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Effect of fatty acids on arenavirus replication: inhibition of virus production by lauric acid

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Summary. To study the functional involvement of cellular membrane properties on arenavirus infection, saturated fatty acids of variable chain length (C10–C18) were evaluated for their inhibitory activity against the multiplication of Junin virus (JUNV). The most active inhibitor was lauric acid (C12), which reduced virus yields of several attenuated and pathogenic strains of JUNV in a dose dependent manner, without affecting cell viability. Fatty acids with shorter or longer chain length had a reduced or negligible anti-JUNV activity. Lauric acid did not inactivate virion infectivity neither interacted with the cell to induce a state refractory to virus infection. From mechanistic studies, it can be concluded that lauric acid inhibited a late maturation stage in the replicative cycle of JUNV. Viral protein synthesis was not affected by the compound, but the expression of glycoproteins in the plasma membrane was diminished. A direct correlation between the inhibition of JUNV production and the stimulation of triacylglycerol cell content was demonstrated, and both lauric-acid induced effects were dependent on the continued presence of the fatty acid. Thus, the decreased insertion of viral glycoproteins into the plasma membrane, apparently due to the increased incorporation of triacylglycerols, seems to cause an inhibition of JUNV maturation and release.

Introduction

The replicative cycle of enveloped viruses is closely dependent on the characteristics of the host cell membrane, particularly it is relevant at the viral entry, which comprises fusion processes, and at the assembly and budding stages, when the virion acquires the lipid envelope from the host cell. Thus, it is conceivable that alterations in the fluidity and/or the permeability of the plasma membrane may affect the infection with enveloped viruses. On this basis, several agents

disturbing the lipid composition of the cell membrane have been proposed as potential antiviral compounds [22]. Because fatty acids have a prominent role in the lipid bilayer of the cell membrane as components of phospholipids, glycolipids and triacylglycerols, the effect of saturated and unsaturated fatty acids on the replication of various viruses, including herpes simplex virus, human immunodeficiency virus, visna virus, vesicular stomatitis virus, vaccinia virus, Sendai and Sindbis viruses, has been reported. A dual mode of action has been shown for these compounds, either inducing a blockade in the viral cycle or leading to inactivation of the virus particle [3, 14–16, 30].

Junin virus (JUNV) is a member of *Arenaviridae* able to cause a severe disease in humans known as Argentine hemorrhagic fever (AHF) [32]. Virions consist of a bisegmented ambisense RNA genome surrounded by an helical nucleocapsid and enclosed in an envelope derived from the infected cells. After adsorption to the cell surface, JUNV internalizes by an endocytic mechanism mediated by a low-pH-induced fusogenic activity of a viral glycoprotein [4], and then the replicative cycle proceeds until progeny viruses are released by budding at the plasma membrane. The glycoprotein presence at the cell surface is required for maturation of infectious JUNV particles. Previous reports have shown that the optimal expression of JUNV glycoproteins in the cell membrane requires the cleavage of the glycoprotein precursor GPC into GP1 and GP2 at the trans-Golgi network [8]. The acquisition of a complex form of the envelope glycoprotein oligosaccharide chains is not essential for JUNV infectivity [28], whereas protein myristoylation is relevant in the interaction of glycoproteins with membranes during virus maturation [7]. To further determine the functional involvement of cellular membrane properties on JUNV infection, and to establish whether it is a possible approach to antiviral chemotherapy, the influence of alterations in the lipid composition of the host cell has been studied, analyzing the effect of fatty acids on JUNV replication.

Materials and methods

Chemicals and antibodies

Fatty acids were purchased from Sigma Chemical Co. (USA) and prepared as 1% solution in ethanol. Rabbit polyclonal anti-JV serum was prepared in our laboratory [28] and the monoclonal antibody GB03-BE08 reactive to JUNV glycoprotein [24] was kindly provided by Dr. A. Sanchez (Centers for Disease Control, Atlanta, USA). Fluorescein labelled goat anti-mouse IgG and anti-rabbit IgG were from Sigma Chemical Co. EXPRE^{35}S^{35}S (sp.activity 1175 Ci/mmol) was purchased from New England Nuclear (USA).

Cells and viruses

Vero and CV1 cells were grown as monolayers in Eagle's minimum essential medium (MEM) (GIBCO, USA) supplemented with 5% inactivated calf serum. The human diploid foreskin fibroblast cell line PH was provided by Dr. G. Carballal (CEMIC, Buenos Aires, Argentina) and propagated in MEM supplemented with 10% fetal calf serum. For maintenance medium (MM) the serum concentration was reduced to 1.5% for Vero and CV1 cells, and to 5% for PH cells.

The following arenaviruses were used: the naturally attenuated IV4454 strain of JUNV obtained from a mild human case [6], the prototype pathogenic XJ strain of JUNV isolated from a severe human case of AHF [21], its avirulent derivative XJC13 [11], the host-range mutant of JUNV named C167 attenuated for newborn mice [25], and the TRLV 11573 strain of Tacaribe virus (TACV) [10]. Virus stocks were prepared and titrated by plaque formation in Vero cell cultures.

