Prostaglandin A_1 inhibits the replication of Sindbis virus in monkey kidney and mosquito cells

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Abstract
The present study reports the effect of prostaglandin A_1 (PGA_1) on the replication of Sindbis virus in monkey kidney and mosquito cells. In PGA_1 treated cells we observed a severe reduction of virus yield. In both cells lines the highest nontoxic concentration of PGA_1 (10 ìg/mL) decreased virus replication, dose dependently, by more than 90%. SDS-PAGE analysis of [^{35}S] methionine labeled proteins showed that viral proteins (E_1/E_2 and C) were normally synthesized in PGA_1 treated Vero cells, and induction of stress proteins (HSP70 and HSP90) was detected in uninfected and infected cells. In Vero cells the inhibition of virus replication was accompanied by a decrease in [^{3}H] glucosamine incorporation into the virus glycoproteins.

Keywords: Prostaglandin A_1 - Sindbis virus - Vero cells - Aedes albopictus cells; Sindbis virus–PGA_1; Vero cells - Sindbis virus - PGA_1 - Proteins synthesis–Glycoproteins; Aedes albopictus cells - Sindbis virus - PGA_1 - Proteins synthesis – Glycoproteins.

INTRODUCTION

Prostaglandins (PGs) are potent eicosanoid lipid mediators, derived from phospholipase-released arachidonic acid, which are involved in numerous homeostatic biological functions and in inflammation. PGs have been shown to function as microenvironmental hormones and intracellular signal mediators and also to participate in the regulation of a large variety of physiological and pathological processes (FUNK, 2001).

It is now widely accepted that PGs of the A and J types (cyclopentenone prostaglandins) play a role in cellular defense mechanisms against viral infections (FUKUSHIMA, 1990; SANTORO, 1997). The mechanisms by which PGs can interfere with viral replication have been intensively investigated and different results have been reported for several virus-cell systems. Cyclopentenone prostaglandins inhibit virus replication by acting

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on multiple cellular and viral targets, by modifying the synthesis, maturation and intracellular translocation of virus proteins. A relationship between heat-shock protein (HSP) synthesis induced by PGs (types A and J) and virus replication has also been described (SANTORO, 1997). Recently, it was reported that cyclopentenone PGs are potent inhibitors of nuclear transcription factor NF-kB and of NF-kB-dependent HIV-1 transcription in human cells (AMICI et al., 2004).

Sindbis virus (SINV) a member of the Togaviridae family; Alphavirus genus is propagated in nature through a horizontal cycle involving vertebrate hosts and mosquito vectors. The 12-Kb genome of SINV is an RNA molecule of positive polarity which is capped at the 5' terminus and polyadenylated at the 3'terminus. The structural proteins of SINV are encoded in a single large open reading frame which specifies a polyprotein with a relative mass (Mr) of 130 K. The polyprotein is clevead to originate C, E_1 and PE_2, the precursor to E_2. The C protein interacts with the genomic RNA to form the nucleocapsid and the glycoproteins E_1 and E_2 comprising the spikes inserted in the lipid envelope surrounding the nucleocapsid (STRAUSS, J.H.; STRAUSS, E.G., 1994).

MATERIAL AND METHODS

Cell culture and virus

Vero cells, were grown at 37°C in Dulbecco's Modified Eagle's medium (DMEM, Life Technologies, USA) supplemented with 8% of fetal calf serum (FCS). Aedes albopictus cells, clone C6/36, were grown at 28°C in Leibovitz's (L-15) medium, supplemented with 0.2 mM non-essential amino acids, 0.3% tryptose phosphate broth, 0.02% L- glutamine, 5% FCS, penicillin (500 U/ml), streptomycin (100 mg/ml) and amphotericin B (Fungizone, 2.5 mg/ml). SINV was obtained from Instituto Oswaldo Cruz, Fiocruz, RJ, Brazil. The virus stock was prepared in Vero cells and stored at - 70°C. Stock solution of PGA_1 (1mg/ml, Sigma Chemical Co) was made in ethanol and was stored at - 20°C. The stock solution was diluted in growth medium to proper concentration and the control medium contained the same concentration of ethanol diluent (0.02%) which was shown not to affect cell growth or virus replication.

