

THE EFFECTS OF INTERFERON ON THE FATTY ACIDS IN UNINFECTED CELLS

K. APOSTOLOV and W. BARKER

Department of Virology, Royal Postgraduate Medical School, Hammersmith Hospital, London W12 0HS, England

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1. Introduction

The main biological activities of interferon are the capacity to confer on cells resistance to virus infection, and the inhibition of growth of malignant cells *in vivo* and *in vitro* [1–4]. In addition, a variety of other effects have been described [1]. We were attracted to the subject by a report describing an increase in the buoyant density of the plasma membrane of mouse L cells after 18 h treatment with mouse interferon [5]. We have found that the fusogenic properties of Newcastle disease virus (NDV) and Sendai virus are selectively inhibited by treatment with free iodine [6]. The fusogenic property of these viruses is their capacity to induce haemolysis and fusion of cells, and includes virus entry (infection) by fusion of the virus envelope and the cell membrane [7]. We went on to show that there was a direct correlation between the inhibition of haemolysis and the degree of saturation of the fatty acids of the virus by iodine [8]. We concluded that the fusogenic properties of these viruses are dependent on the fluidity of the virus membrane. The saturation of fatty acids decreases the fluidity and mobility of the biological membranes [9]. This concept was also supported by the finding that virulent strains of NDV, which are more fusogenic than the avirulent ones, induce a relative increase in unsaturated fatty acids (UFA) in infected cells as well as in the virions released [10]. Here, we show that interferons are involved in the metabolism of the fatty acids of infected cells, especially in the modification of the degree of saturation of the 18 carbon fatty acids (C18 FA).

2. Materials and methods

Human fibroblasts (MRC5) were obtained from Porton Down and used at the 27th passage. Primary

bovine kidney cells were kindly supplied by Mr D. Luther, ARC Institute for Research on Animal Diseases, Compton, Newbury, Berks.

The cells were grown at 37°C on medium 199 (Flow Labs.), supplemented with 7.5% fetal calf serum and 2 mM L-glutamine and containing antibiotics penicillin and streptomycin at 100 IU and µg/ml. Experiments were performed in 25 cm² tissue culture flasks with the cells at 75% confluence.

Human leucocyte interferon (Hu IFN-α (Ly)), and quantitated antiserum to this interferon, was kindly supplied by Dr C. Fantes, Wellcome Research Laboratories, Beckenham, Kent. Human fibroblast interferon (Hu IFN-β), was kindly supplied by Dr T. Cartwright, Searle Laboratories, High Wycombe, Bucks. Interferons were stored frozen at –20°C in PBS containing 1% serum albumen.

Time-dependent kinetic and dose–response experiments: The growth medium was removed and the cell sheet washed with PBS. Cells were incubated for 1 h at 37°C overlaid with serum-free medium containing the relevant concentration of interferon. Unadsorbed interferon was washed away after this time and the cells incubated at 37°C for the required time, overlaid with serum-free medium. Harvesting of the cells was accomplished by freezing the flasks at –20°C and the cells stored at this temperature until required for lipid extraction.

Antibody neutralisation tests: interferon was incubated with a 10 × excess of antibody at room temperature for 2 h. After this time the equivalent of 1000 units/ml interferon of this mixture, in serum-free medium, was applied to the cells for 1 h (100 units/ml in the case of Hu IFN-α on PBKC). The cell sheet was then washed and the medium replaced with serum-free medium for a subsequent 12 h incubation, when the cells were frozen for fatty acid extraction.

Interferon controls were similarly set up, without pretreatment of the interferon, and antibody controls, without interferon.

Lipid extraction and fatty acid identification: cells were disrupted by 3 cycles of freezing and thawing. The total lipids were extracted and the fatty acid methyl esters obtained as in [8].

The fatty acid methyl esters were separated by gas-liquid chromatography (GLC) on 3% OV1 on 100/120 Gas Chrom. Q. Matched 1.4 m \times 4 mm i.d. glass columns (Pye Unicam) were used with a temperature programme comprising a 4 min isothermal period at 160°C followed by a programmed rise of 4°C/min to 240°C on a Pye Unicam series 204 chromatograph. Nitrogen was used as the carrier gas at a flow rate of 30 ml/min and detection of the fatty acids was by flame ionisation. Identification of the fatty acids was by comparison of retention times and co-chromatography with authentic fatty acid methyl esters (Appl. Sci. Labs., Sigma).

