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Inhibition of Mayaro virus replication by prostaglandin A_1 and B_2 in Vero cells

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Abstract

The effect of prostaglandins (PGA_1 and PGB_2) on the replication of Mayaro virus was studied in Vero cells. PGA_1 and PGB_2 antiviral activity was found to be dose-dependent. However, while 10 µg/ml PGB_2 inhibited virus yield by 60%, at the same dose PGA_1 suppressed virus replication by more than 90%. SDS-PAGE analysis of [^{35}S]-methionine-labelled proteins showed that PGA_1 did not alter cellular protein synthesis. In infected cells, PGA_1 slightly inhibited the synthesis of protein C, while drastically inhibiting the synthesis of glycoproteins E_1 and E_2.

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Prostaglandins (PGs) are a class of naturally occurring cyclic 20 carbon fatty acids, synthesized from polyunsaturated fatty acid precursors by most eukaryotic cells. These compounds have been shown to function as microenvironmental hormones and intracellular signal mediators and to participate in the regulation of a large variety of physiological and pathological processes (1).

Prostaglandins inhibit viral replication in cultured cells, but different types of prostaglandins produce different effects on viral replication in several virus-host systems. As with other aspects of prostaglandin action, their antiviral effects are dependent on dose and structure of the cyclopentane ring, and vary for different types of viruses and host cells (2). Viral transformation has been shown to affect prostaglandin biosynthesis in cultured cells, either increasing or decreasing it, depending on the cell type (3). Prostaglandins of the E series (PGE) were shown to inhibit the production of parainfluenza 3 virus in WISH cells (4), and to decrease the yield of measles virus in Vero cells (5). On the other hand, PGE and PGF increased the size of plaques and the yield of herpes simplex virus in Vero cells (6). PGs of the A series are potent inhibitors of virus replication in three different systems: Sendai virus in African green monkey kidney cells (7), and vaccinia virus and vesicular stomatitis virus in mouse fibroblast cells (8).

Mayaro virus (genus Alphavirus, family *Togaviridae*) is an arthropod-borne virus antigenically related to Semliki Forest virus (9). Mayaro virus, like other *Togaviridae*, is perpetuated in nature by its ability to infect and replicate both in vertebrate and invertebrate cells (10,11). In Brazil, this virus has been isolated from human and other mammalian species in the Amazon region. Clinical manifestations of human infection were described as a feverish illness, followed by headache, epigastric pain, backache, chills, nausea and photophobia (9).

Previous results from our laboratory (12) showed that six viral proteins could be de-

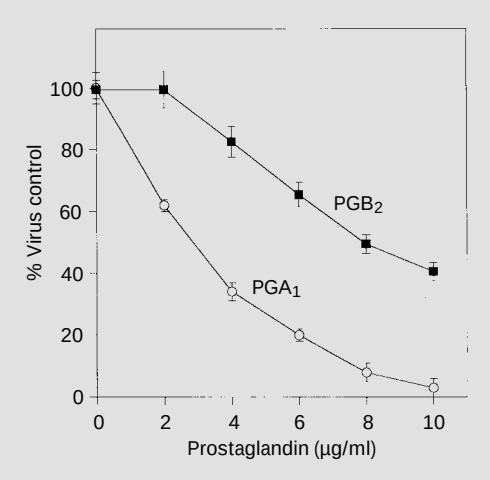

Figure 1 - Effect of PGA_1 and PGB_2 on Mayaro virus infection of Vero cells. Vero cells were infected with Mayaro virus (1 PFU/cell) and treated with different concentrations of prostaglandins. One set of cells was used as untreated control. Supernatants from treated and untreated cells were then tested for their ability to form plaques. Data are reported as means ± SD of triplicate samples.

tected in Mayaro virus-infected BHK-21 cells and *Aedes albopictus* cells. Three of them are the structural proteins P_1 (54 kDa), P_2 (50 kDa) and P_3 (34 kDa) and the other three are precursors of the viral structural proteins, with molecular weights of 110 kDa, 64 kDa and 62 kDa. By analogy with other alphaviruses, P_1 and P_2 correspond to the glycoproteins E_1 and E_2, and P_3 corresponds to the non-glycosylated capsid protein (C protein). In the present report, we describe the effect of PGA_1 and PGB_2 on the replication of Mayaro virus in Vero cells assessed by their effects on viral protein synthesis. Possible mechanisms of this antiviral action were investigated.

