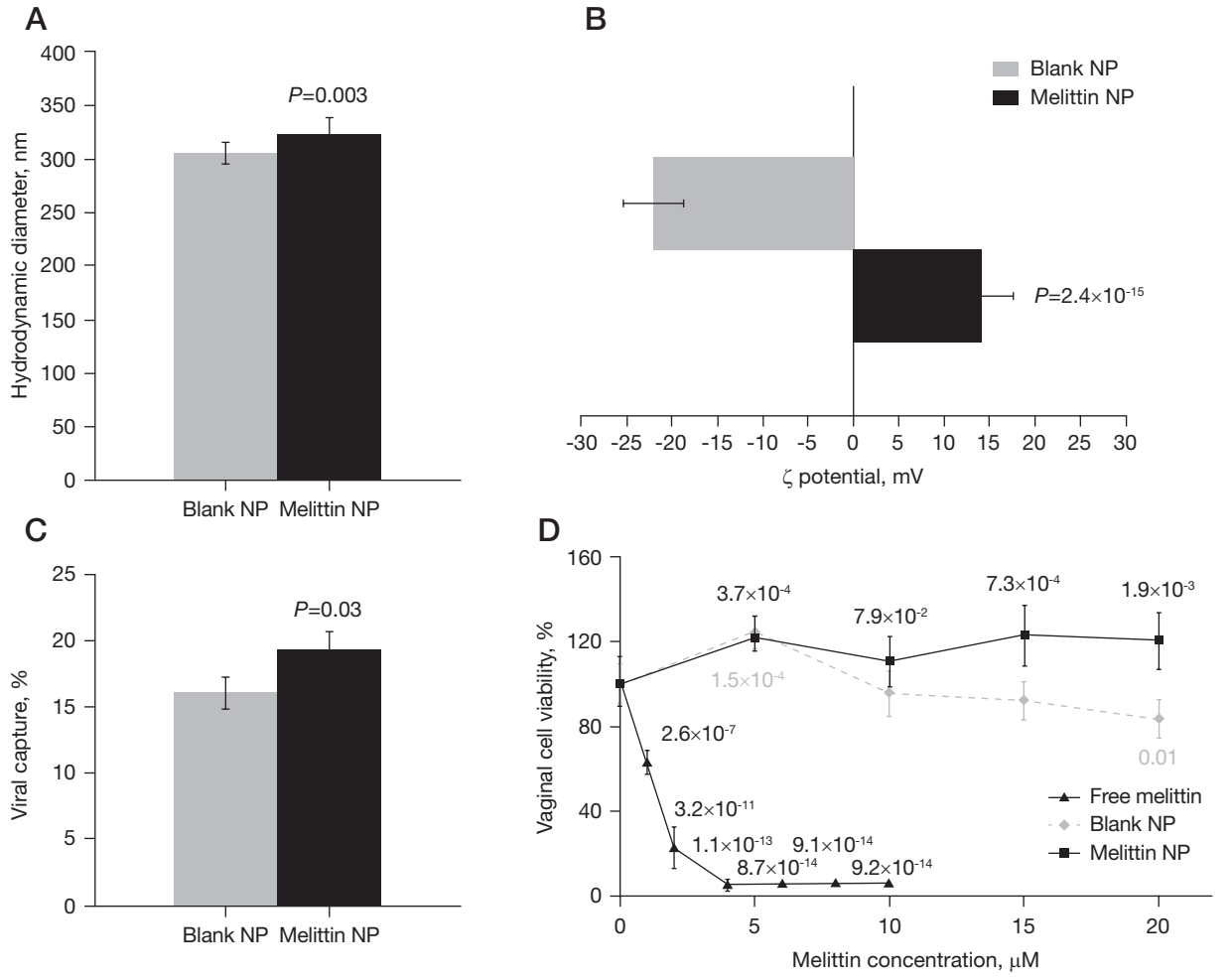
This report demonstrates the first proof-of-concept for therapeutic nanoparticle-mediated inhibition of HIV-1 infectivity (Figure 5). Free melittin can inhibit CXCR4- and CCR5-dependent HIV-1 infection, but can be toxic to normal cells. However, melittin formulated as a nanoparticle appears completely unreactive against vaginal epithelial or reporter cells *in vitro*, and still prevents infection by both CXCR4 and CCR5 viral strains. The lack of melittin toxicity toward normal cells when presented in the nanoparticle format *in vivo* has been illustrated previously, even for multiple consecutive intravenous administrations that exceed the accepted 50% lethal dose for free melittin [2]. Herein, we further demonstrated that pegylated melittin nanoparticles were not cytotoxic to vaginal cells (Figure 3D). Previous studies support our vaginal cytotoxicity findings in that pegylation of nanoparticles prevents their interaction and uptake by vaginal and mononuclear cells making them safe for use as topical vaginal microbicides [15].