Cell viability assay

Cellular viability was measured with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method [18] in confluent cell cultures grown in 96-well plates and exposed during 48 h at 37 °C to two-fold dilutions of the compounds, with three wells for each dilution. The cytotoxic concentration 50% (CC_{50}) was calculated as the compound concentration required to reduce the MTT signal by 50% compared with controls.

Virus yield inhibition assay

Cells grown in 24-well plates were infected at a multiplicity of infection of 0.1, and after 1 h adsorption cells were washed and refed with MM containing various concentrations of the compounds. Two replicates per dilution of each compound were tested. After 24 h of incubation at 37 °C, supernatant cultures were harvested and extracellular virus yields were determined by plaque assay. The inhibitory concentration 50% (IC_{50}) was calculated as the drug concentration that reduced virus yield by 50% in the compound-treated cultures compared with the untreated ones. To determine cell-associated virus, the cells remaining after harvesting supernatant cultures were washed, frozen and thawed twice, centrifuged at 1000xg and the supernatant was assayed by plaque formation.

Time of addition or removal experiments

Vero cells grown in 24-well plates were allowed to adsorb JUNV strain IV4454 at a moi of 0.1 for 60 min at 4 °C. After removal of the inocula, the cells were washed twice with PBS and then MM containing 250 μM lauric acid was added to infected cells at various times after infection and further incubated at 37 °C. Another set of infected cultures were incubated with MM containing compound immediately after adsorption and the drug was removed by medium change at 2, 4 and 6 h p.i. In all cases, extracellular virus yields were measured at 24 h postinfection.

Direct action of lauric acid on cells and virions

To assay virucidal activity, a virus suspension containing 1×10^6 PFU of JUNV was incubated with an equal volume of MM with or without 250 μM fatty acid for 2 h at 37 °C. Then mixtures were chilled, diluted 1:100, and inoculated to Vero cells. To determine the ability of lauric acid to induce a cell refractory state, Vero cells were incubated with 250 μM lauric acid or MM for 2 h at 37 °C and were then washed extensively with PBS prior to inoculation with JUNV. After 24 h of infection, virus yields were titrated by plaque formation.

Indirect immunofluorescence

Vero cells grown on glass coverslips were infected with JUNV at a moi of 0.1 and 250 μM lauric acid was added to MM after adsorption. For cytoplasmic staining, cells were washed with PBS at 24 h p.i. and fixed with methanol for 15 min at −20 °C. Then, cells were washed with PBS and reacted with immunoglobulins purified from hyperimmune rabbit serum reactive against all JUNV proteins, followed by an incubation with a fluorescein-conjugated

goat anti-rabbit IgG. For membrane immunofluorescence, cells were first incubated with the monoclonal antibody GB03-BE08 reactive against GP1 and its precursor GPC, then fixed with 4% formaldehyde (freshly prepared from paraformaldehyde) and incubated with fluorescein-labelled goat anti-mouse IgG. After a final washing with PBS, the cells were mounted in a glycerol solution containing 1,4-diazabicyclo[2.2.2] octane (DABCO). The percentage of fluorescent cells in each preparation was calculated from 20 randomly selected microscope fields.

Protein radiolabelling, immunoprecipitation and gel electrophoresis

Vero cells were infected with JUNV at a moi of 1. At 44 h p.i. infected cells were incubated in methionine-cysteine-free medium for 1.5 h and then labelled with 100 μCi/ml EXPRE^{35}S^{35}S for 3.5 h. Lauric acid (250 μM) was added with the methionine-cysteine-free medium and maintained during the labelling period. After labelling, cells were washed three times in cold PBS and lysed in radioimmunoprecipitation assay (RIPA) buffer consisting of 0.15 M NaCl, 0.1% SDS, 1% Triton X-100, 0.4 mM phenylmethylsulphonylfluoride and 1% sodium deoxycholate in 0.01 M Tris-HCl pH 7.4. Samples of the clarified cell lysates were mixed with polyclonal rabbit anti-JUNV serum and incubated for 30 min at 37 °C and 90 min at 4 °C. Antibody-antigen complexes were collected with protein A-sepharose, washed three times in RIPA buffer and solubilized by boiling for 2 min in SDS-PAGE sample buffer. Viral polypeptides were then electrophoresed on 12% SDS-polyacrylamide gels. The protein bands on the gels were visualized by fluorography and exposition on AGFA-Gevaert Curix X-ray film.

Quantification of triacylglycerols

Fatty acid-treated and untreated cells were washed twice with cold PBS, scraped from the dishes and collected by centrifugation. Total lipids were extracted in a mixture of methanol, chloroform, and water in the proportions 1.2:0.6:0.2. The lipids were separated from the water-soluble material by diluting the extraction mixture with 1 volume of chloroform followed by 0.7 volume of water. After centrifugation, the chloroform layer was removed and dried. Lipids were resuspended in isopropanol and triacylglycerols were determined with an enzymatic assay kit (TG Color GPO/PAP AA, Wiener Lab., Argentina).

