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Evaluation of the effects of Quercetin and Kaempherol on the surface of MT-2 cells visualized by atomic force microscopy

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ABSTRACT

This study investigated the anti-viral effects of the polyphenolic compounds Quercetin and Kaempherol on the release of HTLV-1 from the surface of MT-2 cells. Atomic force microscopy (AFM) was used to scan the surface of the MT-2 cells. MT-2 cells were fixed with 100% methanol on round glass lamina or cleaved mica and dried under UV light and laminar flow. The images were captured on a Multimode equipment monitored by a NanoScope IIId controller from Veeco Instruments Inc operated in tapping mode and equipped with phase-imaging hardware. The images demonstrated viral budding structures 131 ± 57 nm in size, indicating profuse viral budding. Interestingly, cell-free viruses and budding structures visualized on the surface of cells were less common when MT-2 was incubated with Quercetin, and no particles were seen on the surface of cells incubated with Kaempherol. In summary, these data indicate that HTLV-1 is budding constantly from the MT-2 cell surface and that polyphenolic compounds were able to reduce this viral release. Biological samples were analyzed with crude cell preparations just after cultivation in the presence of Quercetin and Kaempherol, showing that the AFM technique is a rapid and powerful tool for analysis of antiviral activity of new biological compounds.

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1. Introduction

Human T-lymphotropic virus 1 (HTLV-1) was the first human retrovirus to be characterized (Popovic et al., 1982; Osame et al., 1986; Gessain et al., 1985). After its discovery, HTLV-1 was also found to be the causative agent of adult T-cell lymphoma/leukemia (ATL) (Hinuma et al., 1981; Takatsuki, 2005) and HTLV-1-Associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP) (Osame et al., 1986; Gessain et al., 1985).

HTLV has some peculiarities, such as poor infectivity as a cell-free virus (Jones et al., 2008; Derse et al., 2001; Morozov and Weiss, 1999) and no observed cytopathic effect in cell culture (Vahlne, 2009). The fact that cell-free enveloped virions of HTLV-1 are undetectable in the serum of individuals infected with HTLV-1 and during in vitro culture of lymphocytes infected naturally strongly supports these HTLV biological characteristics (Vahlne, 2009; Poiesz et al., 1980; Gallo, 2005). For that reason, cell lines derived from peripheral blood mononuclear cells (PBMCs) of ATL and HAM/TSP patients are used frequently for the analysis of virus-induced pathogenesis, viral replication and morphogenesis, as well as for the evaluation of anti-HTLV-1 antiviral effects of drugs.

Concerning the research on new antiviral therapeutic approaches, the majority of reports are based mostly on new protocols for inhibition of receptor-binding assays (Kampani et al., 2007) and the assessment of classical molecular and immunological alterations in vitro and in vivo. For in vitro studies, the cell lines MT-2, C91/PL, C5/MJ, HUT 102 and others have been evaluated (Balestrieri et al., 2008).

MT-2 is a well-described HTLV-1-positive cell line that has been used widely for investigations of HTLV-1 infection. MT-2 cells exhibit profound expression of HTLV-1 proteins and mRNA coding for HTLV-1 polyproteic precursors, and the molecular assessment of HTLV-1 using MT-2 as a model has yielded frequently reliable

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and consistent results (Morozov and Weiss, 1999; Balestrieri et al., 2008; Coelho-dos-Reis et al., 2009).

Nevertheless, there is a lack of simple and efficient methods to evaluate morphological changes in cells infected with HTLV-1 and alterations in HTLV-1 structure that are applicable to pathogenesis and antiviral therapy studies. In this respect, atomic force microscopy (AFM) is a powerful nanomicroscopy technique that allows the determination of the dimensions of virus particles and their mechanical properties. The potential of AFM to characterize biological samples has been well demonstrated over the last 17 years. Although it is a relatively new technique, AFM has been used in many studies on bacteriophages, animal and plant viruses, viral protein structures and biomolecule interactions. This technique has contributed to knowledge on the biomedical and biotechnological applications of viral isolates around the world (Cidade et al., 2003; Gladnikoff et al., 2009; Malkin et al., 2003; Kuznetsov et al., 2008; Trindade et al., 2007).

Polyphenolic compounds are among the most ancient compounds ever described and studied. Quercetin and Kaempherol, two polyphenolic compounds of the bioflavonoids group, have been studied extensively in terms of their antioxidant, anti-inflammatory and antiviral effects (Sonoda et al., 2004; Hämäläinen et al., 2007; Min et al., 2007; Rogerio et al., 2007; Sharma et al., 2007; Chiang et al., 2003; Yu et al., 2009). Quercetin [IUPAC name: 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one] and Kaempherol [IUPAC name: 3,5,7-trihydroxy-2-(4-hydroxyphenyl)chromen-4-one] are bioflavonoids distributed widely in the plant kingdom and are common constituents of most edible fruits and vegetables (Chiang et al., 2003; Cao et al., 2009). A previous study has shown that a plant extract rich in polyphenolic compounds induces the expression of pro-apoptotic protein levels and down-regulates anti-apoptotic protein expression in cell lines infected with HTLV-1 (Harakeh et al., 2006). Nonetheless, the specific polyphenolic compounds to which these effects should be attributed remain unknown. Induction of apoptosis by Quercetin was also demonstrated in murine leukemia virus cell lines (Yu et al., 2009).

