PGJ₂, A New Antiviral Prostaglandin: Inhibition of Sendai Virus Replication and Alteration of Virus Protein Synthesis

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SUMMARY

Prostaglandin J₂ (PGJ₂) was found to suppress dramatically Sendai virus replication in African green monkey kidney cells in culture. PGJ₂ was not toxic at the active dose to uninfected cells and did not significantly inhibit macromolecular synthesis, but it specifically stimulated the synthesis of a polypeptide of 74000 mol. wt. In Sendai virus-infected cells, PGJ₂ partially inhibited virus protein synthesis and caused an alteration in the mobility of the virus glycoprotein HN in SDS-PAGE, corresponding to a decrease of about 4000 in its mol. wt. We propose that the PGJ₂-induced alteration in the molecular structure of the HN protein prevents the insertion of this protein into the cell membrane thereby blocking virus maturation. The α,β-unsaturated carbonyl group in the cyclopentane ring of the PGJ₂ molecule may be necessary for antiviral activity.

Prostaglandins (PGs) have been shown to be involved in the regulation of many physiological phenomena, including cell growth and differentiation (Santoro & Jaffe, 1987), immune function (Goodwin & Webb, 1980) and interferon action (Pottathil et al., 1980).

We have previously demonstrated that PGs of the A series are potent inhibitors of virus replication in three different systems: Sendai virus in African green monkey kidney (AGMK) cells (Santoro et al., 1980, 1981) and vaccinia virus and vesicular stomatitis virus in mouse L fibroblasts (Santoro et al., 1982a, 1983; Benavente et al., 1984). The antiviral action was found to be specific for PGs of the A series (both PGA₁ and PGA₂), whereas PGs of the B, E and F series, prostacyclin, 6-keto PGF₁α and thromboxane B₂ were inactive (Santoro et al., 1980). PGAs have also been shown to suppress encephalomyocarditis virus replication in mouse L cells (Ankel et al., 1985).

In the present report we describe the effect of prostaglandin J₂, a dehydration product of PGD₂ in aqueous solution (9-deoxy-Δ⁹-PGD₂) (Fukushima et al., 1982b), on the production of Sendai virus by an AGMK cell line (37RC) in culture.

The establishment and culture of 37RC cells have been previously described in detail (Santoro et al., 1981). Confluent monolayers of 37RC cells were infected with egg-grown Sendai virus [10 haemagglutinating units (HAU)/10⁵ cells] and, 1 h after infection, virus inocula were removed and culture medium containing PGJ₂ or control diluent was added. Sendai virus production was determined by measuring both the HAU present in the medium and haemadsorption (HAD) by infected monolayers of cells, as previously described (Santoro et al., 1981). For HAD measurements, human O Rh⁺ erythrocytes were used. After incubation at 4 °C for 60 min the monolayers were extensively washed and after cell lysis occurred the number of erythrocytes absorbed was quantified by haemoglobin (Hb) determination according to the technique of Crosby & Furth (1956). PGJ₂ did not interfere with either the haemagglutination (HA) or the HAD test. Preincubation of the virus with PGJ₂ (from 1 to 10 μg/ml) or PGJ₂ administration (4 μg/ml) at the time of testing did not alter HA; treatment of Sendai virus-infected AGMK
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Fig. 1. Effect of PGJ2 on Sendai virus production by AGMK cells. Confluent monolayers of AGMK cells, grown in RPMI-60 medium supplemented with 5% foetal calf serum (FCS, Gibco) and antibiotics, were infected with 10 HAU/10^6 cells (Santoro et al., 1980). One h p.i., virus inocula were removed and culture medium containing PGJ2 (O) or diluent control (●) was added. PGJ2 was stored as 100% ethanolic stock solution (1 mg/ml) and control medium contained the same concentration of absolute ethanol (0.02%); this had no effect on virus replication. (a) Sendai virus production (HAU/2 x 10^5 cells/ml) was determined at different times p.i. (b) dose–response curve. Data are expressed as mean ± S.D. of at least duplicate samples.

monolayers with PGJ2 (4 µg/ml) just before and/or during the HAD test had no effect on HAD (control, 99.6 ± 0.1; PGJ2, 96.3 ± 3.8 µg Hb/2 × 10^5 cells).

Fig. 1 (a) shows the effect of PGJ2 treatment (4 µg/ml) on Sendai virus production (HAU/ml) at different times after infection (p.i.). PGJ2 was administered only once, after the 1 h infection period, and culture medium was not changed in the following 72 h. PGJ2 was found to suppress Sendai virus production by more than 93% at 24, 48 and 72 h p.i. In four separate experiments PGJ2 at 4 µg/ml was able to inhibit Sendai virus production by 93 to 100%, without being toxic to uninfected AGMK cells. PGJ2 did not prevent the Sendai virus-induced cytopathic effect, but generally delayed it for 10 to 12 h.