Results

Saturated fatty acids of variable chain length (C10–C18) were evaluated for their inhibitory activity against the multiplication of JUNV strain IV4454 in Vero cells. As can be seen in Fig. 1, virus replication was inhibited in a concentration-dependent manner by the monocarboxylic acids, but with a variable degree of efficacy according to the chain length. The most active inhibitor was the medium chain length lauric acid (C12), which reduced the JUNV progeny released into the medium from Vero cells by more than two log units at concentrations higher than 250 μM (Fig. 1C). Treatment of infected cells with monocarboxylic acids with shorter or longer chain length was less effective. JUNV yields after 24 h of treatment with capric (C10), myristic (C14), palmitic (C16) and stearic (C18) acid were reduced only in the range 0.1–0.5 log units at the maximum concentrations tested (Fig. 1A, B).

Simultaneously, the lack of toxicity of the five compounds for Vero cells was investigated by assessing their effects on cell viability by the MTT method.

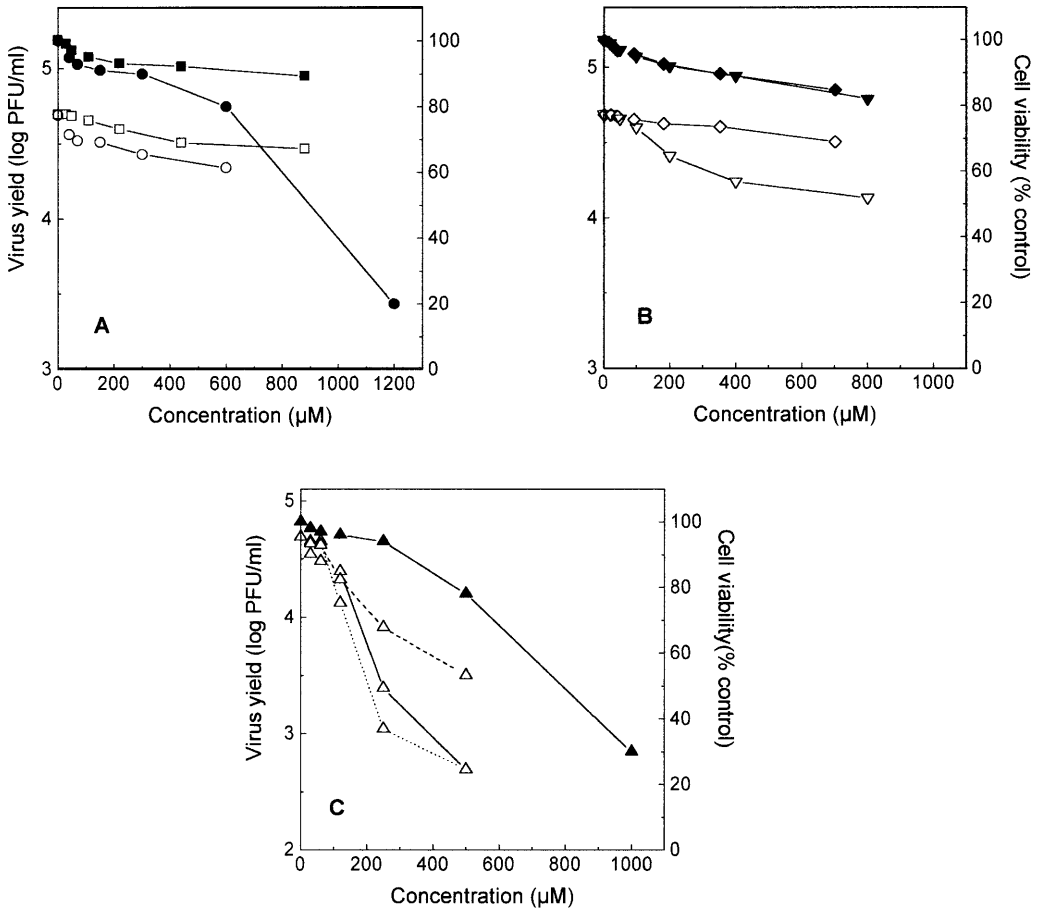

Fig. 1. Dose-response curves of the effect of saturated fatty acids on JUNV multiplication and cell viability. Cells were incubated for 48 h in the presence of different concentrations of the fatty acids and then cell viability was determined by the MTT method (solid symbols). Other set of cultures were infected with JUNV strain IV4454 (m:0.1) and after 24 h of infection in the presence of the compounds, extracellular virus yields were determined (open symbols). **A** Capric acid (●, ○), myristic acid (■, □) in Vero cells. **B** Palmitic acid (▼, ▽), stearic acid (◆, ◇) in Vero cells. **C** Lauric acid (▲, △) in Vero cells (——), PH cells (- - - -) and CV-1 cells (. . .). Each point is the mean value of duplicate determinations

No significant cellular alterations were observed in the range of concentrations assayed and the cell viability was greater than 80% for all the fatty acids (Fig. 1) with the only exception of capric and lauric acid at the highest concentration (1200 μM and 1000 μM for C10 and C12, respectively).

