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Syncytia formation in HIV-1 infected cells is associated with an increase in cellular oleic acid

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Infection of cells with the human immunodeficiency virus type-1 (HIV-1) usually results in the formation of giant multinuclear cells (syncytia) [(1986) Nature 322, 470–474; (1986) Nature 322, 725–728; (1985) Hum. Pathol. 18, 760–765; (1987) Ann. Neurol. 21, 490–496]. The appearance of syncytia is associated with an increase in the monounsaturated oleic acid content. This report describes experiments which compare the activity of known antiviral agents with that of saturated fatty acid derivatives in inhibiting oleic acid and syncytia formation. A concept is introduced which proposes that infection of cells with the human immunodeficiency virus causes a rise in cellular oleic acid which leads to increased membrane fluidity.

Antiviral therapy; Fatty acid; HIV; (Syncytia)

1. INTRODUCTION

A range of cytopathic effects (CPE) are induced by viruses in vitro. The two most common types of CPE observed are those caused by the 'lytic' viruses which produce a rounding and detachment of adherent cells from the culture substratum; and those viruses described as 'fusogenic' which induce syncytia formation. These phenomena usually precede cell death. These effects are associated with significant changes in stearic (C18:0; m.p. 69°C) and oleic acid (C18:1; m.p. 13.9°C) acid metabolism of the host cell [5–8]. In mammalian cells the balance between these two fatty acids is controlled by the enzyme complex Δ-9-desaturase whereby the monounsaturated oleic acid is produced by desaturation of the stearic acid [9]. The stearic to oleic acid ratio is designated as the saturation index (SI) [10]. This index decreases in cells infected with fusogenic viruses (i.e. a rise in the oleic acid content is observed) and increases with lytic viruses [5–8]. The data show that AZT, α-interferon and dihydroxy stearic acid reduce the desaturation of stearic acid in cells infected with HIV when compared with untreated infected controls. The known inhibitory effect of AZT [11] and å-interferon [12] on HIV replication supports the concept that unrestrained HIV infection in tissue culture disturbs the stearic/oleic balance of cell membranes, an effect which may play a part in fusion and cell death.

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2. METHODS

2.1. *Lipid extraction*

Lipids were extracted by a modification of the Folch method [13]. Briefly free fatty acids were liberated by alkali saponification with 15% (w/v) methanolic potassium hydroxide and followed by acidification with 4 M hydrochloric acid; methyl esters were produced by reaction with freshly prepared diazomethane. Fatty acid methyl esters (FAMEs) were resolved by gas-liquid chromatography on a 2 m × 2 mm i.d. silanized glass column packed with 3% SP-2310/2% SP-2300 on chromosorb WAW (Supelco Chromatography Supplies). A temperature programme of 160°C to 260°C at 4°C · min^{-1}, with nitrogen at 20 ml · min^{-1} as the carrier gas, was used. Detection

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was by flame ionisation and individual FAMEs were identified and quantitated by comparison of retention times and co-chromatography, with authenticated FAME standards (Sigma Chemical Co. Ltd).

2.2. *Virus culture*

HIV 1 (HTLV III RF) was grown in H9 cells with RPMI supplemented with 10% fetal calf serum (FCS). Cell debris was removed by low speed centrifugation and the virus was stored at −70°C until required. C8166 lymphoid cells were infected with $10TCID_{50}$ HTLVIII (RF) for 1.5 h. For each kinetic experiment approximately 10^7 cells in 15 ml of medium were cultured in the presence of either 10 μM azido-thymidine (AZT), Roche Products UK Ltd; 10^4 U/ml α-interferon (α-IFN), Wellcome; or 100 μM dihydroxy stearic acid (DHSA), Cadburys for 48 h. Infected and uninfected cells were included as controls. At 18, 24 and 40 h, 10^6 cells (triplicate samples) were removed from each culture, washed three times in phosphate buffered saline (PBSA) and the cell pellet stored at −20°C until analysed. These experiments were carried out on three different occasions.