3. Results and discussion

The human leucocyte (Hu INF- α (Le)), lymphoblast (Hu INF- α (Ly)), and fibroblast (Hu INF- β) interferons were used for treatment of human fibroblast (MRC-5) cells and also primary bovine kidney cells (PBKC) [11]. Chick chorioallantoic interferon induced by Sendai virus was used for treatment of chick fibroblasts. After extraction of the interferon-treated cell and GLC separation of the fatty acids, we chose the ratio of the peak heights of the 18 carbon saturated fatty acid (C18s) to the 18 carbon unsaturated fatty acids (C18u) as a marker for the effects of the interferons. This ratio was used because the changes in the 18 carbons were consistent and reproducible, in contrast to the other major group, the 16 carbon fatty acids. In addition, the 18 carbon fatty acids represent >60% of all the fatty acids extractable from the cell. Moreover, changes in saturation of FA are reflected in abrupt changes of melting points, with a consequent change of buoyant density and fluidity in the constituent lipids. In the GLC programme which we are using the unsaturated C18 FA are grouped in one peak and stearic acid in another.

In fig.1 we show the time-dependent kinetics of the C18s:C18u ratio in human fibroblast and PBKC after treatment with human interferons. There is a marked relative increase in stearic acid (C18s), rising

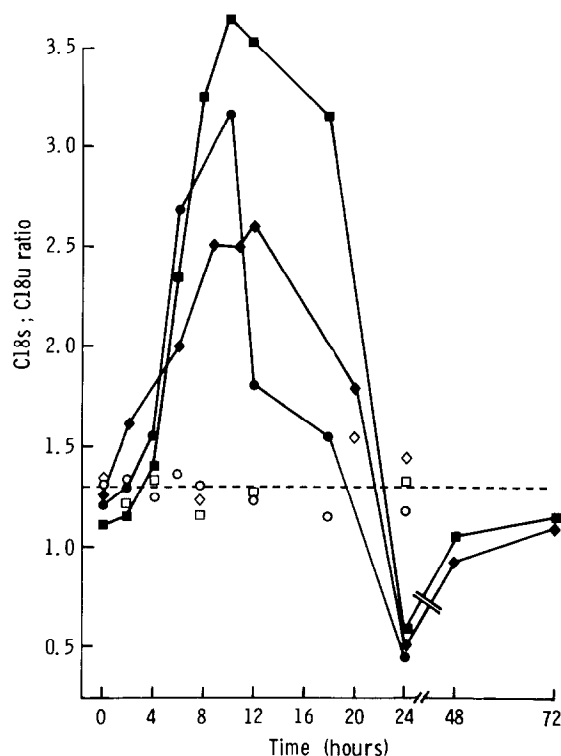


Fig.1. Kinetics of the effect of interferon on the C18s:C18u ratio in MRC5 and PBKC cells: MRC5 cells treated with 1000 units/ml Hu INF- α (Ly) (■); PBKC treated with 100 units/ml Hu INF- α (Ly) (●); MRC5 cells treated with 100 units/ml Hu INF- β (◆). Open symbols represent untreated controls and the broken line represents the control levels.

to a maximum at 10–12 h, followed by a return to control values at 20–22 h. However, there is a subsequent relative increase in the unsaturated C18 fatty acids, reaching a maximum at 24–30 h with a gradual return to control values by 72 h. This effect was achieved with (Hu INF) concentrations >100 units/ml in human fibroblasts, and in primary bovine kidney cells with Hu INF- α at >10 units/ml but not with Hu INF- β . The higher sensitivity of PBKC to Hu INF- α and the lack of effect with Hu INF- β in PBKC, are in agreement with the results on the higher sensitivity of PBKC to Hu INF- α as demonstrated by antiviral activity [12]. Incubating the cells in the continued presence of interferon, rather than washing away unadsorbed material after 1 h (not shown), produced similar results to those described above, indicating that it is the initial interferon concentration present which determines the reaction of the cell, rather than

Table 1

Antibody neutralisation: the effect on the C18s:C18u ratio of Hu IFN- α and Hu IFN- β on MRC5 cells and PBKC, together with the effect of pre-treatment of these interferons with Hu IFN- α antiserum before application to the cell

	MRC5						PBKC					
	Control		Hu IFN- α (Ly)		Hu IFN- β		Control		Hu IFN- α (Ly)		Hu IFN- β	
	-Ab	+Ab	-Ab	+Ab	-Ab	+Ab	-Ab	+Ab	-Ab	+Ab	-Ab	+Ab
C18s:C18u	1.23	1.30	3.21	1.28	2.63	2.03	1.10	1.07	2.98	1.15	1.07	1.12
% Inhibition	—	—	—	97.5	—	42.9	—	—	—	97.3	—	0

the continued action of interferon. Using crude chick interferon on chick fibroblasts a similar kinetic response in the C18s:C18u ratio was obtained (not shown), but the changes were smaller.