From data in Fig. 1 the CC_{50} and IC_{50} values were calculated for each compound. Furthermore, the inhibitory action of fatty acids on JUNV multiplication was evaluated with several virus strains, including attenuated isolates such as the above mentioned IV4454 and the XJC13 and C167 strains as well as the highly pathogenic XJ strain. Results obtained confirmed the effectiveness of lauric acid

Table 1. Spectrum of antiviral action of fatty acids against arenaviruses

Compound	$CC_{50}(\mu M)$[a]	$IC_{50}(\mu M)$[b]				
		JUNV				TACV
		IV4454	XJ	XJCl3	Cl67	
Capric acid	900	423	ND	>580	ND	>580
Lauric acid	792	124	188	129	46	67
Myristic acid	>876	>876	263	>876	>876	630
Palmitic acid	>780	218	105	186	>780	712
Stearic acid	>703	>703	ND	>703	ND	>703

[a]Compound concentration required to reduce cell viability by 50%, as determined by MTT assay

[b]Compound concentration required to inhibit virus yield by 50%

ND Not determined

as JUNV inhibitor and also other arenavirus, the antigenically related TACV was susceptible to this compound (Table 1).

The inhibitory effect of lauric acid against arenaviruses was not confined to one cell type. Other cell lines different from Vero cells, such as the monkey kidney line CV1 and the human foreskin cell line PH showed a similar level of sensitivity to the antiviral action of lauric acid against JUNV (Fig. 1C), with IC_{50} values of 106.3 and 112.3 μM in virus yield inhibition assays in CV1 and PH cells, respectively. Furthermore, the production of cell associated virus was as sensitive to lauric acid as extracellular virus formation (data not shown).

To test the possibility that the compound acted directly either on the virus particles as inactivating agent or on the cells to be infected by inducing a state refractory to virus infection, separated treatment was investigated. Preincubation of JUNV with lauric acid during 2 h prior to infection had no significative effect on the virus yield (Table 2). In fact, when the suspension of JUNV was directly titrated immediately after treatment with lauric acid no reduction in remaining infectivity respect to the untreated control was detected (data not shown). When Vero cells were pretreated with lauric acid for 2 h and subsequently infected with JUNV, the yield of infectious virus after 24 h of incubation in compound-free MM was not affected (Table 2). By contrast, after 24 h in the simultaneous presence of JUNV and lauric acid, virus yield was reduced as previously shown in Fig. 1C. These results demonstrated that lauric acid did not inactivate virion infectivity neither interacted with the target cell to block virus infection, and consequently its inhibitory action is due to an interference during the virus multiplication cycle.

To obtain information on the compound-sensitive step during JUNV infection, the dependence of the inhibitory effect of lauric acid on the time of drug addition or removal during a growth cycle was next examined. As shown in Fig. 2, a similar level of inhibition was observed if 250 μM lauric acid was added immediately

Table 2. Effect of pretreatment of virions or cells with lauric acid on the multiplication of JUNV

Treatment	Virus titer (PFU/ml)		Inhibition (%)
	control	lauric acid	
Cells[a]	4.2×10^4	3.8×10^4	9.5
Virions[b]	5.1×10^4	4.2×10^4	17.6
Cells+virions[c]	5.0×10^4	3.0×10^3	94.0

[a]Vero cells were incubated with 250 μM lauric acid or MM for 2 h at 37 °C and were then washed with PBS prior to inoculation with JUNV

[b]JUNV (10^6 PFU/ml) was mixed with 250 μM lauric acid or MM. After 2 h incubation at 37 °C, the mixtures were diluted 1:100 and inoculated to cells.

[c]Vero cells were infected with JUNV and after adsorption MM containing or not 250 μM lauric acid was added. For all three experimental methods, virus yields were determined at 24 h p.i.

after virus adsorption (time 0) or at 4 h p.i. Even when the fatty acid was added at 6–8 h p.i., extracellular virus yields were decreased as compared to those of untreated cells, with approximately 1 log reduction in virus titers. In another set of experiments the compound was added immediately after adsorption and then removed at various times by washing. When present only for a limited period of time after infection (either 2, or 4 or 6 h), the fatty acid was ineffective to inhibit JUNV replication (Fig. 2). Thus, the results shown in Fig. 2 indicate that lauric acid inhibits a late step of the replicative cycle of JUNV.

To investigate the mechanism of virus inhibition in more detail, the effect of lauric acid on viral protein synthesis was first examined. The fluorography of ^{35}S-labelled and immunoprecipitated proteins from treated and untreated infected cells showed no differences in the intensities and migration of the bands corresponding to the main JUNV proteins (data not shown).