The present study investigated the surface of MT-2 as well as the HTLV-1 released from those cells in the presence of Quercetin and Kaempherol to evaluate the antiviral effects of these compounds. A standard AFM technique was developed and used to scan the MT-2 surface.

2. Materials and methods

2.1. Preparation of samples for AFM

2.1.1. MT-2 culture

MT-2 cells were grown in a RPMI-1640 medium with 20% fetal bovine serum suspension supplemented with L-glutamine, gentamicin and penicillin at 37 °C, 5% CO_2. After 72 h of growth, the suspension of cells was homogenized vigorously to dissolve clumps, and differential centrifugation (100 × *g*, 10 min, 25 °C) was used to remove the remaining cell clusters. The cells were washed twice with phosphate buffer solution and centrifuged (1000 × *g*, 10 min, 4 °C). The cells were resuspended in RPMI 20% fetal bovine serum suspension supplemented as described before, and the suspension was adjusted to approximately 1.0×10^6 cells/ml for all the assays performed.

2.1.2. Quercetin and Kaempherol

The effects of Quercetin and Kaempherol on MT-2 cells were assessed after incubating the cells with either Quercetin or Kaempherol at 100 μM for 24 h, resuspending the cells at a final concentration of 1×10^6 cells/ml and seeding them in 24-well plates.

2.1.3. Glass slides and mica preparation

Prior to microscopy, 13-mm round glass slides were washed exhaustively with neutral detergent and incubated in distilled water at 60 °C overnight to remove the remaining dirt and grease. The glass slides were rinsed again with cold Milli Q water and rinsed subsequently with 100% ethanol and dried under laminar flow. After drying, 500 μl of 1% poly-L-lysine was added to the slides (Sigma–Aldrich, USA), and the slides were dried again under laminar flow. Cleaved mica (Ted Pella, Inc.) was also used for cell analysis. MT-2 cells incubated with or without drugs were centrifuged in a cytospin centrifugation apparatus (Jouan, USA) (1000 × g, 10 min, 4 °C; 5.0×10^5 cells) onto the glass slide or cleaved mica. After centrifugation, the cells were dehydrated successively with 30%, 50% and 70% ethanol. The slides were then fixed with cold methanol and dried under laminar flow.

2.2. AFM scanning

AFM imaging was performed at room temperature on equipment monitored by a NanoScope IIId controller from Veeco Instruments Co. (Plainview, NY, USA) operated in tapping mode and equipped with phase-imaging hardware. Commercial tapping mode tips from Nanosensors™ with 228 μm long cantilevers, resonance frequencies of 75–98 kHz, spring constants of 3.0–7.1 N/m and a nominal tip curvature radius of 5–10 nm were used. The regions scanned included both cells and matrix, but emphasis was placed on the cell surface, considering the aim of this study. The majority of AFM images presented are phase images because they display the greatest amount of contrast and detail.

2.3. Data analysis for AFM scanning

The sizes, height and depth of structures, indicating HTLV-1 viral budding, free viral particles and membrane invaginations on the surface after Quercetin or Kaempherol treatment, were measured using the NanoScope software. This study was undertaken as three independent experiments.

3. Results

3.1. Establishing the topological profile of the MT-2 cell surface by AFM

AFM was utilized to investigate the effects of Quercetin and Kaempherol on the surface of MT-2 cells. Cells were incubated for 24 h with these drugs at a concentration of 100 μM.

To characterize the MT-2 cell surface and HTLV-1 particles in the presence or absence of Quercetin and Kaempherol, AFM images were taken at different magnifications. Fig. 1a shows a whole MT-2 cell. In the MT-2 cell control (untreated cells), round structures presenting an inward curve co-localized with spherical structures resembling virus particles, indicating profuse HTLV-1 budding, as shown in Fig. 1b, these structures were named viral budding units. After 24 h treatment with Kaempherol, no HTLV-1 free particles or viral budding units were seen on the MT-2 cell surface (Fig. 1c). Fig. 1d represents the MT-2 cell surface analysis after a 24-h treatment with Quercetin. Abundant pores formed by invagination of the membrane were seen; however, a large number of the pores did not co-localize with spherical structures, indicating diminished HTLV-1 budding compared to the untreated MT-2 cells. These pores were also seen in MT-2 cells treated with Kaempherol. The use of AFM allowed the reconstruction of a 3D image of this membrane invagination displaying the diameter, height and depth of a representative pore sample, as illustrated in Fig. 1e (12.04 nm in depth).