Virus titration

Confluent monolayers of cells were infected with SINV. After incubation for 1h, virus inocula were removed and monolayers were washed with PBS and incubated with growth medium containing PGA_1 or control medium. Twenty four hours post infection the cell culture supernatants were recovered and used for titration of extracellular infective virus. The latter was performed by plaque assay in Vero cells that had just reached confluence. The monolayers were overlaid with growth medium, supplemented with 10% FCS and 50% Karaya gum (Sigma Chemical Co), and were further incubated in an atmosphere of 5% CO_2 at 37°C for 3 days. The monolayers were then stained with crystal violet (1%) and the virus plaques were counted. (CAMPOS et al., 2003)

Analysis of virus proteins

For polyacrylamide gel electrophoresis confluent monolayers of Vero cells were labeled with [^{35}S]-methionine (60 mCi/ml) in methionine-free medium for 1 hr at 37°C as described previously (CAMPOS et al., 2003). Vero cells were also labeled with [^{3}H] glucosamine hydrochloride (20 mCi/ml, Amersham) in glucose-free medium for 5 hrs at 37°C. The medium was then removed and the cellular proteins were analyzed by SDS-PAGE using the buffer system describeb by Laemmli (1970). The gel was stained with comassie blue to visualize the proteins warkers, dried and autoradiographed using Kodak X-Omat (YAR-S) film. Relative molecular mass (Mr) of proteins was determined by proteins standards (Life Technologies). Autoradiograms were quantified densitometrically by using a laser bean densitometer (Ultroscan 2202 LKB Instruments, Sweden), and bands were expressed as relative peak areas determined by the intensity of signal (Sion Image computer program). The inhibition of virus proteins by PGA_1 treatment was calculated considering the intensity of signal in untreated cells as 100%. For immunoblot analysis an equal amount of protein from each sample was separated by PAGE, as

described above, and blotted onto nitrocellulose. The blots were developed with monoclonal anti-HSP70 mouse antibody, mouse ascites fluid, clone BRM-22 (Sigma Chemical Co) and horseradish peroxidase-linked whole antibody from mice (ECL, Amersham). (MASTROMARINO et al., 1993).

RESULTS

The effect of PGA_1 on SINV replication was examined in Vero and A. albopictus cells in the presence of various concentrations of this compound. The result of this experiment revealed that in both cell lines the treatment of PGA_1 for 24h decreased virus yield dose-dependently (FIGURE 1). At the concentration of 10 mg/ml, the virus yield was inhibited by 93%. In all concentrations used, PGA_1 was not toxic to the cells, as demonstrated by the dye exclusion test using trypan blue (data not shown).

In order to verify the virus protein synthesis after the PGA_1 treatment, confluent monolayers of Vero cells were infected with Sindbis virus as described above. PGA_1 was added at 1h after infection and continued for the following 24h. The cells were then labeled with [^{35}S]-methionine during 1h and processed for SDS PAGE and autoradiography. The infection produced a typical protein profile consisting of virus membrane glycoproteins E_1/E_2, the precursor protein PE_2 and the capsid protein C. The protein B, described by Duda (1975), and Hernandez and others (2003) is also seen. In uninfected cells PGA_1 did not alter the general protein profile, however, it induced two proteins of approximate 70 K and 90 K (FIGURE 2-A, lane 2). The 70 k protein could be identified as a heat-shock protein by comparing lanes 1 and 2 and by immunoblot analysis using monoclonal antibodies against HSP70 (FIGURE 2-B). Figure 2-A also shows that PGA_1 treatment did not affect significantly the synthesis of virus structural proteins E_1, E_2 and C and the precursor (FIGURE 2-A, lane 3), while it prevented the virus-induced shut-off of cellular protein synthesis (FIGURE 2-A, lane 4).

PGA_1 induces the synthesis stress proteins in Vero cells (FIGURE 2) and in A. albopictus cells (BARBOSA; REBELLO, 1998). We also found that in SINV infected cells, heat treatment (39 ºC for Vero cells and 30 ºC for A. albopictus) inhibited virus replication by 98% in both cell lines. These

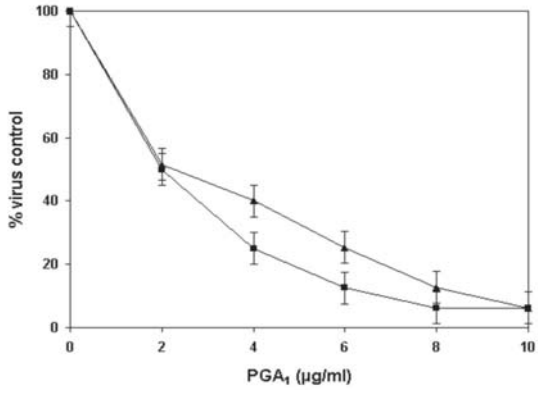

Figure 1 - Effect of PGA_1 on SINV production.
Notes: - Cells were infected with SINV (1 PFU/cell) and treated with different concentrations of PGA_1 for 24h. One set of cells served as untreated control. Supernatants from treated and untreated cells (triplicate samples) where harvested and virus titers determined at 24 h. p.i. - Vero cells (²%); ***A. albopictus*** cells (%).