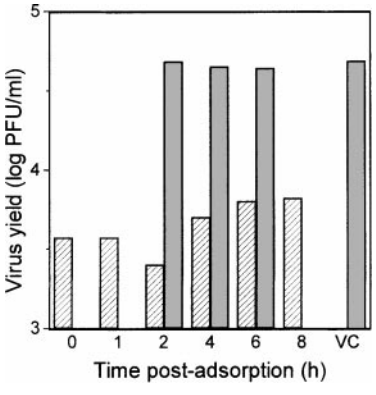

Fig. 2. Effect of the time of addition or removal of lauric acid on JUNV replication. Vero cells were allowed to adsorb JUNV (m:0.1) for 1 h at 4 °C. After removal of the inocula, cells were washed with PBS and then MM containing 250 μM lauric acid was added at 0, 1, 2, 4, 6 and 8 h post-adsorption and incubated further at 37 °C (dashed bars). Other set of infected cultures were incubated with lauric acid immediately after adsorption and then drug was removed at 2, 4 or 6 h (grey bars). *VC* Virus control yield, without compound treatment (empty bar). Extracellular virus yields were determined at 24 h p.i. in all cultures. Each value is the mean of duplicate determinations

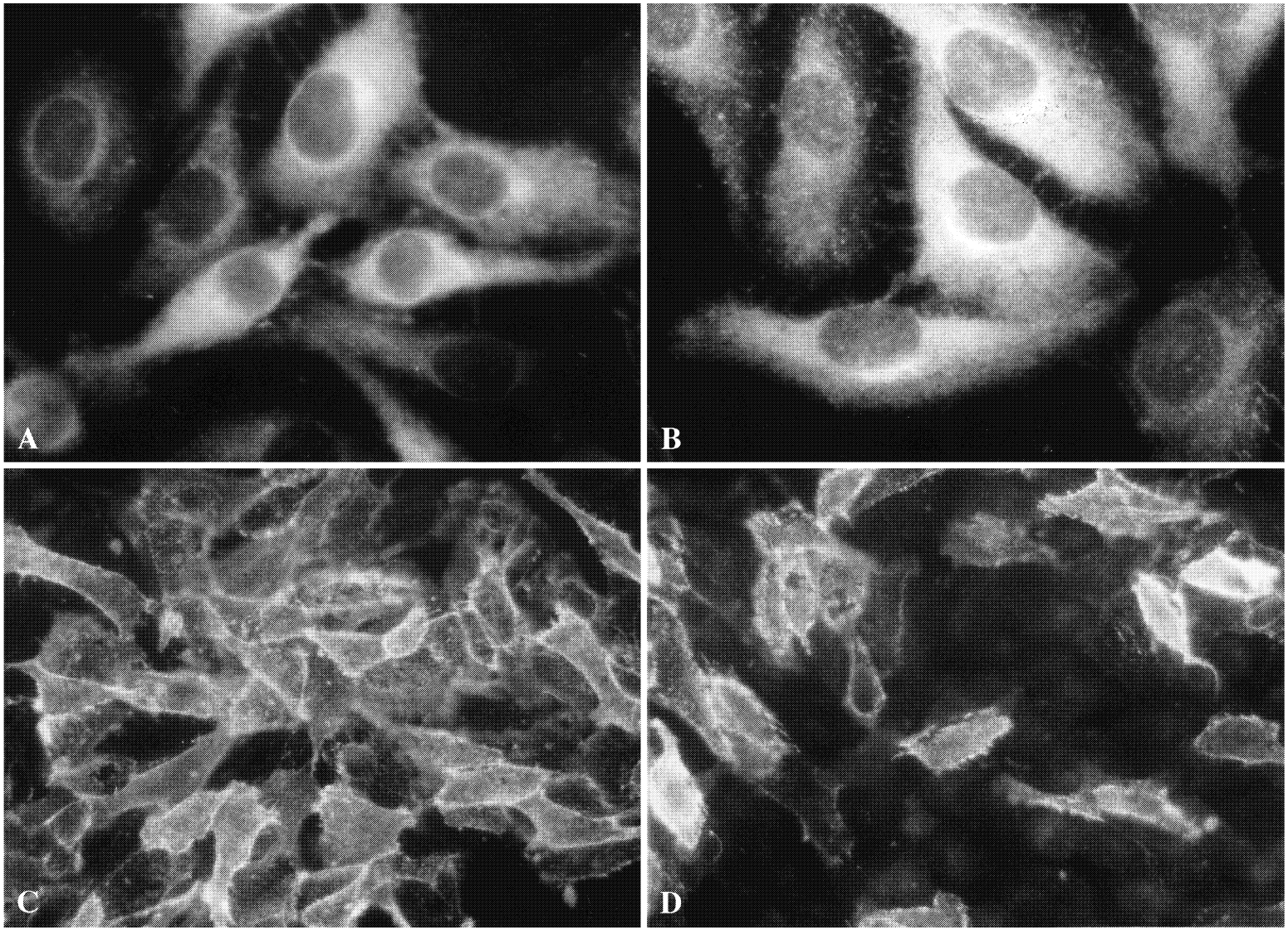

Fig. 3. Action of lauric acid on expression and distribution of JUNV proteins. Vero cells were infected with JUNV during 24 h in the absence (**A**, **C**) or in the presence (**B**, **D**) of 250 μM lauric acid. Cytoplasmic immunofluorescence staining (**A**, **B**) was carried out using anti-JUNV immunoglobulins from hyperimmune serum, and for membrane staining (**C**, **D**) the monoclonal antibody GB03-BE08 reactive against GP1 and the precursor GPC was used

Although virus protein synthesis was not impaired by lauric acid, an alteration in the cellular distribution of the viral proteins may occur and be responsible of a blockade in the assembly and/or budding of the viral progeny. Therefore, the location of JUNV proteins in Vero cells was examined by cytoplasmic and membrane immunofluorescence. In control infected cells, at 24 h p.i. JUNV proteins were found to be disseminated throughout the cytoplasm with a very bright staining (Fig. 3A). When infected cells were treated with lauric acid, no difference in the intensity and distribution pattern of cytoplasmic staining was observed (Fig. 3B). When immunofluorescence staining of viral glycoproteins in the membrane of infected cells was performed, untreated cells exhibited a linear pattern of bright fluorescence along the cell surface (Fig. 3C), whereas the intensity staining of membrane-associated JUNV glycoproteins and the number of positive cells was notoriously diminished in lauric acid-treated cells (Fig. 3D).

