INTRODUCTION

HIV is one of the main health risks in today's society in many regions of the world. Most current methods of treatment are based on HIV-reverse transcriptase and protease inhibition to reduce the rate of HIV replication. There is however a clear need for more effective treatment methods and drug candidates to effectively treat the disease. Some of these target the interaction between virus and T-cell, the primary cell type infected by HIV. Whereas classical virology studies show interesting antiviral effects, it remains unclear whether or not the drug candidate may modulate the capability of CADA. T-cells were infected with HIV in the presence of different doses of CADA. What do you mean? Conversely, uninfected T-cells were treated with the same doses of CADA — i.e. as a parallel — uninfected T-cells were treated with the same doses of CADA and CADA were analyzed flow-cytometrically and subsequently denatured with RapiGest™ SF surfactant (0.1%) (Waters). The MW of the Leu3a-FITC staining is depicted for the different doses of CADA (line).

METHODS

Sample preparation

To study the effect of CADA on human CD4+ memory T-cells treated with the compound, the biosynthesis and the CADA bioavailability. CADA experiments were conducted using a 1:15:1 sample and control (uninfected) of cell culture medium and CADA-exposed biosynthesis were analyzed by SDS-PAGE as illustrated in Figure 1. The effect of CADA was further evaluated in infected cultures (see Figure 3).

UPLC-MS conditions

LC-MS experiments were conducted using a 1.5 h column (Finnpipette 200 µL, 0.1% HCOOH (0.1% formic acid) at 25°C (60°C). The mass spectrometer used was the Waters® Protein Expression System, using a scan time of 1.5 s per function over the m/z range 50-1990. Protein identifications and quantitative information were generated by the use of dedicated algorithms (Waters® Protein Expression Software).

Atlantis® 3μm C18 75 μm x 15 cm nanoscale LC column (Waters Corp.). An Xcalibur® 2.1 MS Control Software and MassLynx® version 4.1.1 was used, with all samples in triplicate. Typical sample volume was 5 µL of the aqueous digest — which is a ten-fold extension of the calibrated sample volume — i.e. no significant peptide and protein regulation has been observed by means of the employed label free UPLC-MS technique.

The results presented here are from a label-free quantitative LC-MS analysis of human T cell-line proteins. The effect of the treatment was monitored by utilizing the relative quantitation results, which provided invaluable input for the investigation on the mechanism of action of the compound on targeted and non-targeted cellular components.

RESULTS & DISCUSSION

Cell Expression

Figure 1. CD4 down-modulation in human T-cells after incubation with CADA (5 µM). Cell surface CD4 expression of untreated and CADA-treated cells was monitored by staining with the specific CD4 monoclonal antibodies. Horizontal references lines are included between batches. An uptake control is included to monitor the background staining.

Figure 2. Relaxation of CADA-induced CD4 down-modulation in human T-cells after incubation with CADA (5 µM). Cell surface CD4 expression of untreated and CADA-treated cells was monitored by staining with the specific CD4 monoclonal antibodies. Horizontal references lines are included between batches. An uptake control is included to monitor the background staining.

Figure 3. Correlation between anti-HIV potency and CD4 down-modulation of CADA-treated T-cells. The data show that the anti-HIV potency of CADA is positively correlated with the CD4 down-modulation. The results suggest that the anti-HIV effect of CADA is mediated through CD4 down-modulation.

Figure 4. Example low (bottom pane) and elevated energy mass spectra for the detection of abundant and low-energy peptides. The mass spectra show that the high-energy information is utilized for qualitative, whereas the low-energy information is used for the quantification of the peptides and subsequently proteins, which is a ten-fold extension of the calibrated sample volume — i.e. no significant peptide and protein regulation has been observed by means of the employed label free UPLC-MS technique.

Figure 5. Example low (bottom pane) and elevated energy mass spectra for the detection of abundant and low-energy peptides. The mass spectra show that the high-energy information is utilized for qualitative, whereas the low-energy information is used for the quantification of the peptides and subsequently proteins, which is a ten-fold extension of the calibrated sample volume — i.e. no significant peptide and protein regulation has been observed by means of the employed label free UPLC-MS technique.

Figure 6. Mass-pair distribution close-up of the protein “signal” peptide from the CADA-treated T-cells. The results suggest that the anti-HIV effect of CADA is mediated through CD4 down-modulation.

Figure 7. Relative protein expression (top) and total intensity of identified peptides in both conditions — control and treated — normalized to human proteins (2 ≥ fragment ions/peptide) and identified replicate 2 ≤ 2 fold. For the treated sample the cumulative protein intensity was ≥ 2 fold.

CONCLUSION

The results shown in Figures 9 and 10 suggest that the regulation of the human CD4 target protein is very mild and that the number of regulated peptides is very low. The results also indicate that the detection of CADA-induced CD4 down-modulation is limited to the use of the employed label free UPLC-MS technique.

Figure 8. Relative protein expression (top) and total intensity of identified peptides in both conditions — control and treated — normalized to human proteins (2 ≥ fragment ions/peptide) and identified replicate 2 ≤ 2 fold. For the treated sample the cumulative protein intensity was ≥ 2 fold.

REFERENCES


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