A PGJ2 dose–response curve is shown in Fig. 1 (b). PGJ2 was added 1 h after virus infection and Sendai virus titres were determined 48 h p.i. PGJ2 antiviral activity was found to be dose-dependent; 50% inhibition of virus replication was obtained with doses of 1 µg/ml and 4 µg/ml was the most active dose that was non-toxic to AGMK cells. Similar dose–response curves have been previously described for PGA1 and PGA2 antiviral activity in the same system (Santoro et al., 1981).

Unlike interferon, which inhibits virus replication only if the host cell has been treated prior to virus infection, pretreatment of the host cell was not required for the PGJ2 antiviral effect to occur. Moreover, PGJ2 was not acting by inactivating the virus itself or by inhibiting virus adsorption by the cells, since neither the pretreatment of Sendai virus with PGJ2 (4 µg/ml) for 15 min before infection, nor the presence of PGJ2 (4 µg/ml) during the 1 h infection period, had any effect on virus replication in AGMK cells (at 24 h p.i., the HA titres were: control, 16.0 ± 2.0; PGJ2 virus pretreatment, 16.0 ± 0.0; PGJ2 during adsorption, 16.0 ± 2.0 HAU/ml; HAD measurements at 24 h were: control, 99.8 ± 0.1; PGJ2 virus pretreatment, 94.9 ± 4.9; PGJ2 during adsorption, 96.3 ± 3.8 µg Hb/2 × 10^5 cells).

At the dose at which more than 93% of virus production was inhibited, treatment with PGJ2 of uninfected AGMK cells did not produce any toxic effect, as determined by either microscopic examination or by vital dye exclusion.

The effect of PGJ2 treatment on macromolecular synthesis in uninfected as well as infected AGMK cells is shown in Fig. 2. DNA, RNA and protein synthesis were measured by the incorporation into acid-insoluble material of [3H]thymidine, [3H]uridine and [35S]methionine, respectively. A 24 h treatment of uninfected cells did not alter the uptake of [3H]thymidine, but it slightly inhibited DNA synthesis. RNA synthesis, as well as [3H]uridine uptake, were instead
Fig. 2. Effect of PGJ₂ on macromolecular synthesis in uninfected or Sendai virus-infected AGMK cells. DNA, RNA and protein synthesis were measured in confluent monolayers of cells infected or not with 10 HAU/10⁶ cells of Sendai virus and treated with PGJ₂ (4 μg/ml) or control diluent. Cells were labelled for 18 h, starting 5 h p.i., with 5 μCi/ml/2 × 10⁵ cells of [³H]thymidine, [³H]uridine or [³⁵S]methionine (Amersham) for DNA, RNA or protein synthesis respectively, and the radioactivity incorporated into acid-soluble and -insoluble material was determined (Santoro et al., 1982a). Briefly, cells were washed three times with phosphate-buffered saline and 0.4 ml 5% TCA was added to each culture. After 1 h, radioactivity in acid-soluble material was counted. Acid-insoluble radioactivity was measured after washing the TCA precipitates with ethanol three times, drying under an infrared lamp and dissolving the samples in 0.4 ml of a solution containing 0.1 M NaOH, 0.5% SDS. (a) Uptake of precursors; (b) incorporation of precursors into DNA, RNA or proteins. Data represent the mean ± S.D. of triplicate samples. □, Control; ■, PGJ₂; III, infected control; □, infected PGJ₂.

increased, while there was no significant change in protein synthesis or [³⁵S]methionine uptake (Fig. 2a).

In Sendai virus-infected cells PGJ₂ treatment caused changes in DNA and RNA synthesis and uptake of precursors similar to the alterations described in uninfected cells (Fig. 2b). Viral (actinomycin D-resistant) RNA synthesis was not altered by PGJ₂ in this system. Treatment with actinomycin D (5 μg/ml) caused a 90.2% inhibition of RNA synthesis in infected cells. No difference was found either in the uptake or the incorporation into RNA of [³H]uridine after a 20 h treatment with PGJ₂ (uptake: control, 37.2 ± 1.4; PGJ₂, 40.8 ± 2.8; incorporation: control, 0.77 ± 0.04; PGJ₂, 0.95 ± 0.11). PGJ₂-treatment caused a 17.9% inhibition of protein synthesis in Sendai virus-infected cells.

The pattern of host and virus protein synthesis was analysed after labelling with [³⁵S]methionine by separation in SDS-polyacrylamide gels and autoradiography. PGJ₂ did not alter the pattern of protein synthesis in uninfected cells, but specifically stimulated the synthesis of a polypeptide of 74000 mol. wt. (PJp74).