The effect of lauric acid on JUNV protein expression was quantitatively determined by counting the number of fluorescent cells. The inhibition in the number of positive cells for cytoplasmic and membrane staining was 9 and 55%, respectively, confirming that viral protein synthesis is not affected by lauric acid, but the insertion in plasma membrane is diminished.

In various transformed and normal cell lines, it has been reported that medium chain length monocarboxylic acids stimulate the *de novo* synthesis of triacylglycerols (TAG), and are also incorporated into the synthesized TAG [13]. Thus, the effect of fatty acid treatment in the system JUNV-Vero cells used in this report was examined by evaluating the TAG content of infected cells. Lauric acid at a concentration of 250 μM simultaneously induced an increase in the level of TAG by 80% respect to normal untreated cells (Fig. 4) and, as previously shown, a 92% reduction in virus infectious release (Fig. 1C), whereas monocarboxylic acids with a different chain length had no enhancing effect on TAG content together with a reduced or negligible anti-JUNV activity (Figs. 1 and 4).

To confirm the apparent correlation between the inhibition of JUNV replication and the stimulation of TAG content induced by lauric acid, an experiment was designed to examine the reversibility of both effects. Vero cells were infected

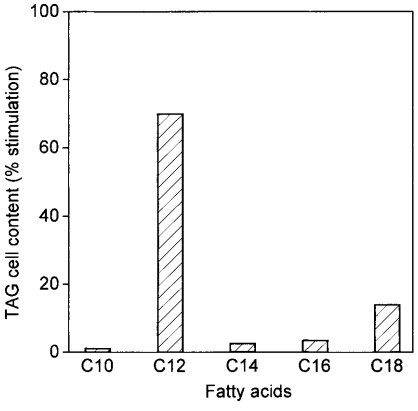

Fig. 4. Influence of the chain length of monocarboxylic acids on the cellular content of TAG. Vero cells were infected with JUNV, strain IV4454 (m:0.1) and, after adsorption, MM containing 250 μM fatty acid was added. At 24 h p.i., the total lipids were extracted and TAG content was determined. Results are expressed as % stimulation: [(TAG concentration in treated culture – TAG concentration in control culture)/TAG concentration in control culture] × 100. Each data is the mean of duplicate determinations

with JUNV and treated with lauric acid for 24 h, then the culture fluid was replaced with fresh MM without compound and cells were incubated for a further 24 h. At 24 and 48 h p.i., supernatant virus yield and cellular TAG content were assayed. As seen in Table 3, after drug removal virus infectivity and normal TAG content were both partially recovered, indicating that the degree of reduction of infectious virus in the medium induced by lauric acid was associated with the TAG increase in the cells.

Discussion

The involvement of lipids in different phases of the infection with enveloped viruses is presently a subject of review. Lipid molecules can mainly alter the properties of cell membranes and, consequently, influence the initial virus binding to cell receptors, the membrane fusion events during internalization, the insertion of viral proteins in the cell membrane and the final stages of virion assembly and budding. Furthermore, fatty acylation of viral glycoproteins is also required in a wide range of viruses to target the modified protein to a membrane or to stabilize protein-protein interactions, affecting assembly and/or eggress from the infected cells. Finally, it is also possible a direct modification on the composition of the virion envelope leading to destabilization and inactivation of the infectious virus particle.

The results reported in this study have shown that the effect of saturated fatty acids against arenaviruses is highly dependent on the length of the hydrocarbon chain. Lauric acid, the compound with 12 carbon atoms, was the most effective inhibitor of JUNV and TACV replication in a concentration-dependent form, whereas cell viability was not impaired (Fig. 1). From mechanistic studies, it can be concluded that lauric acid blocked a late stage in the JUNV replicative cycle, without affecting the synthesis of viral proteins but interfering with glycoprotein insertion at the plasma membrane. Thus, the reduced expression of the viral glycoproteins at the cell surface is the apparent cause leading to the inhibition of virus production.

