

Antiproliferative effect of indomethacin in Aedes albopictus (mosquito) cells

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Abstract

Non-steroidal and anti-inflammatory drugs (NSAIDs) are a class of pharmacological agents that are traditionally used for their anti-cyclooxygenase properties in the treatment of inflammation and other associated diseases. Recently, however, it has been demonstrated that several NSAIDs exhibit antiproliferative effects in various animal models and in vitro experiments. In this paper we demonstrated that treatment of Aedes albopictus cells (mosquito) with indomethacin results in a reduction of normal growth rate. Cells treated with 100 µM indomethacin exhibited a profound time dependent reduction in their proliferation rate over the 96 h test period.

Keywords: *Indomethacin – Antiproliferative effect – Aedes albopictus cells.*

INTRODUCTION

Indomethacin is a non-steroidal anti-inflammatory drug (NSAID) used in the treatment of inflammation and related diseases. The biochemical mechanism generally ascribed to these effects have been the inhibition of cyclooxygenase (COX) enzymes. (CROFFORD, 1997; FUNK, 2001).

COX is the key enzyme of conversion of arachidonic acid to eicosanoids (prostaglandins, tromboxanes and leucotrienes). Prostaglandins, which is largely produced during inflammation, function as a microenvironmental hormone and intracellular signal mediators and also participate in the regulation of a large variety of physiological and pathologic processes (HINMAN, 1972; FUNK, 2001).

While eicosanoids are very well known in mammalian systems, there is an increasing recognition of the importance of these compounds in insects and other invertebrates. Insects elaborate

defense response to microbes infection (humoral and hemocytic) which involves the presence of eicosanoids. Inhibition of eicosanoid formation in larvae of the insect of the tobacco hornworm Manduca sexta, using specific inhibitors of phospholipase A₂ (dexamethasone), cyclooxygenase (indomethacin) and lipoxygenase (esculetin) reduces the ability of larvae to clear the bacterium Serratia marcescens from their hemolymph. The reduced capacity to remove bacteria is associated with the increased of mortality due to this bacteria (STANLEY-SAMUELSON et al., 1991; MILLER; NGUYEN; STANLEY-SAMUELSON, 1994).

Besides the anti-inflammatory effects of indomethacin, several reports have been published showing that this compound attenuate tumor growth (ELI et al., 2001). Results obtained in this paper demonstrate that indomethacin has an antiproliferative action in a mosquito (Aedes albopictus) cell line.

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MATERIALS AND METHODS

Cell culture

Aedes albopictus cells, clone C6/36, were used in this study. This cell line was a gift from the Arbovirus Research Unit, Yale University, USA. The cells were maintained at 28°C in Leibovitz's (L-15) growth medium, supplemented with 0.2 mM non-essential amino acids, 0.3 % tryptose phosphate broth, 0.02% L-glutamine, 10% fetal bovine serum, penicillin (500 U/mL), streptomycin (100 µg/mL) and amphotericin B (fungizone, 2.5 µg/mL).

Indomethacin treatment

Indomethacin, (1-p-chlorobenzoyl)-5-methoxy-2-methyl indole-3 acetic acid, was obtained from Sigma Chemical CO (St. Louis, MO). This compound was prepared as a stock solution (2.5 mM) in ethanol and diluted in growth medium to the indicated concentrations. Cell numbers were counted in a hemocytometer and cell viability was determined by the vital dye exclusion technique (0.1 % trypan blue). Control medium contained the same concentration of ethanol diluent (0.02%), which did not affect cell viability or metabolism.

Data are expressed as a mean \pm SD, and *p* values of less than 0.05 were considered significant.

RESULTS AND DISCUSSION

To investigate the effect of indomethacin in *A. albopictus* cells proliferation, logarithmically growing cells (2×10^5 cells/mL) were plated in petri dishes and treated with different concentrations of indomethacin. After 48 h cells were detached from the petri dishes by trypsinization and were counted (Figure 1). In these conditions indomethacin inhibited cell proliferation dose-dependently beginning at a concentration of 10 µM. Concentrations of indomethacin as high as 100 µM inhibited cell proliferation without altering cell viability.

Aedes albopictus cells treated with indomethacin (100 µM) immediately after plating or after 24 h, exhibited a profound time dependent reduction in their proliferative rate

over the 96 h test period (Figure 2). A significant decrease in cell growth, compared to control, was observed starting at 48 h on treatment with indomethacin which exerted their inhibitory effect without being toxic to the cells.

Cell viability after 96 h, was 97% in both control and indomethacin treated cells. Lack of toxicity was also shown by the ability of *A. albopictus* to recover their growth potential after the removal of indomethacin from the culture medium by washing the cells.

As we demonstrated in this paper the treatment of *A. albopictus* cells with indomethacin drastically reduces cellular proliferation. Indomethacin can inhibit the activity of both cyclooxygenase (COX) isoforms, COX-1 and COX-2, thereby blocking the production of prostaglandins and other eicosanoids (TAVARES; BENNET, 1993). These compounds are well known for their importance in mammalian physiology (HINMAN, 1972; FUNK, 2001). However, recent work has revealed the presence and biological actions of eicosanoids in insects and other invertebrate animals. In insects, eicosanoids mediate cellular immunity to microbial challenge (STANLEY, 2006). In addition, it was demonstrated that prostaglandin A_1 reduces the replication of Mayaro virus and vesicular stomatitis virus (VSV) in cultured *A. albopictus* cells (BARBOSA; REBELLO, 1995; BURLANDY; FERREIRA; REBELLO, 2004).