The surprising disparity between melittin-mediated decreases in HIV-1 infectivity and preserved mammalian cell viability could result from differential interactions of melittin nanoparticles with cells and enveloped viral particles. It is clear that the incorporation of melittin in nanoparticles generates only a modest increase in affinity for HIV-1 (Figure 3C), less than might be expected given the large gap in electrokinetic potential between blank and melittin nanoparticles (Figure 3B). This disparity might be explained in part by the fact that even blank nanoparticles have a slightly more positive ζ potential (-22 ±3 mV; Figure 2B) than cultured HIV-1 (-28.2 mV). This small difference might be enough for melittin and blank nanoparticles to have similar affinities for HIV-1 (Figure 2C).

However, the most likely explanation is that melittin is known to be a fusogenic peptide in addition to a pore-forming toxin [1]. We have shown previously that cancer cells demonstrate fusion with melittin-containing nanoparticles as a mechanism of melittin delivery and therapeutic efficacy [2]. Thus, we anticipate that HIV-1 particles interact with melittin nanoparticles by traditional lipid-to-lipid membrane hemi-fusion events, similar to those that occur between the lipid membrane bilayers of liposomes and melittin nanoparticle monolayers [1,2]. This mechanism greatly facilitates effective transport of melittin from nanoparticle lipid monolayers to HIV-1 envelope bilayers, followed by expected melittin aggregation and pore formation, which deactivates the viral package.

Interestingly, even blank nanoparticles were able to modestly decrease infectivity, particularly for the CXCR4 strain (Figure 4A). Given the simplicity of the blank nanoparticle structure, this result begins

Figure 3. Formulation and effect of melittin nanoparticles on HIV-1 capture and vaginal cell viability



