Effect of Indomethacin on Epstein-Barr Virus Early Antigen Induction

Larry W. Daniel, Georg Bauer, and Harald zur Hausen

Institut für Virologie, Zentrum für Hygiene, Universität Freiburg, Hermann-Herder-Strasse 11, 7800 Freiburg, West Germany

ABSTRACT

The authors suggested that prostaglandins are involved in the induction of EBV-EA. We also compare the effects of indomethacin treatment on EBV-EA induction by chemicals with the induction by superinfection, since the two methods of induction appear to follow different pathways.

MATERIALS AND METHODS

Cells and Virus. The origin and maintenance of P3HR1 and Raji cells have been described elsewhere (24). All cells were grown in RPMI 1640 medium (Flow Laboratories, Irvine, Scotland) supplemented with 10% fetal calf serum, penicillin (100 IU/ml), and streptomycin (100 µg/ml). The cells were subcultured once or twice weekly. EBV from P3HR1 cells, kindly provided by Dr. G. W. Bornkamm, was prepared and concentrated 100-fold as described previously (8). The concentrated virus was used in superinfection assays in concentrations between 0.1- and 10-fold that of the original P3HR1 culture supernatant.

RESULTS

Reversible Inhibition of Raji Cell Growth by Indomethacin. Chart 1 illustrates the effect of indomethacin (100 µg/ml) on the growth of Raji cells. Addition of indomethacin completely inhibited growth, but the effect was reversed by removing the indomethacin and culturing the cells in fresh medium. There was a lag time before growth resumed following the removal of indomethacin; a similar delay was seen when cells were subcultured in the absence of indomethacin. When indomethacin was added to logarithmically growing cells, without subculturing, proliferation was rapidly inhibited.

Inhibition of Viral Early Antigen Induction by Indomethacin. Synthesis of EBV-EA by Raji cells was markedly reduced by indomethacin treatment at the time of stimulation with TPA (Chart 2). The inhibition was dose dependent, and a 100-µg/ml dose of indomethacin resulted in greater than 50% inhibition. When added after TPA stimulation, indomethacin was less effective in inhibiting EBV-EA induction (Chart 3). However, a significant inhibition was seen when the indomethacin was added as late...
Chart 1. Effect of indomethacin on the growth of Raji cells. Raji cells were cultured in RPMI 1640 medium (3.5 x 10^5 cells/ml), and cell counts were performed at daily intervals. Cells were incubated with (O—O) or without (•—•) indomethacin (100 μg/ml). Some cultures were incubated with indomethacin for 2 days then washed and incubated without indomethacin (O—O), or were incubated without indomethacin for 2 days, then washed and incubated with indomethacin (B—B).

Chart 2. Effect of indomethacin on the TPA-induced induction of EBV-EA antigen. Raji cells were incubated with 20 ng TPA/ml and simultaneously treated with various concentrations of indomethacin. At 48 hr after treatment, EBV-EA synthesis was measured as described in "Materials and Methods." A, percentage of total cells; B, percentage of non-indomethacin-treated control. EA, early antigen.

Chart 3. Effect of varying the time of addition of indomethacin (100 μg/ml). Raji cells were treated with TPA (20 ng/ml) at 0 hr, and indomethacin was added at the times indicated. Synthesis of EBV-EA was measured at 24 hr poststimulation. A, percentage of total cells; B, percentage of non-indomethacin-treated control. EA, early antigen.

Chart 4. Inhibition of EBV-EA synthesis by indomethacin, effect of various inducers. Raji cells were treated with various inducers alone or in combination, and the synthesis of early antigen (EA) was measured at 24 hr poststimulation. A, activated serum, 5% (v/v); B, IdUrd 50 μg/ml; C, n-butyrinic acid, 10 mM; D, activated serum plus IdUrd; E, TPA, 20 ng/ml; F, activated serum plus TPA; and G, EBV from P3HR1 cells. The cells were treated with (•) or without (O) indomethacin (100 μg/ml).

Our results confirm the previous observation that indomethacin inhibits the growth of Raji cells (15) and extends their results to show that the inhibition of growth is readily reversible. Bayer and Beaven (5) have made similar observations with rat hepatoma cells in culture. These authors found that indomethacin was cytostatic at pharmacological concentrations (100 μg/ml). The relatively high concentration required may have been due to binding of indomethacin to serum proteins, since it has been shown that 80% of the drug is bound in culture medium (6). Indomethacin is largely ionized at the pH used for cell culture, and this may also decrease the effective drug concentration due to impaired incorporation into the cell (5).

The rat hepatoma cells are inhibited by indomethacin and proceed synchronously when the drug is removed (6). In preliminary experiments, using flow cytofluorimetry to measure DNA content per cell, we have shown that Raji cells are also inhibited in G0 rather than during DNA synthesis.

Indomethacin inhibited the induction of EBV-EA synthesis in Raji cells and in all other cell lines tested when used at 100 μg/ml; however, low concentrations (1 μg/ml) were ineffective. Prostaglandin synthesis in other cell types is inhibited by 1 μg/ml, and this may indicate that the effect of indomethacin on EBV-EA induction is not due to inhibition of prostaglandin synthesis. We do not detect prostaglandin synthesis in TPA-treated Raji cells using previously published procedures (9). In these experiments, Raji cells were prelabeled with [3H]arachidonic acid treated with TPA and analyzed for labeled prostaglandins. Therefore, the

5 L. W. Daniel, unpublished data.
effects of indomethacin on Raji cells do not appear to be due to the inhibition of prostaglandin synthesis.

Inhibition of EBV-EA synthesis due to indomethacin may be related to the arrest of the cells in G1. Previous studies have shown that DNA synthesis is not required for EBV-EA induction (21); therefore, inhibition of cellular DNA synthesis is probably not responsible for the effects of indomethacin. Rather, the transition into the S phase may be required to render the cells competent for viral induction.

Indomethacin, like retinoic acid (21), did not prevent EBV-EA induction by superinfection and thus provides further evidence that the induction of viral early antigen synthesis by chemical inducers follows a different pathway from induction by superinfection. Further studies will be required to determine the effects of cell cycle phase on the molecular events leading to the derepression of the latent viral genome in EBV-positive cell lines.

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