In mammalian models it was demonstrated that NSAIDs given "in vivo" to rodents and humans can inhibit tumor growth (LUNDHOLM et al., 1994). An explanation for the antineoplastic properties was suggested by Adolphe, Deysson e Lechat (1972) who reported that certain NSAIDs were capable of inhibiting the proliferation of Hela cells by causing cell cycle arrest. Recently, other groups have shown that NSAIDs can induce apoptosis in tumor cell lines (ADOLPHE; DEYSSON; LECHAT, 1972).

A key question is whether NSAIDs exert their anticarcinogenic effects "in vivo" by a mechanism that is dependent of their capacity in inhibit COX activity. Data in literature are still insufficient to suggest a clear mechanism for the antiproliferative action of NSAIDs.

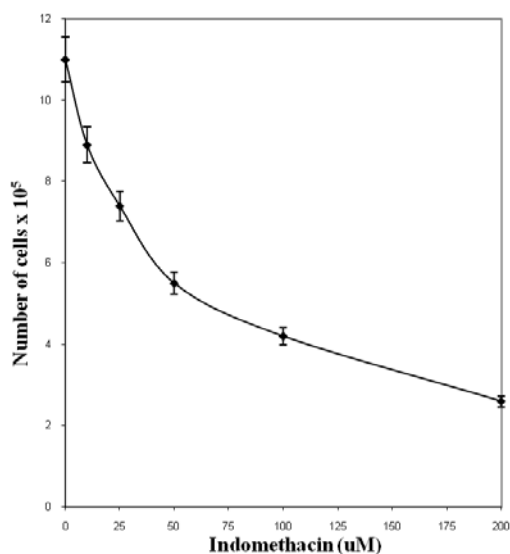


Figure 1 - Concentration-dependent effect of indomethacin on the growth of *A. albopictus* cells.

Notes: - cultures *A. albopictus* cells, plated at a density of 2.5×10^5 cells/mL, were treated at different concentrations of indomethacin; - cell numbers were determined 48 h after plating as described in Material and Methods.

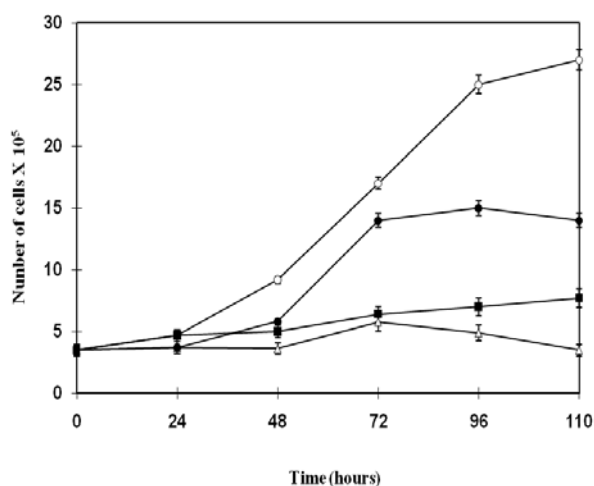


Figure 2 - Time-dependent effect of indomethacin (100 μ M) on the growth of *A. albopictus* cells.

Notes: - *A. albopictus* cells were plated at a density of 2.5×10^5 cells/mL and cell numbers counted at 24 h intervals; - control (o) indomethacin treated just after cell plating; (●) indomethacin treated after 24 h cell plating (■) and indomethacin treated just after cell plating and after 24 h cells washed and maintained in growth medium (Δ); - numbers of cells were determined as described in Material and Methods.

CONCLUSION

In this paper we described the antiproliferative effect of indomethacin in a mosquito cell line, *Aedes albopictus* clone C6/36.

This compound at a non toxic concentration (100 μ M) inhibits the normal proliferation of these cells. In our knowledge this paper described for the first time the antiproliferative effect of indomethacin in a mosquito cell line.

Efeito antiproliferativo da indometacina em células de *Aedes albopictus* (mosquito)

Resumo

Os antiinflamatórios não esteróides (AINEs) pertencem a uma classe química de agentes farmacológicos que são tradicionalmente utilizados devido às suas propriedades anti-ciclooxigenase, no tratamento da inflamação e de outras doenças relacionadas. Recentemente, tem sido demonstrado que diversos AINEs apresentam também efeito antiproliferativo em vários modelos animais e em experiências *in vitro*. Neste trabalho, demonstramos que o tratamento de células de mosquito (*Aedes albopictus*) com indometacina resultou na redução do ritmo normal de crescimento da cultura. Em células tratadas com indometacina (100 μ M), verificamos uma profunda redução, tempo-dependente, da proliferação celular durante 96 h de período experimental.

Palavras-chave: Indometacina – Efeito antiproliferativo – Células de *Aedes albopictus*.

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