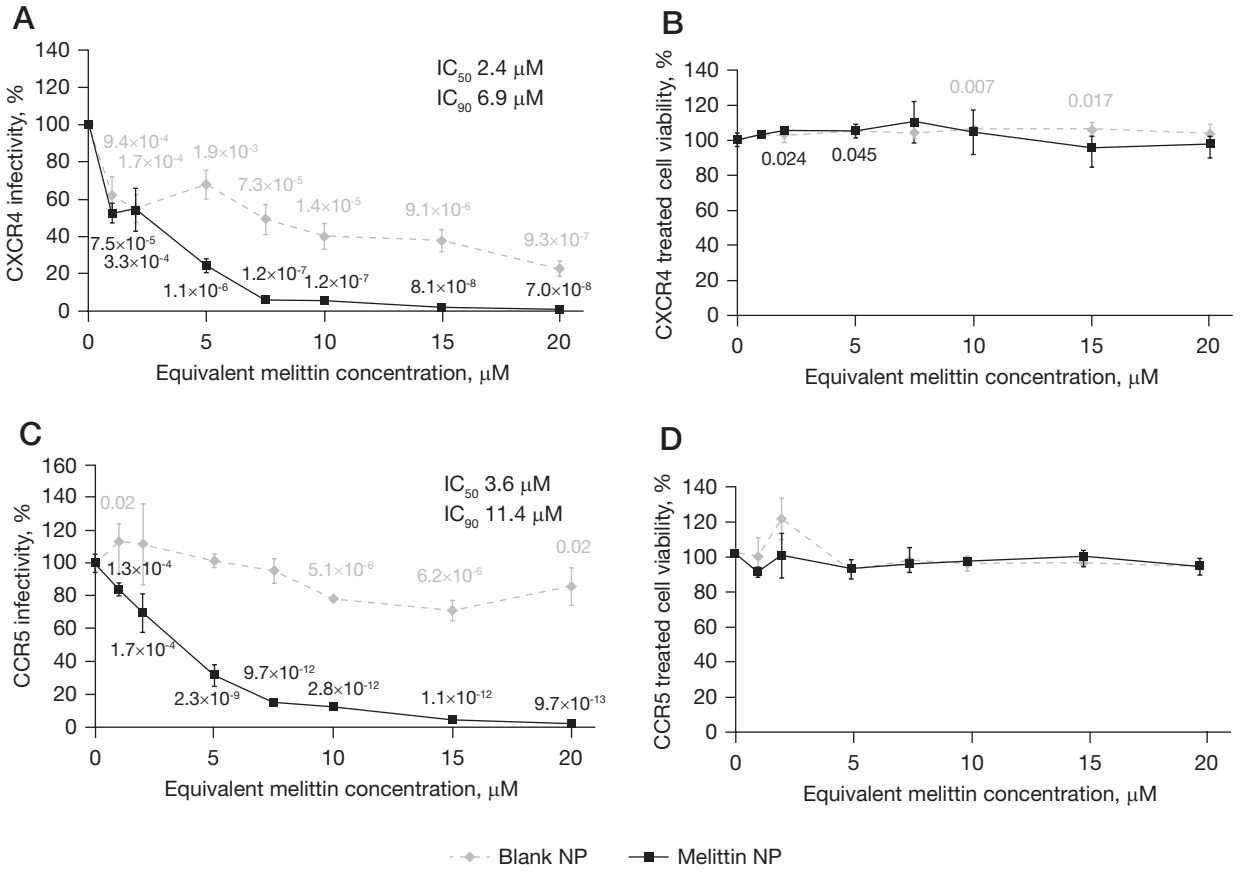
(A) Nanoparticle (NP) sizing by dynamic light scattering (n=10). **(B)** NP ζ potential (n=10). **(C)** Percentage HIV-1 CCR5 tropic viral capture by blank and melittin NP (n=3). **(D)** Effect of free melittin, blank NP and melittin NP on VK2 vaginal cell viability (n=6). Data represent the mean ±SD. Statistically significant P-values (P<0.05) are listed above or below error bars.

to suggest that the potential 'fusion' energy stored between the lipid shell and perfluorocarbon core of the blank nanoparticles is sufficient to encourage some viral fusion with the nanoparticles whether melittin is present or not. This finding warrants future investigations to determine whether even more finely tuned 'inert' nanoparticles might be constructed to serve as decoys for HIV-1 fusion.

In contrast to enveloped virus, normal cells have a larger lipid membrane surface area and can rapidly repair membrane defects. Moreover, viral cargo (RNA) is under far greater tension than cell content and is thus more sensitive to rupture. Combined, these differences may explain the disparity between viral and mammalian cell toxicity for the effective doses tested. We suggest that these data justify further investigations into antiviral prophylactic approaches in a vaginally applied gel suspension that might be used to capture, disrupt and prevent initial infection with HIV-1 or other enveloped viruses. Additionally, in terms of scalability, production of these nanoparticles is more than sufficient to conduct clinical trials. These simple colloidal nanoparticles are quickly and easily made at low cost in large 30 ml batches or greater with simple-to-insert additives, such as targeting ligands or melittin added post-production.