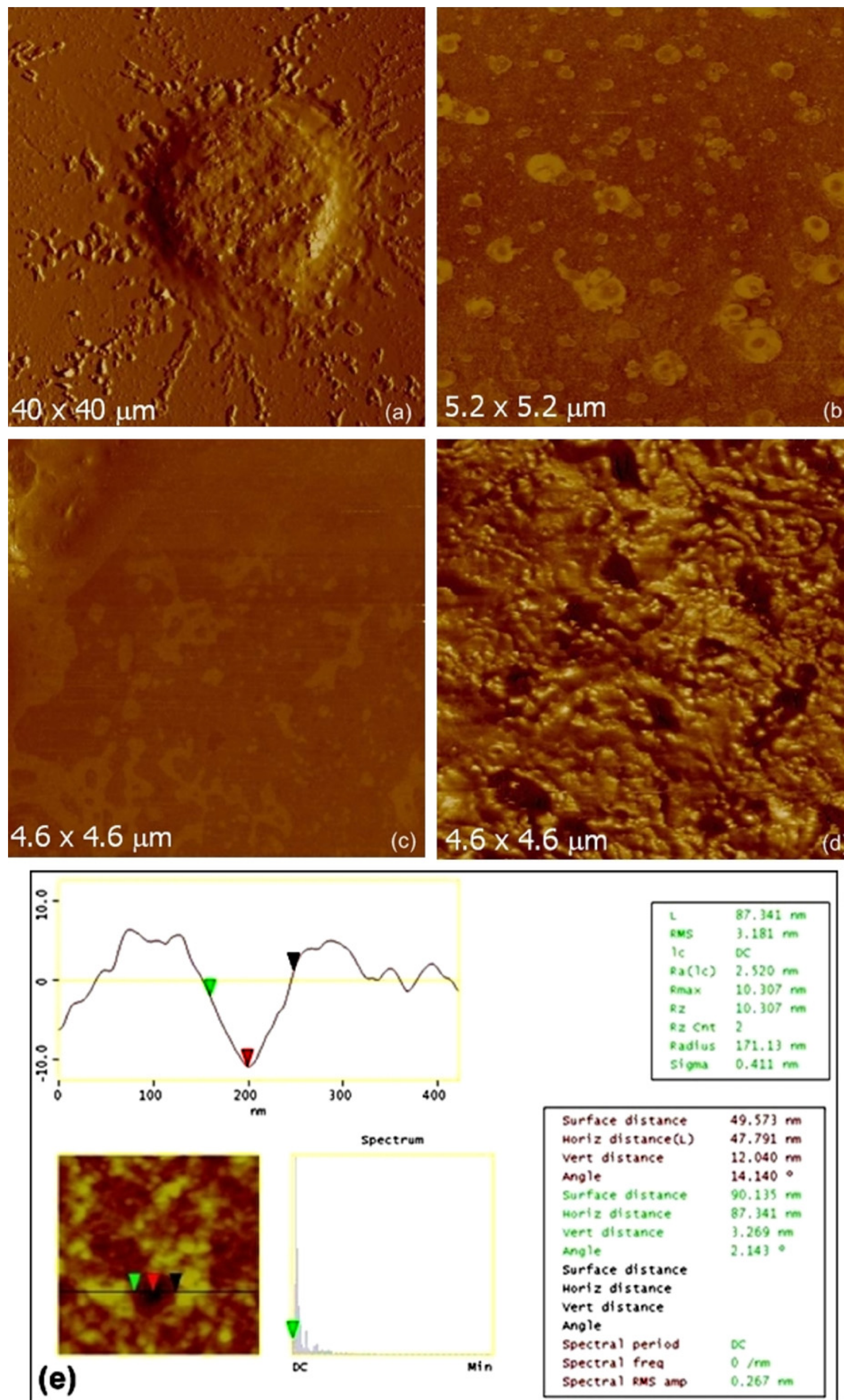

Fig. 1. Gallery of AFM images from MT2 cells, a cell lineage that contains HTLV-1 provirus integrated (Human T-lymphotropic virus 1) and sheds virus particles. (a) Whole MT2 cell surface area (40 × 40 μm), amplitude image; (b) MT2 cell control untreated surface showing rounded budding structures and free virus particles (5.2 × 5.2 μm), phase image; (c) MT2 cell surface after 24 h Kaempherol treatment – 100 μM; no viral particle is seen in this picture (4.6 × 4.6 μm), phase image; (d) MT2 cell after 24 h Quercetin treatment – 100 μM, phase image; pores formed by invaginations of the membrane are seen on entire surface of MT2 cell (4.6 × 4.6 μm); (e) MT2 cell treated with Kaempherol showing topographic profile of pore formed by membrane invagination with 12.04 nm of depth, height image.