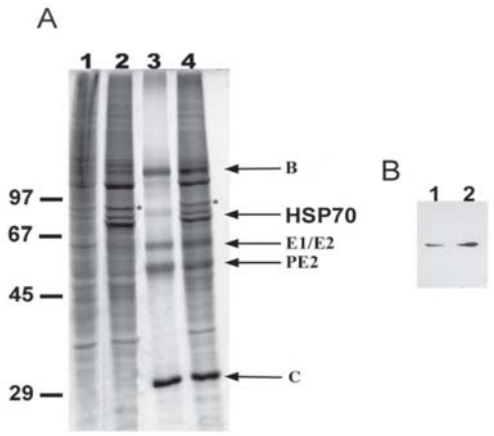

Figure 2 - Effect of PGA_1 on the synthesis of cellular and viral proteins.
Notes: A) Vero cells were infected (lanes 3 and 4) with SINV, 5 PFU/cell or mock infected (lane 1) and maintained in growth medium for 18h in the presence of PGA_1, 8 mg/ml (lanes 2 and 4). After this period, the cells were labeled with [^{35}S] methionine (60 mCi/ml) for 1h and cellular extracts were subjected to PAGE. The positions of structural (E2, E1 and C) and precursors of virus proteins are indicated. (*) HSP90.
B) Identification of HSP70. Cells from lane 1 (control) and lane 2 (PGA_1)were processed for immunoblot analysis using anti-HSP70 monoclonalantibodies.

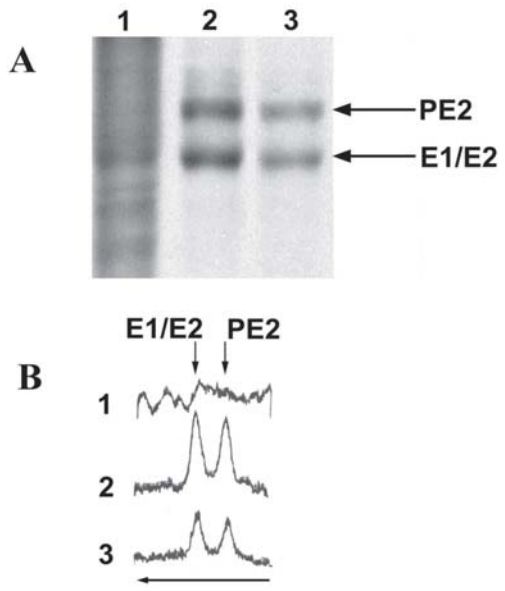

Figure 3 - Effect of PGA_1 on the glycosylation of SINV proteins.

Notes: A) Vero cells were infected with Sindbis virus (5 PFU/cell) in the same conditions described in Fig 2. At 18 hpi cellswere labeled with [^{3}H]-glucosamine (20 mCi/ml) for 5 hours and samples were processed to SDS-PAGE and fluorography. Mock-infected cells (lane 1), infected cells (lane 2) and infected and PGA_1 (8mg/ml) treated cells (lane 3). B) Densitometric analysis of autoradiographic patterns. Direction of migration is indicated by the arrow at the botton of the figure.

effects were followed by the induction of heat shock protein synthesis (date not shown).

Next, we examine the effect of PGA_1 on SINV glycoproteins synthesis. Glycosylation of virus proteins was examined by labeling with ^{3}H-glycosamine in the presence of PGA_1 and analyzed by SDS-PAGE and fluorography. At the concentration of 8 µg/ml, PGA_1 inhibits the incorporation of ^{3}H-glycosamine into PE_2 and E_1/E_2 proteins. Densitometric analysis indicate that this inhibition was 45% for PE_2 and 42% for E_1/E_2.

DISCUSSION

The results presented in this paper show that PGA_1 inhibits the replication of SINV in Vero cells and in A. albopictus cells. While eicosanoids are very well known in mammalian systems, there is increasing recognition of the importance of these compounds in insects and other invertebrates. Eicosanoids have physiological roles in insect reproduction, barnacle hatching, snail egg production , bivalve spawning and sea star oocyte maturation (STANLEY-SAMUELSON, 1994). Additionally, results presented by Stanley-Samuelson (1991) strongly support the hypothesis that eicosanoids are mediators in the invertebrate immune response. In fact, in the tobacco hornworn Manduca sexta, selective inhibition of eicosanoid biosynthesis compromise the horworm's immune response to bacterial infection. In mosquito cells, eicosanoids biosynthesis may be an important step on the control of arbovirus replication.