Formulation of HIV-1 prophylactics is not unprecedented as evidenced by recent trials of tenofovir gel systems [16]. However, a potential drawback for tenofovir use is the requirement for active infection. By contrast, melittin-loaded nanoparticles offer an alternative

Figure 4. Attenuation of HIV-1 infectivity by melittin nanoparticles



(A) Comparison of melittin nanoparticles (NP) and an equivalent number of blank NP in reducing HIV-1 CXCR4 tropic viral infectivity. **(B)** Comparison of the effect of melittin NP and an equivalent number of blank NP on the viability of reporter cells treated with HIV-1 CXCR4 tropic virus. **(C)** Comparison of melittin NP and an equivalent number of blank NP in reducing HIV-1 CCR5 tropic viral infectivity. **(D)** Comparison of the effect of melittin NP and an equivalent number of blank NP on the viability of reporter cells treated with HIV-1 CCR5 tropic virus. Data represent the mean ±SD of n=6 determinations. Statistically significant P-values (P<0.05) are listed above or below error bars.

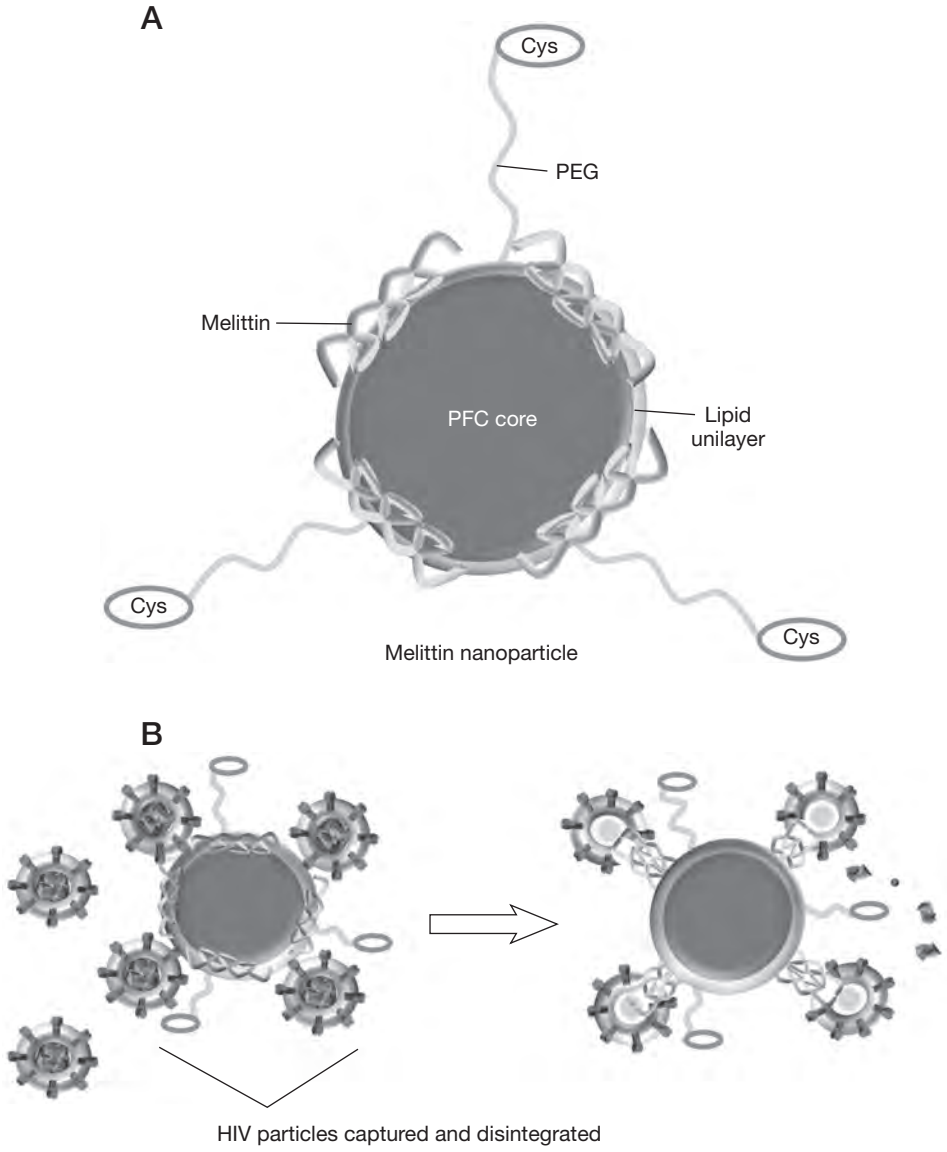
prophylactic option that is aimed at preventing the initial infection. Accordingly, melittin-mediated disruption of viral envelopes represents a unique approach to inhibition of viral entry through mucosal portals. Furthermore, this approach, which combines HIV-1 neutralization with permanent virion destruction, is preferable to neutralization only approaches such as sCD4. Although laboratory culture strains of HIV-1 are often sensitive to neutralization by sCD4 alone or bifunctional sCD4 immunoglobulin chimeras, primary isolates tend to be more resistant [17–21]. Moreover, some reports have demonstrated that sCD4 may increase HIV-1 infectivity [22,23] or facilitate fusion [24] of certain mutant HIV-1 strains.