3.2. HTLV-1 on the surface of MT-2 cells visualized by AFM

Whereas most viruses spread by releasing large numbers of virions from each infected cell, HTLV-1 spreads efficiently between T cells via a tight and highly organized cell–cell contact known as the virological synapse. T lymphocytes infected with HTLV-1 store transiently viral particles as carbohydrate-rich extracellular assemblies that are held together and attached to the cell surface by virally induced extracellular matrix components, as shown recently by Pais-Correia et al. (2010), and referred to as HTLV-1 biofilm-like structures.

An AFM image of the HTLV-1 budding structures and HTLV-1 free particles is shown in Fig. 2. All the cells scanned in the independent experiments showed large numbers of these structures with

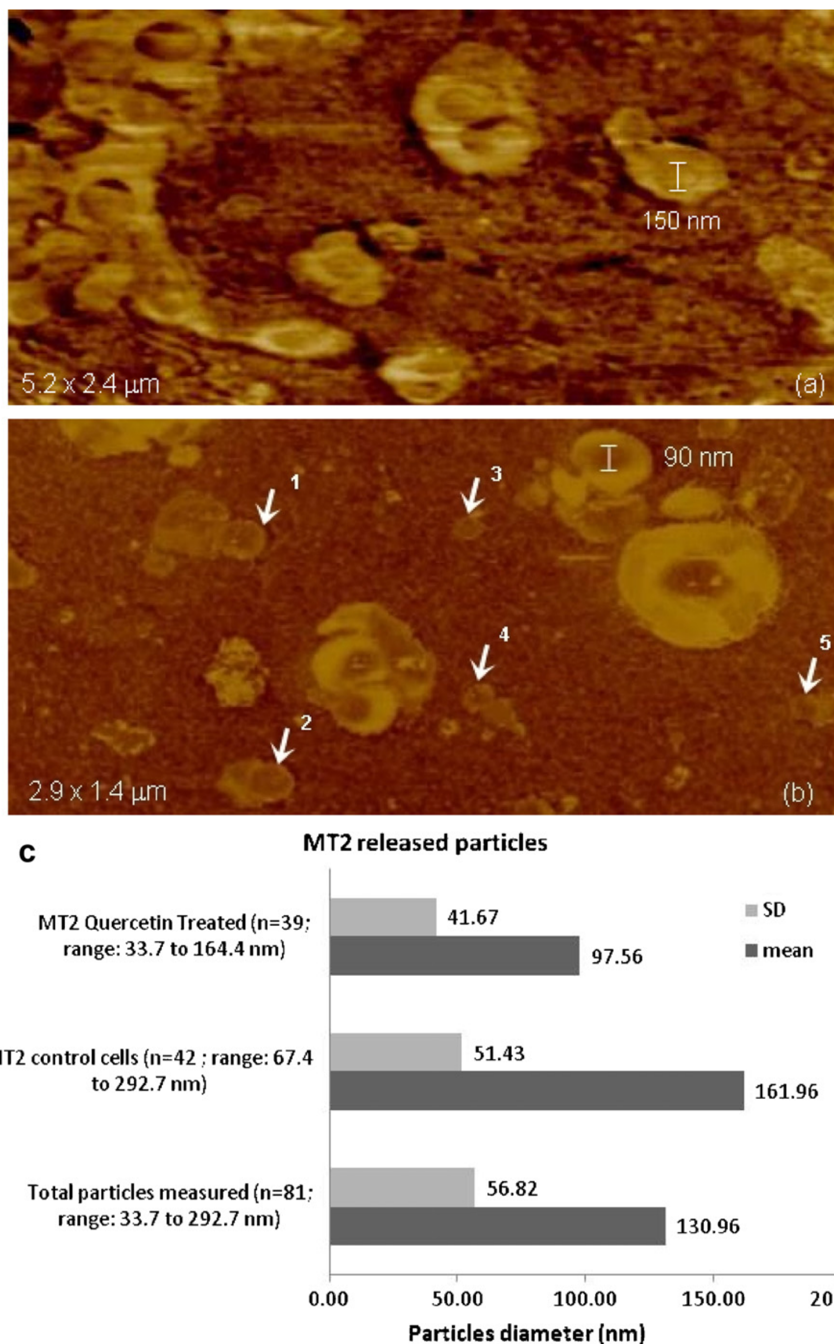

Fig. 2. Analisys of HTLV-1 budding structures and viral free particles on MT2 cell surface. (a) MT2 control cell surface showing many rounded viral budding structures with viral like particles inside, in the right side of the figure, a spherical particle resembling virus is measured as an example (150 nm diameter) (5.2 × 2.4 µm); (b) viral budding structures and virus free particle (indicated with arrows – AFM measurements: 1 – 128 nm; 2 – 134 nm; 3 – 78 nm; 4 – 90 nm and 5 – 95 nm; 2.9 × 1.4 µm); (c) distribution of virus particles diameter in MT2 control cell and MT2 treated with Quercetin. A total of 81 particles were examined (mean = 131 ± 57 nm). In Quercetin treated cells, virus particles showed shortest measurements compared to MT2 control untreated. All images displayed are phase images.