Vero cells were grown in Dulbecco's modified Eagle's medium (DMEM, Life Technologies, Grand Island, NY) supplemented with 8% calf serum and antibiotics, at 37°C in a 5% CO_2 atmosphere. PGA_1 and PGB_2 (Sigma Chemical Co., St. Louis, MO) were stored as a stock solution (1 mg/ml) in absolute ethanol and were diluted to the indicated concentrations. Control medium contained the same concentration of ethanol diluent (0.02%) which did not affect cell growth or virus replication. Mayaro virus was obtained from the American Type Culture Collection, Rockville, MD. The virus stock was prepared in BHK-21 cells and stored at -70°C. Virus titrations were performed by plaque assay in Vero cells. Briefly, a virus dilution (0.5 ml) was added to confluent cell monolayers on 60-mm diameter Petri dishes. After 60 min at 37°C, unadsorbed virus was removed by aspiration and the monolayers were overlaid with 4 ml of growth medium supplemented with 10% fetal bovine serum and 0.95% agar Noble (Difco Laboratories, Detroit, MI) and were incubated in 5% CO_2 at 37°C. Two days later, the monolayers were stained with neutral red (25 µg/ml) and the virus plaques counted.

For the protein synthesis studies, confluent cell monolayers were labelled with [^{35}S]-methionine (20 µCi/ml) in methionine-free medium. After labelling, cells were washed and lysed in lysis buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol and 0.001% bromophenol blue). Samples were then heated for 5 min at 95°C and subjected to electrophoresis on one-dimensional 12.5% polyacrylamide gels, using the SDS buffer system of Laemmli (13) at room temperature. The dried gels were exposed to Kodak X-Omat (YAR-S) film. The molecular mass of the protein was determined by co-electrophoresis of standard proteins (Pharmacia, Uppsala, Sweden). Protein sythesis was quantitated by densitometric analysis of autoradiographic patterns using a laser-beam densitometer (Ultroscan 2202, LKB Instruments, Bromma, Sweden) and measuring the area of each peak. Each virus protein as measured by the densitometric analysis is reported as a percentage of total virus proteins.

The antiviral effects of PGA_1 and PGB_2 on the growth of Mayaro virus were measured in Vero cells by the plaque reduction assay. None of the compounds tested were toxic to the cells at the concentrations used, as demonstrated by the absence of a cytopathic effect or by vital dye exclusion. PGs were administered after a 1-h period of infection, and culture medium was not changed during the following 24 h. A PG dose-re-

sponse curve is shown in Figure 1. The antiviral activity of PGA_1 and PGB_2 was dose-dependent; 50% inhibition of Mayaro virus replication was obtained with doses of 3 µg/ml for PGA_1 and 8 µg/ml for PGB_2. Unlike interferon, which is efficient only if the treatment occurs prior to Mayaro virus infection, pretreatment of Vero cells was not required for PGA_1 and PGB_2 to block Mayaro virus replication. PGA_1 could be added after infection of Vero cells and still inhibited virus yield. There was no difference in antiviral potency when PGA_1 and PGB_2 were added together with Mayaro virus as compared to adding them 1 or 2 h later. However, the antiviral action was decreased by 30, 40 and 60% if PGA_1 was added at 3, 4 and 5 h post-infection, respectively. At later stages, PGA_1 had no effect on Mayaro virus replication. Thus, events occurring after virus adsorption and penetration during the initial stages of virus replication are the target of PGA_1.

