The non-steroidal anti-inflammatory drug, indomethacin, as an inhibitor of HIV replication

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Abstract Indomethacin, a common non-steroidal anti-inflammatory drug (NSAID), has been used to treat rheumatoid arthritis. Although indomethacin has also been used as an immunopotentiator and symptomatic NSAID in AIDS, its effect on HIV replication is unknown. MT-4 lymphocytes were inoculated with HIV in the presence of indomethacin and tested for p24 expression by ELISA. The 50% inhibition (IC_{50}) was 10 μM, corresponding to plasma levels after administration of 50 mg oral indomethacin. The antiviral effect appears to be specific since no toxicity has been observed at the IC_{50} dose, and unrelated NSAIDs have not shown the activity at clinical doses. Indomethacin may, thus, represent a new class of anti-HIV drug.

Key words: AIDS; HIV; Indomethacin; Lymphocyte; Membrane lipid; Prostaglandin

1. Introduction

After fifteen years of the AIDS epidemic no effective anti-HIV therapy has been developed. This is partly due to the fact that the focus of research has been concentrated on a limited number of viral targets, e.g. reverse transcriptase and HIV protease. Despite an enormous wealth of accumulated knowledge about HIV and its relationship with host cells, so far, this information has not been translated into meaningful therapy. However, it is clear that an antiviral strategy needs to be diversified by investigating compounds aimed at cellular targets.

In the past we have investigated several drugs affecting primarily cellular functions [1] and found that they were acting as HIV inhibitors at pharmacologically relevant concentrations without exhibiting the cytotoxicity usually associated with administration of anti-HIV drugs like AZT. These compounds, e.g. estrogen, progesterone, chorionic gonadotropin, coumarins, warfarin, bestatin, levamisole, various plant-derived proteins, etc., were already used in clinics for unrelated therapeutic purposes.

Indomethacin is a non-steroidal anti-inflammatory drug (NSAID) with multiple effects on immune function [2]. This compound is among the drugs of choice for relieving pain and fever. For more than thirty years indomethacin has been used for symptomatic treatment of rheumatoid arthritis and related conditions. The exact mechanism of anti-inflammatory, analgesic, and antipyretic action of indomethacin is not known, although it has been commonly associated with interference of fatty acid metabolism, i.e. inhibition of prostaglandin synthesis by blocking cyclooxygenase (CO) activity.

Indomethacin has been proposed and subsequently used, albeit with mixed results, for the improvement of the immune function of HIV-positive patients [3–5]. However, to the best of our knowledge, there are no published reports investigating the direct effect of indomethacin on HIV replication. In this study we have examined this particular indole derivative for its capacity to prevent de novo HIV infection, and compared it to other structurally related and unrelated NSAIDs in a standard antiviral assay based on measurement of p24 production.

2. Materials and methods

2.1. Drugs

Various antagonists of CO, including indomethacin, indoprofen, naproxen, ibuprofen, acetylsalicylic acid (aspirin), and 5-lipoxygenase (LP) inhibitor, nordihydroguaiaretic acid (NDGA), were purchased from Sigma (St. Louis, MO), stored at 4°C as stock 100 mM solutions in appropriate solvents and used thereafter.

2.2. Virus

Virus inoculum was derived from 0.22 μm-filtered supernatant of H9/IIIB lymphocytes and stored frozen at −70°C as a stock solution at 100 ng p24 per ml. The number of HIV particles in culture supernatant was determined by p24 ELISA and infectious viral titer was determined by limiting end-point dilution assay [6].

2.3. Antiviral assay

The CD4+ T cell line MT-4 was used as the indicator cell for the p24 expression assay. Commercial p24 ELISA kits were purchased from Coulter (Hialeah, FL). All antiviral assays were carried out in 96-well culture plates with each well containing 2×10^4 MT-4 cells in 200 μl RPMI 1640 medium with 10% FCS. Serial ten-fold dilutions of test drugs were added and followed by addition of the virus. The final concentration of the viral inoculum in microwell cells was equivalent to 100 infectious units per lymphocyte. The mixture of cells, virus, and drugs was left for 2 days until tested for p24. The values of p24 corresponding to original viral inoculum left in wells without MT-4 were used as blanks, in order to discriminate newly synthesized virus from residual virus inoculum. Each drug was tested at least three times in triplicate wells.

2.4. Cytotoxicity assay

The toxicity of NSAIDs was determined by colorimetric assay of the viability of MT-4 cells. The assay is based on measuring the conversion of XTT tetrazolium salt by mitochondrial hydrogenases into a color dense formazan product [7]. The culture conditions, culture plates and initial cell number of MT-4 were the same as in the antiviral assay. Serial log concentrations of NSAIDs were added to MT-4, incubated for 2 days, and XTT (1 mg/ml) was added for 3 h. The optical density was then measured in a plate reader (SLT Spectra, Salzburg, Austria) at a wavelength of 450 nm with the reference filter at 620 nm.