In Sendai virus-infected cells, PGJ₂ produced a slight general inhibition of virus protein synthesis; however, the synthesis of the glycoprotein HN was by far the most potently inhibited of all virus proteins. More interestingly, slightly altered mobility, equivalent to a mol. wt. difference of approx. 4000, of the HN protein was observed in [³⁵S]methionine-labelled cell extracts from PGJ₂-treated cells (Fig. 3a). This was also demonstrated by the densitometric analysis shown in Fig. 3(b). Preliminary experiments suggest that the slight reduction in mol. wt.
Fig. 3. Effect of PGJ₂ on protein synthesis of uninfected or Sendai virus-infected AGMK cells. (a) PAGE analysis and autoradiography of polypeptides synthesized by uninfected (lanes 1 and 2) or infected (lanes 3 and 4) cells, (2, 4) treated with PGJ₂ (4 µg/ml) or (1, 3) control diluent. PGJ₂ treatment was started after the 1 h adsorption period. Cells were labelled soon after infection with 10 µCi/10⁶ cells [³⁵S]methionine, in methionine-deprived medium, supplemented with 10% dialysed FCS. After 24 h, the radioactivity incorporated was determined (Santoro et al., 1982a) and samples were analysed by SDS-PAGE in a vertical slab gel apparatus (3% stacking gel, 7.5% resolving gel), using the buffer system described by Laemmli (1970). Gels were washed, fixed in 10% acetic acid, 10% TCA and 30% methanol, dried under vacuum and autoradiographed using DuPont Cronex films. Mol. wt. of polypeptides were calculated using the following ¹⁴C-methylated mixture (Amersham): myosin (200K), phosphorylase b (92-5K), bovine serum albumin (69K), ovalbumin (46K), carbonic anhydrase (30K) and lysozyme (14K). p74 indicates the PGJ₂ induced polypeptide PjP74. Sendai virus proteins P, HN, NP, and M are indicated. F indicates the F₀ protein from cell-grown Sendai virus, identified on the basis of its mol. wt. (b) Densitometric analysis of control (1, 3) and PGJ₂ (2, 4) autoradiographic patterns in (a). Direction of migration is indicated by the arrow at the bottom of the figure. White arrow points to the shift in position of the peak of HN, induced by PGJ₂ treatment. Bars at the bottom of the figure indicate the positions of viral markers P, HN, NP, F and M from unlabelled egg-grown purified Sendai virus.

of the HN protein in PGJ₂-treated cells might be due to an alteration of the glycosylation process.

The inability of Sendai virus to synthesize an active HN protein in the presence of PGJ₂ was also shown by the lack of incorporation of HN into the cell membrane, which can be easily determined because of the haemadsorbing property of this protein. Twenty-four h after Sendai
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virus infection, PGJ₂ treatment caused a 86.1% inhibition in haemadsorption by infected AGMK cells (control, 101·0 ± 4·5; PGJ₂, 14·0 ± 1·6 μg Hb/2 × 10⁵ cells; P < 0·01).

The possibility that PGJ₂ could be causing the production of defective virus particles devoid of HN was examined. AGMK monolayers infected with Sendai virus (10 HAU/10⁵ cells) were treated with PGJ₂ (4 μg/ml) starting after the 1 h adsorption period and were labelled with [³⁵S]methionine (10 μCi/10⁶ cells). Culture medium was harvested 48 h p.i. and, after centrifugation, supernatant proteins were precipitated with ammonium sulphate (280 μg/ml) overnight at 4 °C, in the presence of 1 mM-EDTA, 0·8 mM-N-ethylmaleimide and 0·2 mM-PMSF as proteinase inhibitors, as described by Alitalo et al. (1981). Precipitates were collected by centrifugation, washed twice with 70% ethanol, analysed by SDS-PAGE and processed for autoradiography and densitometric analysis. PGJ₂ treatment was found to reduce the secretion of [³⁵S]methionine-labelled proteins dramatically (control, 28.17 × 10⁴; PGJ₂, 3.04 × 10⁴ c.p.m./10⁶ cells), and to prevent the appearance of virus proteins in the supernatant (data not shown).

PGJ₂ antiviral activity does not seem to be limited to this system. In fact preliminary data have also shown that PGJ₂ can inhibit the multiplication of a strain of herpes simplex virus type 2 isolated in our laboratory.

PGJ₂ appears to act very similarly to prostaglandins of the A series, both in the active dose range and in the mechanism of action of their antiviral activity. PGA₁ has in fact been shown to produce similar alterations in the synthesis of the HN protein in this system (unpublished results) and of the virus G glycoprotein in L cells infected with vesicular stomatitis virus (Santoro et al., 1983). PGA₁ has also been shown to induce the synthesis of a polypeptide of the same mol. wt. as that induced by PGJ₂ in AGMK cells (Santoro et al., 1982b).

Both PGAs and PGJ₂ contain an α,β-unsaturated carbonyl group in their cyclopentane ring, and this may be necessary for the antiviral activity. Interestingly, PGs of the A and D series, as well as PGJ₂, have also been shown to possess a potent antiproliferative activity in several tumour models (Fukushima et al., 1982a,b; Honn et al., 1981; Santoro et al., 1986), and this activity has also been related to the presence of a reactive α,β-unsaturated carbonyl group in the cyclopentane ring of the molecule (Honn & Marnett, 1985).

Aside from the potential chemotherapeutic importance of these compounds, it is of great interest that the antiviral and antineoplastic activity of PGAs and PGJ₂ could be interrelated, or could be mediated by similar mechanisms.

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REFERENCES


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