It has been reported that after supplementation with medium chain fatty acids (11–12 C) the cellular lipid metabolism is altered, and the control of TAG synthesis is the most prominent alteration, with an stimulation of the *de novo* synthesis of TAG from glycerol and fatty acids and an increase in their amount in the cell [13]. The newly synthesized TAG are stored as lipid droplets in the cytoplasm [29], but also certain quantity is intercalated within the lipid bilayer into the plasma membrane, where the amount of TAG is about 6% of the total lipid content [19]. The TAG apparently influence membrane fluidity and permeability and, then, may be directly responsible to modify the interaction of viral glycoproteins with the cell membrane. Here, a direct correlation between the inhibition of JUNV production and the stimulation of TAG content in the system JUNV-Vero cells was demonstrated. Among all the monocarboxylic acids tested, lauric acid was the only compound able to affect simultaneously JUNV yield and cellular TAG amount. In fact, after removal of lauric acid from the infected culture medium,

Table 3. Correlation of inhibition of JUNV replication and stimulation of TAG synthesis

Treatment conditions	Virus yield		TAG content	
	titer (PFU/ml)	inhibition (%)	concentration ($\mu g/10^6$ cell)	stimulation (%)
MM 0–24 h	6.0×10^4	–	9.5	–
Lauric acid 0–24 h	5.6×10^3	90.7	17.3	81.0
MM 0–48 h	6.1×10^5	–	10.8	–
Lauric acid 0–24 h+MM 0–48 h	2.8×10^5	54.1	13.6	26.0

After each treatment, at 24 and 48 h p.i. extracellular virus yields and TAG cell content were determined. Results are expressed as: Virus yield inhibition (%) = [1− (PFU treated culture/PFU control culture)]×100; TAG stimulation (%) = [(concentration treated culture/concentration control culture) − 1] ×100

both levels of TAG and virus infectious particles tend to reach the values of untreated cultures (Table 3), indicating that the two related effects are dependent on the continued presence of the fatty acid.

Our results are similar to those obtained with VSV by Hornung et al [14], who reported an antiviral activity of lauric acid due to a TAG-dependent reduction in the amount of the glycoprotein G and the matrix protein M in the plasma membrane of VSV infected Rita cells after treatment with the compound. It is known that M protein binds to cellular membranes and promotes viral budding by interacting with the G protein and the viral nucleocapsid to assemble the virus particle. In contrast to VSV and other enveloped viruses, arenaviruses do not appear to possess an internal matrix protein [5]. A role analogous to that of the M protein has been proposed in arenaviruses for the Z or p11 protein, a virion protein with Zn-binding properties, but no conclusive experimental evidence is available [23]. The mechanism of the arenavirus assembly process is presently unknown and, due to the lack of a classical matrix protein, it may be unusual in comparison to other viruses. However, it has been reported that the superinfection of JUNV- or Pichinde virus-infected cells with VSV resulted in the production of pseudotypes that were not neutralized by antiserum to VSV, but were neutralized by antiserum to JUNV or Pichinde virus, respectively [9, 26], i.e. pseudotypes formed by VSV particles possessing the external glycoprotein of the arenavirus. These earlier studies suggest that the membrane interaction of arenavirus glycoproteins are compatible with their incorporation into the envelopes of VSV particles, an assembly process dependent on the M protein. Concomitantly, the glycoproteins of both viruses, JUNV and VSV, were similarly reduced in their ability to become inserted into the plasma membrane when it is altered by the lauric acid-induced incorporation of triacylglycerols.

Other similarity between VSV and JUNV is their response to virion treatment with lauric acid, since both virus particles were not inactivated by direct contact with the drug (Table 2) [14]. For other enveloped viruses such as HIV and HSV, it has been reported a strong virucidal activity of medium chain fatty acids and their

1-monoglycerides, causing reduction in virus titer after only 1 min of incubation [15, 16, 30]. Differences in the envelope lipid composition may be responsible for this differential behavior. It is known that the lipid composition of viruses is critical and derives from sequestration of specific lipids from the host cell membrane lipids [1, 20]. No studies on arenavirus lipids have been still performed.

The possible therapeutic use of agents altering the lipid bilayer has regained interest in the recent years. Several types of lipophilic drugs evaluated in vitro [12, 16, 17, 30, 31] and in vivo [2, 3, 27] against enveloped viruses offer the alternative of safe compounds to control virus infections. According to the results presented here, it is also an strategy to be considered for the treatment of viral hemorrhagic fevers.

Acknowledgements

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References

1. Aloia RC, Tian H, Jensen FC (1993) Lipid composition and fluidity of the human immunodeficiency virus envelope and host cell plasma membrane. Proc Natl Acad Sci USA 90: 5181–5185
2. Alrabiah FA, Sacks SL (1996) New antiherpesvirus agents. Their targets and therapeutic potential. Drugs 52: 17–32
3. Bourne N, Ireland J, Stanberry LR, Bernstein DI (1999) Effect of undecylenic acid as a topical microbicide against genital herpes infection in mice and guinea pigs. Antiviral Res 40: 139–144
4. Castilla V, Mersich SE, Candurra NA, Damonte EB (1994) The entry of Junin virus into Vero cells. Arch Virol 136: 363–374
5. Compans RW (1993) Arenavirus ultrastructure and morphogenesis. In: Salvato MS (ed) The Arenaviridae. Plenum Press, New York, pp 3–16
6. Contigiani MS, Sabattini MS (1977) Virulencia diferencial de cepas de virus Junín por marcadores biológicos en ratones y cobayos. Medicina (Bs. Aires) 37 [Suppl 3]: 244–251
7. Cordo SM, Candurra NA, Damonte EB (1999) Myristic acid analogs are inhibitors of Junin virus replication. Microbes Infect 1: 609–614
8. Damonte EB, Mersich SE, Candurra NA (1994) Intracellular processing and transport of Junin virus glycoproteins influences virions infectivity. Virus Res 34: 317–326
9. Damonte EB, Mersich SE, Coto CE (1978) Susceptibilidad viral de células Vero infectadas persistentemente con arenavirus: formación aparente de seudotipos. Rev Asoc Arg Microbiol 10: 78–81
10. Downs WG, Anderson CR, Spence L, Aitken THG, Greenhall AH (1963) Tacaribe virus, a new agent isolated from Artibeus bats and mosquitoes in Trinidad, West Indies. Am J Trop Med Hyg 12: 640–646
11. Guerrero LB, Weissenbacher MC, Parodi AS (1969) Inmunización contra la fiebre hemorrágica argentina con una cepa atenuada de virus Junín. Medicina (Bs. Aires) 29: 1–5