similar and consistent morphology. The surface of MT-2 control cells showed many rounded viral budding structures with virus-like particles inside the membrane projections and invaginations (Fig. 2a); in Fig. 2b, viral budding structures and free virus particles (indicated with arrows and with AFM measurements ranging from 78 to 134 nm) can be seen. A total of 81 particles were examined (mean = 131 ± 57 nm; range 33.7–292.7 nm). In Quercetin-treated cells, the virus particles were smaller (mean = 97.56 ± 41.67 nm; range 33.7–164.4 nm) than those from the untreated MT-2 control cells (mean = 161.96 ± 51.43 nm; range 67.4–292.7 nm). However, for both MT-2 cells treated with Quercetin and untreated cells, over 50% of the total virus particles examined were 97–165 nm in size, with a mean particle size of 133 ± 20 nm. It is important to emphasize the antiviral effect observed with the polyphenolic compounds; Quercetin treatment of MT-2 cells reduced viral budding, and no virus was seen in any of the experiments conducted with Kaempherol.

4. Discussion

The discovery of HTLV represented the first and most important event that paved the way for researchers to understand infec-

tion of humans by retroviruses (Gallo, 2005) and to discover HIV. The development of inhibitors of reverse transcriptase provided a framework for drug development against HIV. Nevertheless, HTLV antiviral therapy still remains obscure and poorly investigated (Oh and Jacobson, 2008). The lack of clear evidence on how HTLV-1 spreads within and between hosts and the absence of consistent and reliable biomarkers of disease progression discouraged the use of antiviral drugs for the control of diseases associated with HTLV-1 (Balestrieri et al., 2008).

Recently, better understanding of direct cell-to-cell transmission of the virus by virological synapses and by proliferation of infected cells has yielded new guidance and perspective for studies aimed at identifying compounds that inhibit efficiently the HTLV-1 replication cycle or even compounds that impair the ability of cells infected with HTLV-1 to persist and generate infected offspring in the host. Recent progress in HTLV-1 transmission, particularly studies demonstrating mechanisms involved in cell-free virus infection of dendritic cells (Jones et al., 2008; Jain et al., 2009), reinforces the importance of identify drugs with both antiviral and immunomodulatory activity.

The MT-2 cell line was derived from bone marrow CD41 T-lymphocytes from a healthy donor after co-cultivation with leukemia cells of an Adult T cell leukemia patient (Miyoshi et al., 1981). While evaluating this cell model (MT-2 cell line transformed by HTLV-1), Morozov and Weiss (1999) showed that there is one complete provirus and seven defective proviruses in MT-2 cells. These investigators purified the viruses using sucrose gradients and provided reliable evidence of a mixed population of virus particles released from MT-2 cells: light particles (density $\sim$ 1.12 g/cm^3) and heavy or classical particles (density $\sim$ 1.16 g/cm^3) containing the standard HTLV-1 protein pattern.

Timar et al. (1987) used immunoelectron microscopy to examine HTLV-1 particles in C91/PL cells (infected permanently with HTLV-1) and HOS/PL cells (a human osteosarcoma cell line derivate producing HTLV-1) and observed particles ranged from 80 to 150 nm in diameter. Recently, Majorovits et al. (2008) visualized HTLV-1-induced virological synapses by electron tomography on CD4$^+$ T cells infected naturally with HTLV-1 and the HTLV-1-infected MS9 cell line. The diameters of HTLV-1 particles measured in the viral synapses on naturally infected CD4$^+$ T cells averaged 105 nm (range 62–173 nm; n = 44), and the particles derived from the MS9 cell line were larger, presenting a mean diameter of 126 nm (range 46–246 nm; n = 151). These findings were very similar to those obtained by AFM in the present study that showed a mean diameter of 131 ± 57 nm (range 33.7–292.7 nm; n = 81). As observed for some retroviruses in many previous studies, the size of HTLV-1 varies from 30 to 237 nm.

After Kaempherol treatment, the MT-2 cell surface examined by AFM showed no viral particle budding. Quercetin treatment diminished the budding in comparison to untreated MT-2 cells, and the virus particles were smaller (mean = 97.56 ± 41.67 nm) than those from untreated MT-2 control cells (mean = 161.96 ± 51.43 nm), which way indicate a defect in particle assembly. Grigsby et al. (2010) developed a codon-optimized HTLV-1 Gag as a model system to analyze HTLV-1 produced in transient infection using a novel biophysical technology, Fluorescence of Fluctuation Spectroscopy (FFS), which provides information about particle size and protein stoichiometry. Considering that Gag polyprotein, the main retrovirus structural protein, is composed of three functional domains whose cleavage results in particle maturation, these investigators have produced immature virus-like particles (VLP) and determined an average Gag copy number of 510 ± 50 (range 300–880). In a similar study conducted with HIV-1, the Gag number increased when defects that can interfere with the particle infectivity, transmission and pathogenesis were introduced during budding (Carlson et al., 2008).

