Long-chain Polyunsaturated Fatty Acids as Anti-HIV Supplementation During Breastfeeding

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SUMMARY

Objective: Breastfeeding by human immunodeficiency virus (HIV)-positive mothers is an unavoidable practice in some very poor countries. It has been suggested that long-chain polyunsaturated fatty acids (LC-PUFAs) in breast milk, such as arachidonic acid, act as natural, protective ingredients against HIV transmission. The objective of this study was to identify the protective mechanism of LC-PUFAs in cells susceptible to HIV infection (e.g. human $CD4^+$ T cells, HeLa cells).

Results: LC-PUFAs are bioactive molecules capable of activating the cellular protective machinery via modulation of endogenous background K^+ or KCNK channels. KCNK channel expression contributes significantly to the stability of the cell membrane potential. During HIV-1 infection, degradation of the KCNK channel is accelerated, and the cell membrane potential becomes pathologically depolarized. From studying functionally distinct KCNK mutants, we found that the degree of membrane potential depolarization was directly proportional to the release efficiency of HIV-1 virions. On the other hand, supplementation of KCNK channel modulators such as arachidonic acid (AA) and docosahexaenoic acid (DHA) at micromolar doses could restore hyperpolarization and stability of the cell membrane potential when endogenous KCNK channels are partially knocked down (mimicking the depolarized state of an HIV-1-infected cell).

Conclusion: The protective mechanism of LC-PUFAs against HIV spread involves stimulation of the endogenous KCNK channels. Our work suggests that supplementation with AA and DHA may be beneficial in reducing the risk of HIV-1 transmission, particularly during the period of breastfeeding. [*Taiwan J Obstet Gynecol* 2009;48(1):65–68]

Key Words: HIV infection, K_{2P} channels, KCNK channels, long-chain polyunsaturated fatty acids, transmission

Introduction

Breastfeeding accounts for up to 40% of mother-to-child transmission (MTCT) of human immunodeficiency virus (HIV) in sub-Saharan Africa. Although the WHO/UNAIDS/UNICEF strongly advises against breastfeeding by HIV-positive mothers, replacement feeding in the pandemic, low-income countries is often not affordable or even feasible because of the lack of clean water or cultural stigmatization [1,2]. Interestingly, a recent nutritional study shows that some natural components in breast milk, such as arachidonic acid (AA) and linolenic acid, are beneficial in reducing the risk of MTCT of HIV [3].

Both AA and linolenic acid are bioactive long-chain polyunsaturated fatty acids (LC-PUFAs) with a diverse range of activities. It was suggested that LC-PUFAs could reduce HIV transmission by inactivating the enveloped viruses or by enhancing the viability of HIV-targeted $CD4^+$ T cells [3–5] (Figure 1). On the other hand, LC-PUFAs are well known for their protective cellular activities against tissue lesions, e.g. during the events of ischemia, strokes or seizures [6]. The mechanism through which LC-PUFAs stimulate the cellular protection machinery primarily involves modulation or activation of two-pore-domain potassium (K_{2P}) channels [6,7] (Figure 2). The gene family of K_{2P} channels was designated KCNK by the Human Genome Organization. The extensive KCNK channel family comprises 18 gene members, and is ubiquitously expressed in a variety of cells, including the major HIV target, $CD4^+$ T cells [8].

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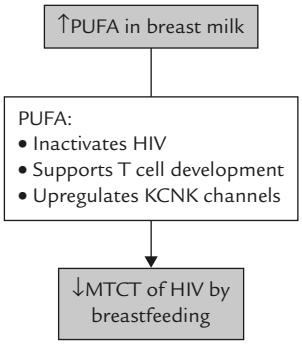

Figure 1. Proposed mechanisms of breast milk long-chain polyunsaturated fatty acids (PUFAs) as a reducer of the risk of human immunodeficiency virus (HIV) mother-to-child transmission (MTCT).

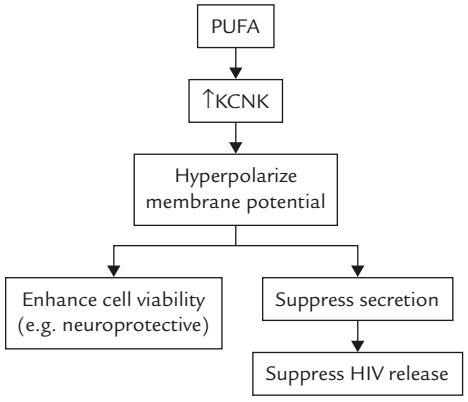

Figure 2. The cell protective mechanism of long-chain polyunsaturated fatty acids (PUFAs) via KCNK channel modulation. HIV = human immunodeficiency virus.