Since PGA_1 was more effective than PGB_2 in inhibiting Mayaro virus production, in the next experiment we used only PGA_1. The effect of PGA_1 treatment on macromolecular synthesis by uninfected and infected Vero cells was measured by the incorporation of [^{3}H]-thymidine, [^{3}H]-uridine and [^{35}S]-methionine into acid-insoluble material. A 24-h treatment did not alter the incorporation of these precursors (data not shown). The pattern of host and virus protein synthesis was analyzed by SDS-PAGE and by autoradiography. Figure 2 (lanes A, B, C and D) shows

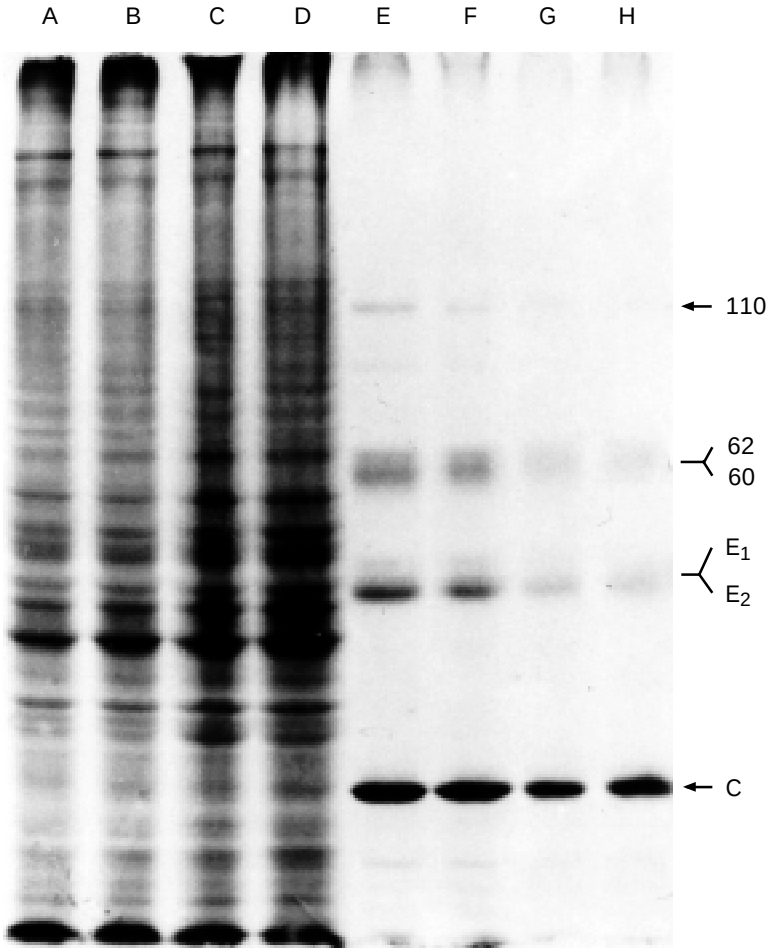

Figure 2 - Effect of PGA_1 on viral and cellular protein sythesis. Vero cells were mock infected (lanes A, B, C and D) and infected (lanes, E, F, G and H) with Mayaro virus (5 PFU/cell) and maintained in growth medium (lane A and E) for 24 h in the presence of PGA_1 at concentrations of 1 µg/ml (lanes B and F), 5 µg/ml (lanes C and G) and 10 µg/ml (lanes D and H). After this period, the cells were labelled with [^{35}S]-methionine (20 µCi/ml) for 1 h and cellular extracts were subjected to polyacrylamide gel electrophoresis.

that PGA_1 did not alter the pattern of protein synthesis in uninfected cells. In Mayaro virus-infected cells (lanes E, F, G and H) there was a strong inhibition of cellular protein synthesis and all the viral proteins were clearly distinguished. We also observed that PGA_1 produced inhibition of virus protein synthesis. Densitometric analysis of the autoradiogram revealed that the C protein synthesis was moderately inhibited (15%) even at a concentration of 10 µg/ml, which inhibited virus production by more than 90% (Figure 1). The synthesis of glycoproteins E_1 and E_2 was by far the most strongly inhibited among all viral proteins. At a concentration of 10 µg/ml, PGA_1 inhibited the synthesis of E_1 and E_2 by more than 50%.