Although HTLV-1 was the first human retroviruses to be discovered, very little is known about its virion morphology and particle assembly. Pais-Correia et al. (2010) reported that T lymphocytes infected with HTLV-1 store transiently viral particles as extracellular assemblies that are held together and attached to the cell surface by virally induced extracellular matrix components, called HTLV-1 biofilm-like structures. According to these researchers, removal of these cell surface particles could reduce the ability of HTLV-1-producing cells to infect target cells. Compounds with the selective cytotoxic effect on infected cells and which diminish the formation of this viral biofilm would have important potential therapeutic application.

The present study provides evidence that Quercetin and Kaempherol were able to diminish and inhibit, respectively, viral release, which was found to be abundant on the surface of MT-2 cells. Ongoing investigations seek to verify whether Quercetin and Kaempherol induce cell death by restoring the apoptotic mechanisms of MT-2 cells. In addition, Quercetin and Kaempherol were found to inhibit viral mRNA and viral proteins, indicating that the compounds may have an antiviral activity that could be responsible for the selective MT-2 cell death (unpublished data).

In recent years, AFM has provided a broad range of new opportunities for viewing and manipulating biological systems in their native environments, which can be useful for investigating host-pathogen interactions. Studies based on viral pathogenesis, and consequently cell–virus interactions, require the knowledge of the three-dimensional structure of the macromolecules involved. In the case of viral infections, such studies involve detailed knowledge of virion composition and architecture.

However, studies focusing on the detailed organization of human and animal viruses can be difficult to perform due to the fragile and often heterogeneous structure of these viruses and the difficulty in obtaining adequate amounts of purified particles (Malkin et al., 2002; Plomp et al., 2002; Kuznetsov et al., 2001). In the case of HTLV-1, it is even more difficult to produce, purify and quantify viral particles because none of the classical virological techniques based on cytopathic effect observation for viral titration are applicable to HTLV. Thus, the AFM technique is especially valuable for virus investigations as it provides a rapid, simple and a safe tool for virus characterization using small and relatively crude preparations (Trindade et al., 2007).

In the present study, for instance, a relatively straightforward and undemanding preparation of sample was conducted for AFM imaging without using toxic and dangerous reagents, such as glutaraldehyde and osmium tetroxide, to dehydrate, fix or stabilize the sample. It is important to mention, however, that rigorous safety procedures are essential when handling an MT-2 cell culture. AFM imaging was shown to be a reliable and consistent technique to perform viral assessment on infected cells, is therefore recommend for future viral research investigations.

The results indicate that HTLV-1 is budding constantly from the MT-2 cell surface, and viral release was visualized successfully by AFM. AFM was also valuable in revealing that two polyphenolic compounds, Quercetin and Kaempherol, were able to reduce this viral release, suggesting their potential use as antiviral compounds, which is valuable for future studies on finding new and alternative treatment approaches for HTLV-1-related diseases.

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References

Balestrieri, E., Matteucci, C., Ascolani, A., Piperno, A., Romeo, R., Romeo, G., Chiacchio, U., Mastino, A., Macchi, B., 2008. Effect of phosphonated carbocyclic 2′-oxa-3′-aza-nucleoside on human T-cell leukemia virus type 1 infection in vitro. Antimicrob. Agents Chemother. 52 (1), 54–64.

Cao, J., Zhang, Y., Chen, W., Zhao, X., 2009. The relationship between fasting plasma concentrations of selected flavonoids and their ordinary dietary intake. Br. J. Nutr. 14, 1–7.

Carlson, L.A., Briggs, J.A., Glass, B., Riches, J.D., Simon, M.N., Johnson, M.C., Müller, B., Grünewald, K., Kräusslich, H.G., 2008. Three-dimensional analysis of budding sites and released virus suggests a revised model for HIV-1 morphogenesis. Cell Host Microbe 4 (6), 592–599.

Chiang, L.C., Chiang, W., Liu, M.C., Lin, C.C., 2003. In vitro antiviral activities of Caesalpinia pulcherrima and its related flavonoids. J. Antimicrob. Chemother. 52 (2), 194–198.

Cidade, G.A., Costa, L.T., Weissmuller, G., da Silva Neto, A.J., Roberty, N.C., de Moraes, M.B., Prazeres, G.M., Hill, C.E., Ribeiro, S.J., de Souza, G.G., da Silva Pinto Teixeira, L., da Costa Moncores, M., Bisch, P.M., 2003. Atomic force microscopy as a tool for biomedical and biotechnological studies. Artif. Organs 27 (5), 447–451.