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Abbreviations: AIDS, acquired immunodeficiency syndrome; CH, cholesterol; CO, cyclooxygenase; HIV, human immunodeficiency virus; LP, 5-lipoxygenase; NGDA, nordihydroguaiaretic acid; PG, prostaglandin; PL, phospholipid.

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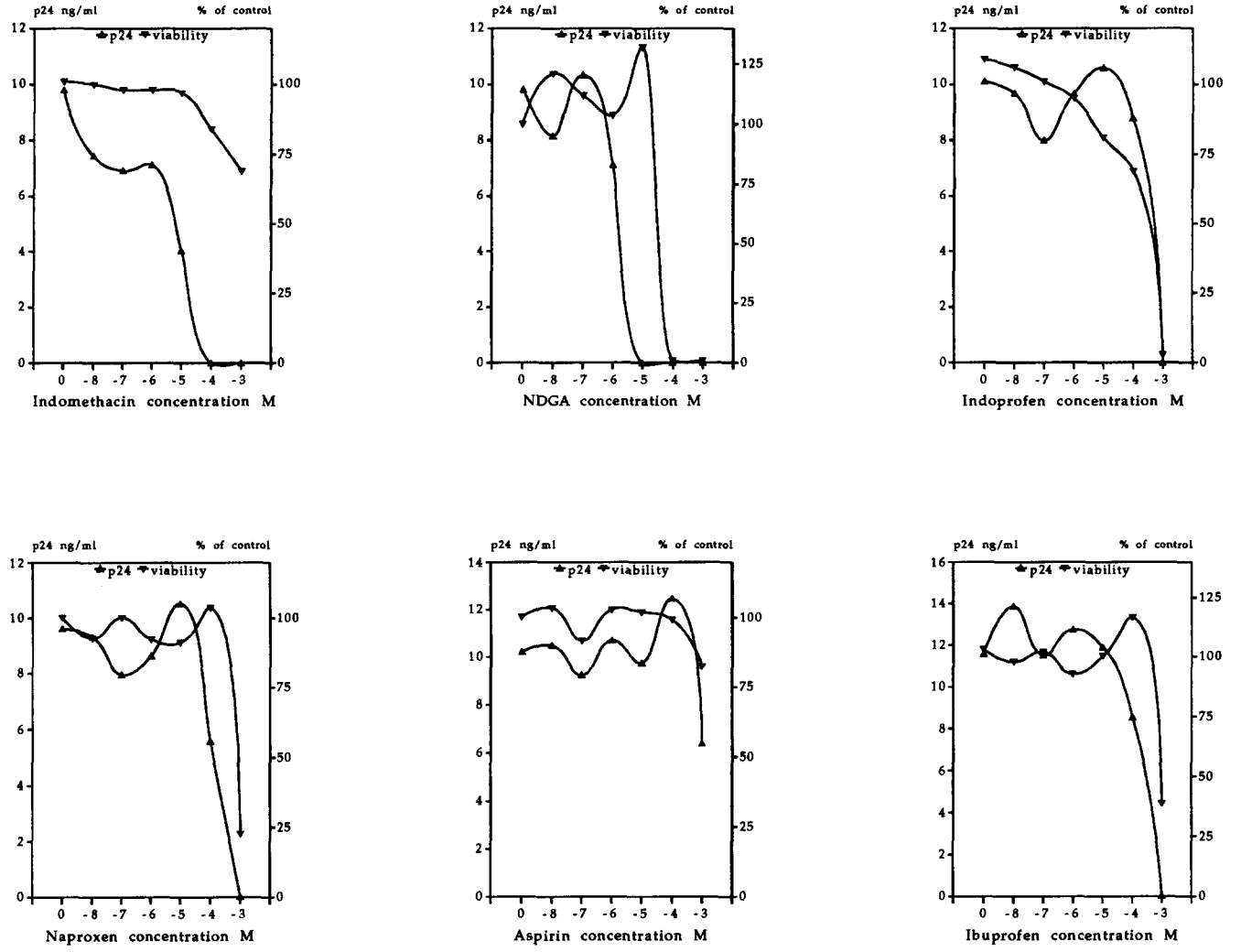

Fig. 1. The effect of various NSAIDs on HIV infection as measured by p24 ELISA of MT-4 culture supernatant on day 2. Effect of NSAIDs on MT-4 viability following 2 days of continuous exposure as measured by XTT assay is shown as percent of controls on the left ordinate. Naproxen, indoprofen, and ibuprofen were effective but at a 1 mM cytotoxic dose. Aspirin had no significant effect even at the highest tested dose. Indomethacin and the experimental lipoxygenase inhibitor, nordihydroguaiaretic acid (NDGA), were able to suppress HIV replication without a concurring negative effect on cell viability.