2.3. *HIV antigen detection*

To assay for anti-HIV activity of DHSA, C8166 cells were incubated with $10TCID_{50}$ HTLV III(RF) at 37°C for 1.5 h. The cells were washed with PBSA three times and 2×10^5 cell aliquots were resuspended in 1.5 ml growth medium in the presence of 25, 50 and 100 μM DHSA and incubated at 37°C for 72 h in a 5% CO_2/95% air atmosphere. An antigen capture ELISA [14] was used to measure HIV in the supernatants taken from the cultures using the steps described by the manufacturers (Organon Teknica). Uninfected and infected untreated controls were included.

3. RESULTS AND DISCUSSION

Table 1 shows the changes in fatty acid composition of the C8166 cells at various times after infection with HIV-1. The combined values of stearic and oleic acids (SUM) constitute 60% of the total resolvable fatty acids: this value remains constant throughout the time course of the experiment but the increase in the unsaturated oleic acid (18:1) relative to stearic acid (18:0) rises by up to 50%. All the other fatty acid components show no significant change during the same time course. In fig.1a the changes in the proportion of these two fatty acids in cells infected with HIV are presented as the SI; the depression in the SI coincides with the appearance of syncytia at about 40 h post-

Table 1

Profile for the fatty acids present in infected C8166 CD4+ cells at various times post-infection

Time (h)	16:0	16:1	18:0	18:1	Sum	18:2	18:3	20.4	Other
0	26	0.6	23.4	31.1	54.4	2.3	9.5	3.7	3.4
18	19.5	2.7	22.1	36.2	58	1.8	1.4	0	16.3
24	17	1.3	16.2	46.2	62	5.7	0.6	1.9	11.1
40	17.2	2.4	13.3	45.9	59.2	4.9	1.5	1.6	13.2
0	15.1	1.7	31.5	32.8	64.3	5.2	1.7	6.1	5.9
18	26.3	1.7	30.1	30.4	60.5	3.6	0.9	3.6	3.4
24	15.3	0.8	25.3	38.8	64.1	5.1	1.1	2.2	11.4
40	15	1.4	6.1	55.4	61.5	4.8	0.5	7.5	9.3
0	21.8	1.7	25.4	33.2	58.6	2.2	0.7	7.9	7.2
18	14	4.7	18.2	32.1	50.3	4.8	2.7	12.1	11.3
24	15.6	2.8	17.3	38.9	56.2	4.8	1.7	8.9	9.8
40	21.1	2.7	13.2	41.4	54.6	4.3	1.8	7.6	7.4
X	18.66	2.04	20.18	38.53	58.64	4.13	2.01	5.25	9.14
SD	4.29	1.11	7.18	7.59	4.21	1.32	2.44	3.63	3.87

Three separate experiments are represented. The mean (X) and the standard deviation (SD) for stearic plus oleic acids (SUM: 18:0 + 18:1) is 58.64 ± 4.21, whereas the values for stearic and oleic acids are 20.18 ± 7.18 and 38.53 ± 7.59, respectively. The % fatty acid composition was calculated for all of the 58 samples analysed in this series of experiments (including the values for infected cells treated with AZT, DHSA and α-IFN). The mean for stearic acid plus oleic acid for all 58 samples is 59.45 ± 4.13. These values are the same as those obtained for stearic plus oleic acids (SUM) from infected cells which are listed in this table