Coelho-dos-Reis, J.G., Martins-Filho, O.A., de Brito-Melo, G.E., Gallego, S., Carneiro-Proietti, A.B., Souza, J.G., GIPH, Barbosa-Stancioli, E.F., 2009. Performance of IgG and IgG1 anti-HTLV-1 reactivity by an indirect immunofluorescence flow cytometric assay for the identification of persons infected with HTLV-1, asymptomatic carriers and patients with myelopathy. J. Virol. Methods 160 (1–2), 138–148.

Derse, D., Hill, S.A., Lloyd, P.A., Chung, Hk., Morse, B.A., 2001. Examining human T-lymphotropic virus type 1 infection and replication by cell-free infection with recombinant virus vectors. J. Virol. 75 (18), 8461–8468.

Gallo, R.C., 2005. The discovery of the first human retrovirus: HTLV-1 and HTLV-2. Retrovirology 2 (1), 17.

Gessain, A., Barin, F., Vernant, J., Gout, O., Maurs, L., Calender, A., De The, G., 1985. Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. Lancet 2, 407–410.

Gladnikoff, M., Shimoni, E., Gov, N.S., Rousso, I., 2009. Retroviral assembly and budding occur through an actin-driven mechanism. Biophys. J. 97 (9), 2419–2428.

Grigsby, I.F., Zhang, W., Johnson, J.L., Fogarty, K.H., Chen, Y., Rawson, J.M., Crosby, A.J., Mueller, J.D., Mansky, L.M., 2010. Biophysical analysis of HTLV-1 particles reveals novel insights into particle morphology and Gag stoichiometry. Retrovirology 20 (7), 75.

Hämäläinen, M., Nieminen, R., Vuorela, P., Heinonen, M., Moilanen, E., 2007. Anti-inflammatory effects of flavonoids: genistein, kaempferol, quercetin, and daidzein inhibit STAT-1 and NF-kappaB activations, whereas flavone, isorhamnetin, naringenin, and pelargonidin inhibit only NF-kappaB activation along with their inhibitory effect on iNOS expression and NO production in activated macrophages. Mediators Inflamm. 2007, 45673.

Harakeh, S., Diab-Assaf, M., Niedzwiecki, A., Khalife, J., Abu-El-Ardat, K., Rath, M., 2006. Apoptosis induction by Epican Forte in HTLV-1 positive and negative malignant T-cells. Leuk. Res. 30 (7), 869–881.

Hinuma, Y., Nagata, K., Hanaoka, M., Nakai, M., Matsumoto, T., Kinoshita, K.I., Shirakawa, S., Miyoshi, I., 1981. Proc. Natl. Acad. Sci. U.S.A. 78, 6476–6480.

Jain, P., Manuel, S.L., Khan, Z.K., Ahuja, J., Quann, K., Wigdahl, B., 2009. DC-SIGN mediates cell-free infection and transmission of human T-cell lymphotropic virus type 1 by dendritic cells. J. Virol. 83 (21), 10908–10921.

Jones, K.S., Petrow-Sadowski, C., Huang, Y.K., Bertolette, D.C., Ruscetti, F.W., 2008. Cell-free HTLV-1 infects dendritic cells leading to transmission and transformation of CD4(+) T cells. Nat. Med. 14 (4), 429–436.

Kampani, K., Quann, K., Ahuja, J., Wigdahl, B., Khan, Z.K., Jain, P., 2007. A novel high throughput quantum dot-based fluorescence assay for quantitation of virus binding and attachment. J. Virol. Methods 141 (2), 125–132.

Kuznetsov, Y., Gershon, P.D., McPherson, A., 2008. Atomic force microscopy investigation of vaccinia virus structure. J. Virol. 82 (15), 7551–7566.

Kuznetsov, Y.G., Malkin, A.J., Lucas, R.W., Plomp, M., McPherson, A., 2001. Imaging of viruses by atomic force microscopy. J. Gen. Virol. 82 (9), 2025–2034.

Majorovits, E., Nejmeddine, M., Tanaka, Y., Taylor, G.P., Fuller, S.D., Bangham, C.R., 2008. Human T-lymphotropic virus-1 visualized at the virological synapse by electron tomography. PLoS One 3 (5), e2251.

Malkin, A.J., McPherson, A., Gershon, P.D., 2003. Structure of intracellular mature vaccinia virus visualized by in situ atomic force microscopy. J. Virol. 77, 6332–6340.

Malkin, A.J., Plomp, M., McPherson, A., 2002. Application of atomic force microscopy to studies of surface processes in virus crystallization and structural biology. Acta Crystallogr. D Biol. Crystallogr. 58, 1617–1621.