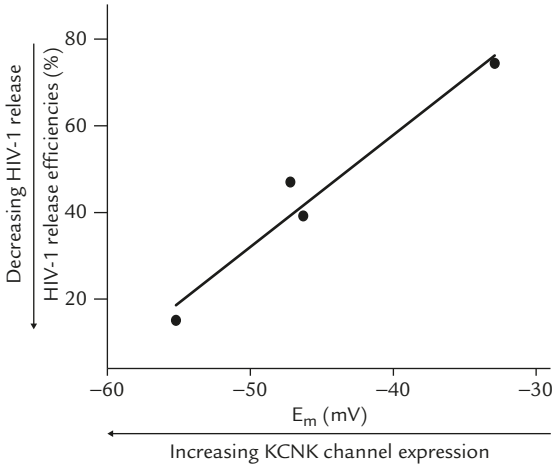

Figure 3. The efficiencies of human immunodeficiency virus (HIV)-1 release were directly proportional to the degrees of membrane potential depolarization. Each dot represents an averaged value for the dual effects of KCNK3 or one of its functionally distinct mutants on membrane potentials (x-axis) and on HIV-1 release efficiency (y-axis). Cell membrane potentials (E_m) in a Ringer-like solution were determined by whole-cell patch-clamp recordings [12]. The inhibitory effects of heterologously expressed KCNK3 channels on viral particle release were compared with the single expression of HIV-1 proviral NL4-3 (set at 100%) in HeLa cells, as previously described [12]. The linear fit between the x and the y parameters indicates a direct relationship between membrane potential depolarization and efficiency of HIV-1 release.

The main function of KCNK channels is to stabilize the cell membrane potential by conducting background K^+ currents which have little time and voltage dependence [9]. KCNK channels stabilize the cell membrane potential by drawing it toward the equilibrium potential for K^+ or E_K (i.e. near –90 mV in most physiologic conditions). Thus, the expression level of endogenous K_{2P} channels is a key determinant of cell viability and excitability. It has been demonstrated that during traumatic episodes of ischemia, activation of KCNK channels by LC-PUFAs could drive membrane potential hyperpolarization and suppress harmful excitability or secretion [6,7,10,11]. Notably, secretion of HIV-1 virions from infected cells could be similarly suppressed by the hyperpolarizing activity of KCNK channels [12] (Figures 2 and 3).

We previously found that the host KCNK3 channel is capable of restricting the release of HIV-1 virions from infected cells. The KCNK3 channel limits viral spread by counteracting the activity of an HIV-1-encoded protein named Vpu [12], whose main function is to enhance the efficiency of viral particle release by up to 100-fold. Because Vpu bears a channel-like structure, this small viral protein has the tendency to self-oligomerize [13] or to promiscuously oligomerize with homologous host channel subunits like KCNK3 [12]. Using a number of protein and functional studies, we identified the protein–protein interaction between host KCNK3 and HIV-1 Vpu in heterologous expression systems (i.e. HeLa and HEK-293 cells), as well as in HIV-1-infected human specimens. The functional consequences of their protein–protein interaction are mutually destructive: Vpu accelerates degradation of the host KCNK3 channel to abolish the background K^+ conductance in HIV-1-infected cells, while endogenous KCNK3 expression restrains the activity of HIV-1 Vpu and viral release to some extent [12]. Further studies using functionally distinct KCNK3 mutants revealed that the efficiency of HIV-1 release was inversely correlated with the degree of membrane potential hyperpolarization sustained by KCNK channel expression (Figure 3). In other words, the expression of background K^+ channels, if not wiped out by Vpu, should be able to halt or decelerate the release of HIV-1 virions from infected host cells.

Once a $CD4^+$ T cell is infected with HIV, its membrane potential and other vital homeostatic functions are disrupted for the purpose of viral spread. An infected cell gradually loses its hyperpolarizing membrane potential until cell death. However, if the expression of background

K^+ or KCNK channels is maintained or even upregulated during HIV infection, viral secretion and spread could conceivably be suppressed by the hyperpolarizing membrane potentials (Figure 3). KCNK3 and KCNK9 are actively expressed in human T lymphocytes [8]. Since LC-PUFAs have been suggested as natural, protective ingredients in breast milk against MTCT of HIV [3] and are capable of stimulating KCNK channels [6], we hypothesized that LC-PUFA supplementation could help sustain KCNK channel activities and cell membrane hyperpolarization, and be developed toward reducing the risk of HIV transmission during breastfeeding.