Differences in potency between PGA_1 and PGB_2 were also reported for vesicular stomatitis virus-infected L-1210 mouse cells (14) and for human immunodeficiency virus-infected human T cell line C8188 (15). Santoro et al. (16) reported that type A prostaglandins potently inhibit the replication of Sendai virus and can prevent the establishment of a persistent infection by this virus in the African green monkey kidney (AGMK) cell line 37 RC. Later, it was shown that a long-acting synthetic analog of PGA_2 (16, 16-dimethyl-PGA_2 methyl ester) and PGJ_2 can suppress influenza A virus replication in mice (17).

Several reports have indicated that PG antiviral activity is associated with specific alterations in the synthesis or maturation of specific virus proteins (1). Santoro et al. (7) showed that PGA_1 (4 µg/ml) inhibited the replication of vaccinia virus by 95% and that the antiviral activity was dependent on the presence of PGA_1 during the early stages of infection. PGA_1 partially inhibited vaccinia virus DNA synthesis and altered both the rate and pattern of virus protein synthesis. Subsequently, Santoro et al. (18) found that PGA_1 strongly suppressed the synthesis of vesicular stomatitis virus glycoprotein G and changed the mobility of this protein in SDS-PAGE. The action of PGA_1 on Sendai virus replication has also been found to be associated with specific alterations in virus proteins. While the non-glycosylated viral polypeptides P, NP and M were normally synthesized in PGA_1-treated cells, the viral glycoproteins HN and Fo were not detected (16). The authors also showed that PGA_1-induced alterations in the HN protein caused a loss of its biological function and prevented the insertion of this protein into the cell membrane, thereby blocking virus maturation.

Our results show that a considerable amount of C protein was synthesized in the presence of PGA_1 at a dose that suppressed virus production by more than 90%. However, the inhibition of glycoproteins E_1 and E_2 by more than 50% probably induced modifications in the viral replications cycle, resulting in a decrease of infectious particles. On the other hand, the addition of PGA_1 during a late phase (5 h) still resulted in inhibition of virus production. This suggests that PGA_1 could also act by blocking a late event in virus maturation or assembly.

Data from the literature are still insufficient to identify a mechanism of action of Pgs as antiviral agents, although recent investigations have shown that the antiviral activity of Pgs is associated with the induction of 70-kDa heat shock proteins synthesis (19). We have previously observed that PGA_1 not only inhibits the replication of Mayaro virus in *A. albopictus* cells, but also stimulates the synthesis of several cellular polypeptides with molecular weights of 70, 57 and 23 kDa (20).

In the present study, we have extended the observation that PGA_1 and PGB_2 inhibit the replication of Mayaro virus to yet another cell type, the Vero cell line, in a dose-dependent manner and have suggested that this effect occurs simultaneously with a decrease in the synthesis of viral proteins E and, to a lesser extent, C.