12. Gunther-Ausborn S, Stegmann T (1997) How lysophosphatidylcholine inhibits cell-cell fusion mediated by the envelope glycoprotein of human immunodeficiency virus. Virology 235: 201–208
13. Hornung B, Amtmann E, Sauer G (1992) Medium chain length fatty acids stimulate triacylglycerol synthesis in tissue culture cells. Biochem Pharmacol 43: 175–181
14. Hornung B, Amtmann E, Sauer G (1994) Lauric acid inhibits the maturation of vesicular stomatitis virus. J Gen Virol 75: 353–361
15. Kohn A, Gitelman J, Inbar M (1980) Unsaturated free fatty acids inactivate animal enveloped viruses. Arch Virol 66: 301–307
16. Kristmundsdottir T, Arnadottir SG, Bergsson G, Thormar H (1999) Development and evaluation of microbicidal hydrogels containing monoglyceride as the active ingredient. J Pharm Sci 88: 1011–1015
17. Moog C, Aubertin AM, Kirn A, Luu B (1998) Oxysterols, but not cholesterol, inhibit human immunodeficiency virus replication in vitro. Antivir Chem Chemother 9: 491–496
18. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65: 55–63
19. Mountford CE, Wright LC (1988) Organization of lipids in the plasma membranes of malignant and stimulated cells: a new model. Trends Biochem Sci 13: 172–177
20. Muñoz-Barroso Y, Cobaleda C, Zhadan G, Shnyrov V, Villar E (1997) Dynamic properties of Newcastle disease virus and their relations with viral hemagglutinin-neuraminidase membrane glycoprotein. Biochim Biophys Acta 1327: 17–31
21. Parodi AS, Greenway DJ, Ruggiero HR, Rivero S, Frigerio MJ, de la Barrera M, Mettler N, Garzón F, Boxaca M, Guerrero LB, Nota NR (1958) Sobre la etiología del brote epidémico de Junín. El Día Médico 30: 2300–2302
22. Raulin J (2000) Lipids and retroviruses. Lipids 35: 123–130
23. Salvato MS (1993) Molecular biology of the prototype arenavirus, lymphocytic choriomeningitis virus. In: Salvato MS (ed) The Arenaviridae. Plenum Press, New York, pp 133–156
24. Sanchez A, Pifat D, Kenyon RH, Peters CJ, McCormick JB, Kiley MP (1989) Junin virus monoclonal antibodies: characterization and cross-reactivity with other arenaviruses. J Gen Virol 70: 1125–1132
25. Scolaro LA, Damonte EB (1997) Glycoprotein-mediated biological properties of a host range mutant of Junin virus. Res Virol 148: 323–331
26. Sengupta S, Rawls WE (1979) Pseudotypes of vesicular stomatitis virus and Pichinde virus. J Gen Virol 42: 141
27. Shafran SD, Sacks SL, Aoki FY, Tyrrell DL, Schlech WF III, Mendelson J, Rosenthal D, Gill MJ, Bader RL, Chang Y (1997) Topical undecylenic acid for herpes simplex labialis: a multicenter, placebo-controlled trial. J Infect Dis 176: 78–83
28. Silber AM, Candurra NA, Damonte EB (1993) The effects of oligosaccharide trimming inhibitors on glycoprotein expression and infectivity of Junin virus. FEMS Microbiol Lett 109: 39–44
29. Spector AA, Mathur SN, Kaduce TL, Hyman BT (1981) Lipid nutrition and metabolism of cultured mammalian cells. Progr Lip Res 19: 155–186
30. Thormar H, Isaacs CE, Kim KS, Brown HR (1994) Inactivation of visna virus and other enveloped viruses by free fatty acids and monoglycerides. Ann NY Acad Sci 724: 465–471
31. Vollenbroich D, Ozel M, Vater J, Kamp RM, Pauli G (1997) Mechanism of inactivation of enveloped viruses by the biosurfactant surfactin from *Bacillus subtilis*. Biologicals 25: 289–297

32. Weissenbacher MC, Laguens RP, Coto CE (1987) Argentine hemorrhagic fever. Curr Topics Microbiol Immunol 134: 79–116

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