Theoretically, melittin nanoparticles are not susceptible to HIV-1 mutational resistance seen with standard HIV-1 therapies, such as nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors (NNRTIs) or pre-exposure prophylactics (PrEPs). The lack of susceptibility of anti-HIV-1 melittin nanoparticles to HIV-1 mutational resistance is particularly significant given new evidence demonstrating significant mutational resistance to PrEPs. Indeed, daily exposure to PrEPs can lead to mutational resistance in as little as 2 weeks [25]. Thus, similar to the overuse of antibiotics selecting for resistant bacteria, long-term use of PrEPs ultimately will select for resistant HIV-1 superstrains for which there will be limited therapeutic options. The spread of these anticipated resistant strains could be catastrophic. Fortunately, anti-HIV melittin nanoparticles target an essentially physical rather than replicative property of HIV-1 by disintegrating the lipid envelope, making HIV-1 less likely to develop resistance to the melittin nanoparticles. We believe that anti-HIV-1

melittin nanoparticle therapy is also well-suited as an alternative, adjunctive or ultimate fail-safe therapy for preventing HIV-1 morbidity and mortality in PrEP or NNRTI-resistant patients.

A similar immunity to mutational resistance would be expected if HIV-1-specific ligands were incorporated into future versions of melittin nanoparticles. Such ligands could be designed to interact directly with HIV-1 envelope proteins to preferentially encourage HIV-1 fusion to melittin nanoparticles rather than cells. Alternatively, the benefits of non-specific targeting might outweigh specific viral targeting if more than 1

Figure 5. A proposed model for the mechanism of action of melittin nanoparticles



(A) Melittin nanoparticles are liquid perfluorocarbon (PFC) core based nanoparticles. A topical melittin antiviral nanoparticle is surrounded by a phospholipid monolayer membrane that contains multiple copies of the 26 amino acid α-helical peptide melittin inserted at an oblique angle as well as polyethylene glycol (PEG) to reduce cellular interactions and cysteine (Cys) amino acid capped linkage points to add specific targeting ligands as necessary. Melittin requires a lipid membrane bilayer to aggregate and self-assemble a lytic pore. Because of its biologically non-reactive PFC core and monolayer lipid membrane, melittin nanoparticles can carry melittin without rupture. **(B)** Melittin nanoparticles are naturally fusogenic with lipid membranes given the potential energy stored between the PFC and lipid interface. Melittin nanoparticles encounter HIV-1 particles through random interactions. Melittin nanoparticle lipids fuse with HIV-1 envelopes resulting in melittin delivery, aggregation and disintegration of HIV-1 lipid membrane bilayers. Having been stripped of its viral envelope, HIV-1 is no longer infectious.

sexually-transmitted enveloped virus could be disrupted with the same simple, safe and effective nanoparticle.

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Disclosure statement

The authors declare no competing interests.

Additional file

Additional file 1: A figure displaying the effect of melittin-loaded PEG-negative nanoparticles on vaginal cell viability can be found at http://www.intmedpress.com/uploads/documents/AVT-12-OA-2513_Hood_Add_file1.pdf

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