The specific nature of the effects of interferons on the C18s:C18u ratio is also shown by the studies on inhibition by antibody (table 1). Antibody to Hu IFN- α (Ly) inhibited almost completely the effects of Hu IFN- α (Ly) but only partially the effects of Hu IFN- β . These findings are in agreement with the studies in which similar results were obtained using virus inhibition as an interferon marker [11–14].

The dose-dependent response effects of interferon on the C18s:C18u are shown in fig.2. After treatment for 12 h the C18s:C18u ratio increases with the higher concentration of interferon. However, as expected, at 24 h the depression in the ratio is bigger with higher doses. A similar dose-dependent response was obtained with Hu IFN- β on MRC5 cells. It is interesting to note that the peak ratios of C16 fatty acids were not changed and did not follow the changes in the C18, which raises the possibility that interferon might act on the C18 FA only.

These results suggest that one effect of interferon on the metabolism of the cell is the modification of the saturation of the fatty acids. The increase in saturation of the fatty acids would lead to integration into the cell membranes of lipids with higher buoyant densities. Mouse L cells, treated with mouse interferon for 18 h show an increase in the buoyant density of plasma membranes, with no change in the membrane mass [5]. An increase in the C18s leads to a decrease of membrane fluidity, but conversely an increase in C18 u leads to a greater membrane fluidity [8,9]. Some of the biological activities of interferon could be explained in terms of the increase and decrease in C18s:C18u ratio. Reduced membrane fluidity could result in inhibition of cell division, fail-

ure of virus maturation and release of enveloped viruses [14–16] and also inhibition of virus replication by inhibition of enzymatic activity [17].

The rebound effect, the relative increase in C18u beyond the control values (fig.1) with higher concentrations of interferon, could explain the phenomenon of refractory effect to interferon [18–21]. Higher concentrations of interferons induced in tissue cul-

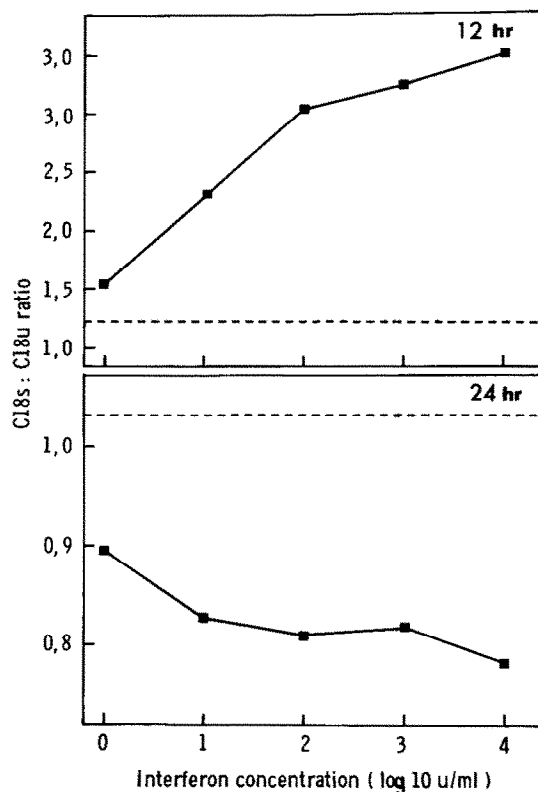


Fig.2. Dose-dependent C18s:C18u ratio after treatment for 12 h and 24 h. PBKC treated with Hu IFN- α (Ly). The broken lines represent the untreated control cell values.

ture a state of refractoriness to further production of interferon at times coincidental with the depression in the C18s:C18u ratio [19]. Also, a coincidental decreased inhibition of virus replication has been described [19]. It is interesting that the response of macrophages to interferon was reported to the biphasic. The initial inhibition of phagocytic activity of 12–18 h was followed by enhancement after 24 h [22].

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