Min, Y.D., Choi, C.H., Bark, H., Son, H.Y., Park, H.H., Lee, S., Park, J.W., Park, E.K., Shin, H.I., Kim, S.H., 2007. Quercetin inhibits expression of inflammatory cytokines through attenuation of NF-kappaB and p38 MAPK in HMC-1 human mast cell line. Inflamm. Res. 56 (5), 210–215.

Miyoshi, I., Kubonishi, I., Yoshimoto, S., Akagi, T., Ohtsuki, Y., Shiraishi, Y., Nagata, K., Hinuma, Y., 1981. Type C virus particles in a cord T-cell line derived by co-cultivating normal human cord leukocytes and human leukaemic T cells. Nature 294, 770–771.

Morozov, V.A., Weiss, R.A., 1999. Two types of HTLV-1 particles are released from MT-2 cells. Virol 255 (2), 279–284.

Oh, U., Jacobson, S., 2008. Treatment of HTLV-I-associated myelopathy/tropical spastic paraparesis: towards rational targeted therapy. Neurol. Clin. 26 (3), 781–797.

Osame, M., Usuku, K., Izumo, S., Ijichi, N., Amitani, H., Igata, A., Matsumoto, M., Tara, M., 1986. HTLV-I associated myelopathy, a new clinical entity. Lancet 1, 1031.

Pais-Correia, A.M., Sachse, M., Guadagnini, S., Robbiati, V., Lasserre, R., Gessain, A., Gout, O., Alcover, A., Thoulouze, M.I., 2010. Biofilm-like extracellular viral assemblies mediate HTLV-1 cell-to-cell transmission at virological synapses. Nat. Med. 16 (1), 83–89.

Plomp, M., Rice, M.K., Wagner, E.K., McPherson, A., Malkin, A.J., 2002. Rapid visualization at high resolution of pathogens by atomic force microscopy: structural studies of herpes simplex virus-1. Am. J. Pathol. 160 (6), 1959–1966.

Poiesz, B.J., Ruscetti, F.W., Gazdar, A.F., Bunn, P.A., Minna, J.D., Gallo, R.C., 1980. Proc. Natl. Acad. Sci. U.S.A. 77, 7415–7419.

Popovic, M., Reitz, M.S., Sarngadharan, M.G., 1982. The virus of Japanese adult T-cell leukaemia is a member of the T-cell leukaemia virus group. Nature 300, 63–66.

Rogerio, A.P., Kanashiro, A., Fontanari, C., Da Silva, E.V., Lucisano-Valim, Y.M., Soares, E.G., Faccioli, L.H., 2007. Anti-inflammatory activity of quercetin and isoquercitrin in experimental murine allergic asthma. Inflamm. Res. 56 (10), 402–408.

Sharma, V., Mishra, M., Ghosh, S., Tewari, R., Basu, A., Seth, P., Sen, E., 2007. Modulation of interleukin-1beta mediated inflammatory response in human astrocytes by flavonoids: implications in neuroprotection. Brain Res. Bull. 73, 55–63.

Sonoda, J., Koriyama, C., Yamamoto, S., Kozako, T., Li, H.C., Lema, C., Yashiki, S., Fujiyoshi, T., Yoshinaga, M., Nagata, Y., Akiba, S., Takezaki, T., Yamada, K., Sonoda, S., 2004. HTLV-1 provirus load in peripheral blood lymphocytes of HTLV-1 carriers is diminished by green tea drinking. Cancer Sci. 95 (7), 596–601.

Takatsuki, K., 2005. Discovery of adult T-cell leukemia. Retrovirology 2, 16.

Timar, J., Nagy, K., Robertson, D., Clapham, P., Weiss, R.A., 1987. Ultrastructure and immunoelectron microscopy of human T-cell leukaemia virus type I-producing lymphoid and non-lymphoid human tumour cells. J. Gen. Virol. 68 (4), 1011–1020.

Trindade, G.S., Vilela, J.M.C., Ferreira, J.M.S., Aguiar, P.H.N., Leite, J.A., Guedes, M.I.M.C., Lobato, Z.I.P., Madureira, M.C., Silva, M.I.N., Fonseca, F.G., Kroon, E.G., Andrade, M.S., 2007. Use of atomic force microscopy as a diagnostic tool to identify orthopoxvirus. J. Virol. Methods 141, 198–204.

Vahlne, A., 2009. A historical reflection on the discovery of human retroviruses. Retrovirology 6, 40.

Yu, C.S., Lai, K.C., Yang, J.S., Chiang, J.H., Lu, C.C., Wu, C.L., Lin, J.P., Liao, C.L., Tang, N.Y., Wood, W.G., Chung, J.G., 2009. Quercetin inhibited murine leukemia WEHI-3 cells in vivo and promoted immune response. Phytother. Res. 24 (2), 163–168.