References

1. Santoro MG, Garaci E & Amici C (1990). Induction of HSP70 by prostaglandin. In: Schlesinger MS, Santoro MG & Garaci E (Editors), *Stress Proteins: Induction and Function*. Springer-Verlag, Berlim, 27-44.
2. Santoro MG (1987). Involvement of protein synthesis in the antiproliferative and the antiviral action of prostaglandins. In: Garaci E, Paoleti R & Santoro MG (Editors), *Prostaglandins in Cancer Research*. Springer, Berlin, Heidelberg, New York, Tokyo, 97-114.
3. Ross P, Lindgren JA & Hammarstrom S (1980). On the mechanism of elevated prostaglandin E_2 production in 3T3 fibroblasts transformed by polyoma virus. *European Journal of Biochemistry*, 108: 279-283.
4. Luczac M, Gurulka S, Szmigielski S & Korbecki M (1975). Inhibition of multiplication of parainfluenza 3 virus in prostaglandin-treated WISH cells. *Archives of Virology*, 49: 377-380.
5. Dore-Duffy P (1982). Differential effects of prostaglandins and other products of arachidonic acid metabolism on measles virus replication in Vero cells. *Prostaglandins, Leukotrienes and Medicine*, 8: 73-82.
6. Harbour DA, Blyth WA & Hill TJ (1978). Prostaglandins enhance spread of herpes simplex virus in cell cultures. *Journal of General Virology*, 41: 87-95.
7. Santoro MG, Carruba G, Garaci E, Jaffe BM & Benedetto A (1981). Prostaglandins of the A series inhibit Sendai virus replication in cultured cells. *Journal of General Virology*, 53: 75-83.
8. Benavente J, Esteban M, Jaffe BM & Santoro MG (1984). Selective inhibition of viral gene expression as the mechanism of the antiviral action of PGA_1 in Vaccinia virus-infected cells. *Journal of General Virology*, 65: 599-608.
9. Casals J & Whitman L (1975). Mayaro virus: a new human disease agent. I. Relationship to other arboviruses. *American Journal of Tropical Medicine and Hygiene*, 6: 1004-1011.
10. Brown DT & Condreay LD (1986). Replication of Alphaviruses in mosquito cells. In: Schlesinger S & Schlesinger MJ (Editors), *The Togaviridae and Flaviviridae*. Plenum Press, New York, 171-207.
11. Mezencio JMS, de Souza W, Fonseca MEF & Rebello MA (1989). Replication of Mayaro virus in *Aedes albopictus* cells: an electron microscopic study. *Archives of Virology*, 104: 299-308.
12. Mezencio JMS & Rebello MA (1993). Mayaro virus proteins. *Memórias do Instituto Oswaldo Cruz*, 88: 299-304.
13. Laemmli UK (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature*, 277: 680-685.
14. Bader T & Ankel H (1990). Inhibition of primary transcription of vesicular stomatitis virus by prostaglandin A_1. *Journal of General Virology*, 71: 2823-2832.
15. Ankel H, Turriziani O & Antonelli G (1991). Prostaglandin A inhibits replication of human immunodeficiency virus during acute infection. *Journal of General Virology*, 72: 2797-2800.
16. Santoro MG, Amici C, Elia G, Benedetto A & Garaci E (1989). Inhibition of virus protein glycosylation as the mechanism of the antiviral action of prostaglandin A in Sendai virus-infected cells. *Journal of General Virology*, 70: 789-800.
17. Santoro MG, Favalli C, Mastino A, Jaffe BM, Esteban M & Garaci E (1988). Antiviral activity of a synthetic analog of prostaglandin A in mice infected with influenza A virus. *Archives of Virology*, 99: 89-100.
18. Santoro MG, Jaffe BM & Esteban M (1983). Prostaglandin A inhibits the replication of vesicular stomatitis virus: Effect on virus glycoproteins. *Journal of General Virology*, 64: 2797-2801.
19. Mastromarino P, Conti C, Petruzziello R, De Marco A, Pica F & Santoro MG (1993). Inhibition of Sendai virus replication by cyclopentenone prostaglandins: a cell-mediated event associated with heat shock protein synthesis. *Antiviral Research*, 20: 209-222.
20. Barbosa JA & Rebello MA (1995). Prostaglandin A_1 inhibits replication of Mayaro virus in *Aedes albopictus* cells. *Brazilian Journal of Medical and Biological Research*, 28: 27-30.