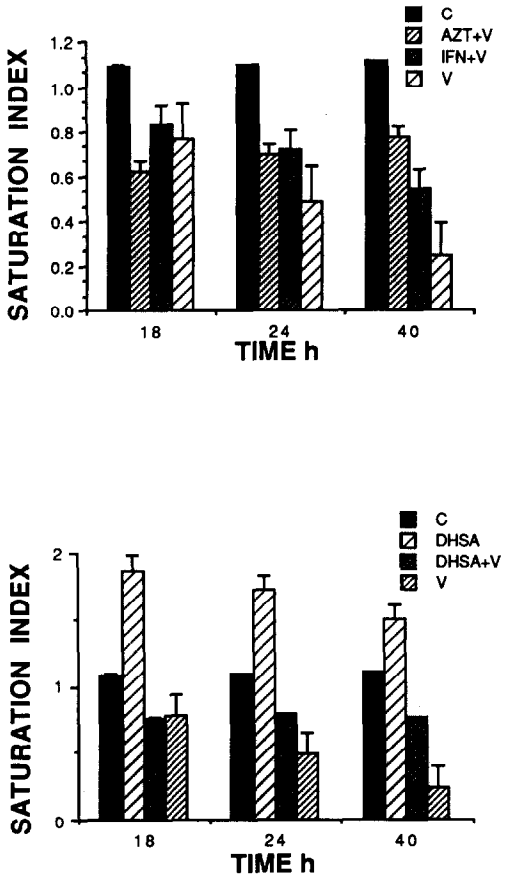

Fig.1. These show the change in saturation index of HIV infected C8166 cells in the presence of AZT (a), α-IFN (a) and DHSA (b). C, uninfected cells; AZT + V, HIV-infected cells treated with AZT; IFN + V, HIV-infected cells treated with α-IFN; DHSA, uninfected cells treated with DHSA; DHSA + V, infected cells treated with DHSA; V, HIV-infected cells.

infection and the SI remains at a low value (0.25). This result indicates that the increase in oleic acid with the presumed increase in membrane fluidity is associated with the fusogenic effect of HIV. This concept is supported by the data for the antiviral agents [11] AZT and α-IFN [12] (fig.1a): both compounds when added to infected cultures reduce the extent of desaturation of stearic acid and syncytia formation. The SI of the uninfected cells (1.1) does not fall during the same period (fig.1a) and no changes in cell morphology are observed. These changes in the membrane composition could explain the recent observation that bile salts have a selective effect on HIV-infected cells [15].

Saturated fatty acids, which are known to decrease membrane fluidity [16], were added to the culture media of infected cells. Stearic acid and its derivatives iodostearic, epoxystearic, sterculic and DHSA all showed a degree of activity in reducing syncytia formation. DHSA was chosen for a detailed study because it was found to be the most active compound. The addition of 100 μM DHSA to the culture media of infected cells reduced desaturation (fig.1b) and inhibited syncytia formation when compared to the infected untreated controls. Moreover, when DHSA was added to uninfected cells a rise in the saturation index (>1.6) was observed (fig.1b). Thus the addition of DHSA to both uninfected and infected cultures produces a rise in the SI when compared to both untreated controls.

The inhibitory effect of DHSA on HIV antigen production was measured in a standard assay [14] on five occasions (data not shown); at 100 μM there is a 78% inhibition. At this concentration DHSA had a slight cytostatic effect; however this effect disappeared when fresh media lacking DHSA was added to the cells. At 50 μM DHSA, a 30% inhibition of HIV antigen production was measured and no toxicity was observed.

These data show that HIV infection of C8166 cells produces a marked desaturation of cellular stearic acid into oleic acid which coincides with the appearance of syncytia. This process can be inhibited by AZT, α-IFN and DHSA, although the mode of action of each compound will be different: AZT and α-IFN are likely to have an effect on HIV replication whereas DHSA would appear to act on the infected cell membranes. HIV infection is known to be linked with changes in phospholipid metabolism [17]; further, increases in cell permeability and membrane fluidity occur in plasma membranes of neuroblastoma cells which have increased levels of oleic acid [18]. Thus HIV cytopathology may be dependent not only on the interaction between the gp120 surface glycoprotein [1,2] and the CD4 receptor molecules [19,20] but on changes in membrane fluidity and perhaps other, as of yet, unidentified factors [21].