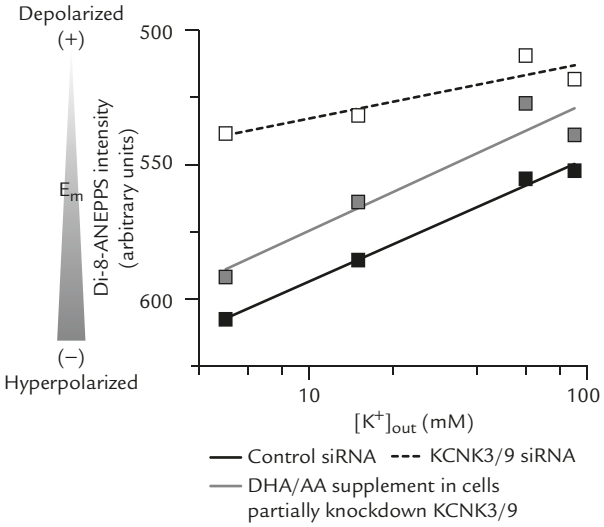

Figure 4. The arachidonic acid (AA) + docosahexaenoic acid (DHA) supplement restored the activity of endogenous KCNK3/9 partially knocked down in HeLa cells. The degree of membrane potential hyperpolarization was assessed using di-8-ANEPPS (Invitrogen, Carlsbad, CA, USA). Partial knockdown of endogenous KCNK3 and KCNK9 depolarized cell membrane potentials (dashed line). The AA + DHA supplement, on the other hand, activated the residual KCNK channel activities and significantly restored cell membrane hyperpolarization (gray solid *vs.* black solid lines). A representative experiment in HeLa cells is shown. siRNA = small interfering RNA.

Results

This work aimed to test if LC-PUFAs could enforce membrane potential hyperpolarization and stability in cells susceptible to HIV infection, thereby lowering the rate of viral particle release during HIV-1 infection. We have previously shown that heterologous expression of the background K^+ channel KCNK3 was capable of maintaining membrane potential stability and suppressing viral release in HIV-1-expressed HeLa cells [12]. As demonstrated in Figure 3, heterologous expression of wild-type KCNK3 suppressed ~50% of viral release from HIV-1-expressed HeLa cells, and maintained the cell membrane potentials at about −50 mV. When expressing a hyperactive KCNK3 mutant, cell membrane potentials became more hyperpolarized and the rate of HIV-1 release was further suppressed to below 20% (the lower left point in Figure 3). On the other hand, when heterologously expressing a nonfunctional KNCK3 mutant, the channel exerted neither any hyperpolarizing effects on the membrane potential nor inhibitory effects on HIV-1 release (the upper right point in Figure 3). From studying the impacts of KCNK3 and its point mutants in cells secreting HIV-1, we observed an inverse relation between the efficiency of HIV-1 particle release and the stability of the cell membrane potential maintained by these background K^+ channels (Figure 3). Based on this relationship, the rate of HIV-1 release could conceivably be suppressed by hyperpolarizing potentials (as in the directions of the horizontal and the vertical arrows in Figure 3).

We thus tested if LC-PUFA supplementation could stimulate endogenous KCNK channel expression and induce membrane potential hyperpolarization in the cells most susceptible to HIV infection. To mimic the depolarized state of HIV-infected cells, endogenous KCNK3 and KCNK9 channels (KCNK3/9) in HIV-1-susceptible cells (i.e. human $CD4^+$ T cells and HeLa cells) were partially knocked down using a low dose of the specific small interfering RNA (siRNA). We then applied AA and docosahexaenoic acid (DHA) at doses equivalent to the recommended dietary intake (1–3.6μM in cell culture) and observed if these LC-PUFAs could restore the activities of endogenous KCNK channels that were partially knocked down. The activity of endogenous KCNK channels was assessed in terms of cell membrane potential at different concentrations of extracellular K^+ ($[K^+]_{out}$). Cells were labeled by polarization-sensitive fluorescent dye di-8-ANEPPS, and their fluorescent signals detected by flow cytometry. In the absence of KCNK knockdown ("control siRNA" in Figure 4), cell membrane potentials were directly correlated with extracellular K^+ content. The correlation was much diminished in the cells with partial KCNK3/9 knockdown. KCNK3/9 knockdown reduced the dependency of the cell membrane potential on extracellular K^+ concentration, as shown by the flattened slope of the membrane potentials with respect to $[K^+]_{out}$. Most importantly, the knockdown of endogenous KCNK3/9 substantially depolarized the cell membrane potential (Figure 4). On the other hand, the reduction in membrane potential hyperpolarization in these KCNK3/9-knockdown cells could be restored by supplementation with LC-PUFAs, such as AA and DHA, to a large extent (Figure 4). The LC-PUFA supplementation also restored the membrane potential

sensitivity to $[K^+]_{out}$, as demonstrated by a slope comparable to that of the control samples (gray solid *vs.* black solid lines in Figure 4). Thus, AA and DHA were capable of stimulating endogenous KCNK channels in the cells most susceptible to HIV-1 infection and restoring their membrane potential stability.

Discussion

Our findings suggest that the mechanism which LC-PUFAs utilize to protect against MTCT of HIV involves modulation of endogenous KCNK channels. Therefore, supplementation with LC-PUFAs, such as AA and DHA, could be effective in suppressing HIV spread (Figures 3 and 4). Although this nutrient supplementation cannot eradicate HIV, it has the potential to reduce the risk of MTCT of HIV when and where breastfeeding by HIV-positive mothers is unavoidable. We are currently assessing the potencies of LC-PUFAs as anti-HIV supplements during breastfeeding, as well as alternative forms of delivery (i.e. nipple paste) in regions where clean water and clean containers are difficult to find. This approach is expected to be feasible even for the very poor regions of the world, since most of the dietary LC-PUFAs are low-cost and have been popular supplements for their other benefits (e.g., enhancement of brain development in neonates and children).

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