3. Results

At two days post-infection, virus-inoculated MT-4 cells started to display signs of cytopathic effect, i.e. syncytia formation. The number of syncytial cells appeared to correlate inversely with the concentration of indomethacin present in treated wells. This phenomenon was not observed in wells treated with equimolar concentrations of other NSAIDs. Instead of relying on counting syncytial cells – a biased and obsolete assay – the effect was quantitated by measuring the levels of p24 antigen by ELISA. The results are summarized in Fig. 1. The 50% inhibitory concentration (IC_{50}) was equivalent to 10 μM of indomethacin and was determined on the basis of three separate experiments with three replicate wells. Other CO inhibitors, i.e. aspirin, indoprofen, ibuprofen, and naproxen, have not shown any appreciable activity at concentrations that were not associated with concurring cytotoxicity. The least toxic drug, aspirin, was the least effective against HIV. In contrast, the LP inhibitor, nordihydroguaiaretic acid, has demonstrated an anti-HIV effect at a non-toxic concentration of 10 μM.

Although some NSAIDs appeared to be cytolytic at effective concentrations, indomethacin was not toxic since the morphology of treated cells was indistinguishable from untreated controls. This was confirmed by cell viability XTT tests (Fig. 1).

4. Discussion

In this study we have evaluated the effect of select inhibitors of cyclooxygenase and lipoxygenase. De novo HIV infection was suppressed in a dose-dependent fashion by a CO antagonist, indomethacin. Nordihydroguaiaretic acid, although less selective, has also shown antiviral activity at non-toxic concentrations. The structurally related and unrelated NSAIDs such

as indoprofen, aspirin, ibuprofen, and naproxen, recognized as equally potent inhibitors of prostaglandin (PG) synthesis, were not effective in the pharmacological non-toxic dosage range. This suggests that the anti-HIV effect is determined by a unique property of indomethacin and agrees with the opinion that the cellular action of indomethacin does not necessarily involve PG inhibition [8]. The anti-HIV role of PGs themselves has been controversial; while one group claimed that PGs were enhancing the replication of HIV [9], others reported the opposite effect [10–12].

Little is known about how viral infection can be prevented by suppressing the function of CO and LP as precursors of prostaglandins and leukotrienes. Schroder et al. [13] have associated the effect of sesquiterpenoid hydroquinone, an experimental HIV inhibitor, with reduced levels of eucosanoids via inhibition of both CO and LP. Other investigators who have observed the suppressive effect of indomethacin on various types of viruses have indicated that the effect could not be attributed to inhibition of PG [14–17]. Furthermore, indomethacin was occasionally shown either to have no effect [18] or to enhance virus yields and pathogenic effects in the host [19,20]. Thus, despite the unusual broad-spectrum activity of indomethacin interfering with replication of many types of viruses, the mechanism remains unclear.

Some researchers have turned their attention to indirect explanations such as an enhancement of cellular immune response to viruses [21]. Others attributed its effect to modification of lipid composition and fluidity of the cell membrane, implicated in both the regulation of immune function and virus infection [22]. Several studies have demonstrated a correlation between the infectious capacity of HIV in vitro and alterations in the plasma membrane lipid composition [23–29]. Phospholipids (PL) and cholesterol (CH) are important components of cell membrane fluidity, prostaglandin and leukotriene synthesis, and cellular metabolic processes such as inflammatory processes, carcinogenesis, and viral infection. Lipid profile alterations leading to increased concentration of membrane CH and/or polyunsaturated lipids causes a decrease in membrane fluidity. The CH/PL ratio is two times higher in HIV-infected than in uninfected cells. The evidence from clinical observations also indicates that patients with AIDS exhibit marked disturbances in lipid, lipoprotein, and triglyceride metabolism [30]. The relationship between HIV infection, changes in membrane fluidity and activation of phospholipase and the arachidonic acid cascade in lymphocytes has been well documented [31,32]. The elevation in the levels of unsaturated fatty acids leads to an increase in cell membrane fluidity and has been associated with enhanced virus–cell fusion and syncytium formation [28,29]. Since indomethacin causes a decrease in membrane fluidity it is possible this mechanism is responsible for the observed effect in our study. In contrast, aspirin, shown to have an opposite effect on the membrane status, is the least active drug in our assay [8,33].

Oral administration of 50 mg of indomethacin generates a peak plasma concentration of the drug ranging from 7 to 11 μM [2], a concentration which inhibits HIV replication in vitro. Indomethacin has already been used in clinics by HIV-infected individuals. While some groups have doubted that the benefit of indomethacin could be ascribed to anything more significant than simple relief from pain and fever [5], others claimed a beneficial immunopotentiating effect such as improved lymphoproliferative response and restoration of the CD4/CD8 ratio [3,4]. Unfortunately, there are no studies aimed at establishing its effect on the levels of viremia and it is unknown whether this drug may have an antiviral effect in vivo.

The concept of manipulating lipid composition and fluidity of the viral or host cell membrane by inhibitors of CO and LP has already been utilized for targeted therapeutic interventions [13,34]. The membrane perturbing amphiphile, indomethacin, represents a new class of molecules which may help to better understand the relationship between HIV and host cells and may serve as the lead compound for designing even more effective anti-AIDS drugs.

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