In conclusion, it is proposed that the formation of syncytia is at least a two stage process. Firstly, HIV glycoproteins and cell receptors form a bridge between adjacent cell membranes; secondly, cell fusion is facilitated by an increase in membrane

fluidity due to the increase in cellular oleic acid. Cell death may be caused by the loss of plasma membrane integrity due to excessive stearic acid desaturation into oleic acid. Finally, it is postulated that HIV after infection of its target cell enhances the activity of the Δ-9-desaturase enzyme complex by an unknown mechanism. Inhibitors of this enzyme could prove to be useful in preventing cell damage caused by HIV infection [22].

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REFERENCES

[1] Sodroski, J., Chun Goh, W., Rosen, C., Campbell, K. and Haseltine, W.A. (1986) Nature 322, 470–474.
[2] Lifson, J.D., Feinberg, M.B., Reyes, G.R., Rabin, L., Banapour, B., Charkrabarti, B., Wong-Stahl, S., Steimer, K.S. and Engelman, E.G. (1986) Nature 322, 725–728.
[3] Sharer, L.R., Eun-Sock, C. and Epstein, L.G. (1985) Hum. Pathol. 18, 760–765.
[4] Pumarola-Sune, T., Navia, B.A., Cordon-Cardo, C., Cho, E.S. and Price, R.W. (1987) Ann. Neurol. 21, 490–496.
[5] Blenkharn, J.I. and Apostolov, K. (1981) J. Gen. Virol. 52, 355–358.
[6] Nozawa, C.M. and Apostolov, K. (1982) Virology 120, 247–250.
[7] Nozawa, C.M. and Apostolov, K. (1982) J. Gen. Virol. 59, 219–222.
[8] Barker, W.R. and Apostolov, K. (1989) Intervirology, in preparation.
[9] Jeffcoat, R. and James, R.T. (1984) in: New Comprehensive Biochemistry 7 (Numa, S. ed.) pp.85–112, Elsevier, Amsterdam.
[10] Apostolov, K. and Barker, W. (1981) FEBS Lett. 126, 261–264.
[11] Mitsuya, H., Weinhold, K.J., Furman, P.J. et al. (1985) Proc. Natl. Acad. Sci. USA 82, 7096–7100.
[12] Ho, D.D., Hartshorn, K.L., Rota, T.R., Andrews, C.A., Kaplan, J.C. and Schooley, R.T. (1985) Lancet i, 602–604.
[13] Folch, J., Lees, M. and Sloane-Stanley (1957) J. Biol. Chem. 226, 497–509.
[14] Kinchington, D., Galpin, S.A., O'Connor, T., Jeffries, D.J. and Williamson, J.D. (1989) AIDS 3, 101–104.
[15] Lloyd, G., Atkinson, T. and Sutton, P.M. (1988) Lancet i, 1418–1421.
[16] Chapman, D. and Quinn, P.J. (1976) Proc. Natl. Acad. Sci. USA 73, 3971–3975.
[17] Lynn, W.S., Tweedale, A. and Cloyd, W.M. (1988) Virology 163, 43–51.
[18] Boonstra, J., Nelemans, O., Feijen, A., Bierman, A., Everardus, J.J., Zoelen, V., Van der Saag, P.T. and De Laat, S.W. (1982) Biochim. Biophys. Acta 692, 321–329.
[19] Dalgleish, A.G., Beverly, P.C.L., Clapham, P.R., Crawford, D.H., Greaves, M.R. and Weiss, R.A. (1984) Nature 312, 763–766.
[20] Klatzmann, D., Champagne, S., Chamaret, J., Gruest, D., Guetard, T., Hercend, J., Gluckman, C. and Montagnier, L. (1984) Nature 312, 767–768.
[21] Somasundaran, M. and Robinson, H.L. (1987) J. Virol. 61, 3114–3119.
[22] Apostolov, K., Barker, W.R., Galpin, S., Jeffries, D.J., Wood, C.B., Habib, N.A., Williamson, R.C.N., Gidley-Baird, A. and Kinchington, D. (1988) Proceedings of the IV International Conference on AIDS, Stockholm, vol. 2, p.148, Abstr. 3528.