The identification of the cellular and viral targets of PGs on the antiviral activity is not completely elucidated. The antiviral activity has been associated with the action of cyclopentenone PGs to function as signals for the induction of HSP synthesis (SANTORO et al., 1982). In several virus models, the target for the antiviral activity appeared to be a late event in the virus replication cycle. PGA_1 and $Ä^{12}$-PGJ_2 was also shown to be a potent inhibitor of SINV replication. The antiviral action is dose-dependent at non-toxic doses which did not cause significant changes in the synthesis of DNA, RNA and proteins of uninfected cells (MASTROMARINO et al., 1993). In addition these authors observed that PGA_1 moderately inhibited the synthesis of the viral structural proteins, and induced the synthesis of a heat shock protein (72 KDa). Actinomycin D treatment completely prevented PGA_1 antiviral activity indicating that a cellular product is responsible for this action. PGA_1 were shown to cause modifications in the synthesis and maturation of virus proteins in vaccinia virus (SANTORO et al., 1982). In Sendai virus infected AGMK 37RC cells, the nonglycosylated viral polypeptides (P, NP and M) were normally synthesized in PGA_1 treated cells, however the viral glycoproteins HN and F_0 were not detected (SANTORO et al., 1983). In L cells PGA_1 suppressed the synthesis of the Vesicular stomatitis virus (VSV) glycoprotein G. (SANTORO; JAFFE; ESTEBAN, 1983). However, in human embryo fibroblasts infected

with herpes virus 1 (YAMAMOTO et al., 1987) and in L-1210 cells infected with VSV (BADER; ANKEL, 1990) PGs block the virus RNA transcription. These findings suggests that the cyclopentenone PGs could be acting at more than one site during the virus replicative cycle.

In the present report we observed that inhibition of SINV production in Vero cells could be obtained (90%) at a concentration of PGA_1 that did not inhibit the synthesis of virus proteins E_1/E_2 and C. These results suggest that PGA_1 could be acting by blocking a late event in virus maturation or virus assembly.

SINV infection did not affect the Vero cell stress response after PGA_1 treatment and high levels of HSP70 synthesis is found after infection (FIGURE 2-A). Several authors have reported that stress proteins could be actively involved in the control of virus replication (SANTORO, 1996).

The results described in this paper also show that treatment of Vero cells with PGA_1 decreased the level of glucosamine incorporation on virus glycoproteins. Thus, at least two different targets of action of PGA_1 exist in the case of SINV infected Vero cells, HSP70 induction and glycosylation of virus proteins. Both events may have consequences for SINV replication.

CONCLUSIONS

The finding that Sindbis virus protein synthesis was not inhibited by PGA_1, suggests that this compound could interfere with a late event in the virus replication cycle, sush as protein processing or virus maturation. In addition ours results also suggest that PGA_1 interfere with Sindbis virus replication by reducing the glycosylation of virus proteins and subsequently the virus infectivity.

Prostaglandina A_1 inibe a replicação do vírus Sindbis em células de rim de macaco e em células de mosquito

Resumo

Neste estudo nós avaliamos o efeito da prostaglandina A_1 (PGA_1) na replicação do vírus Síndbis em células de macaco e em células de mosquito. Nas células tratadas com PGA_1 nós observamos uma redução significativa na produção de partículas virais infecciosas. Em ambas as linhagens celulares tratadas com concentrações não-tóxicas de PGA_1 (1-10 µg/ml) foi observado uma redução da replicação viral, de forma dose dependente, chegando a 90% na maior dose utilizada (10 µg/ml). A análise das proteínas virais e celulares marcadas com [^{35}S]-metionina em SDS-PAGE mostrou que as proteínas virais (E_1, E_2 e C) foram normalmente sintetizas em células Vero tratadas com PGA_1. Além disso, observamos também a indução das proteínas de estresse (HSP70 e HSP90) nas células Vero infectadas ou não infectadas. Em células Vero foi observado uma diminuição na incorporação de [^{3}H] glicosamina nas glicoproteínas virais.

Palavras-chave: Prostaglandina A_1 - Vírus Síndbis - Células Vero - Células de Aedes albopictus; Vírus Síndbis- PGA_1; Células Vero- Vírus Síndbis- PGA_1 - Síntese de proteínas - Glicoproteínas; Células de Aedes albopictus- Vírus Síndbis- PGA_1 - Síntese de proteínas - Glicoproteínas.

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