EFECTUL ANTIMICROBIAN AL UNOR MEDICAMENTE ANTIINFLAMATOARE NESTEROIDIENE

Antimicrobial effect of some non-steroidal anti-inflammatory drugs

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REZUMAT

Medicamentele antiinflamatoare nesteroidiene sunt cele mai prescrise medicamente în toată lumea, fiind prima alegere în tratamentul afecțiunilor inflamatorii degenerative. Din acid arahidonic se sintetizează eicosanoidele, compuşi care au activitate fiziologică şi farmacologică, cunoscuți ca şi prostaglandine (PG), tromboxani (TX), leucotriene (LT) şi lipoxine (LX). Acidul arahidonic este un precursor al eicosanoidelor, care sunt cunoscute ca fiind factori de virulență la specia *C. albicans,* stimulând formarea tubilor germinativi şi inflamația în timpul infecției, el fiind incorporat în fosfolipidele din drojdii. În ultimii ani s-a demonstrat că unele NSAIDs au proprietăți inhibitorii asupra biofilmului şi a viabilității diferitelor specii microbiene. Gradul de inhibiție asupra bacteriilor şi fungilor variază în funcție de tipul de medicament utilizat şi de virulența tulpinilor.

Pentru a evidenția posibillele acțiuni farmaceutice ale unor NSAIDs (aspirină, diclofenac de sodiu, piroxicam şi ibuprofen) a fost studiată acțiunea microbiană a acestor compuşi împotriva mai multor specii bacteriene şi fungice patogene.

Cuvinte cheie: NSAIDs, antibacterian, prostaglandine, drojdii

ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely prescribed drugs worldwide, being the drug of first choice in the treatment of degenerative inflammatory diseases. Arachidonic acid gives rise to eicosanoids, physiologically and pharmacologically active compounds known as prostaglandins (PG), thromboxanes (TX), leukotrienes (LT) and lipoxins (LX). AA is a precursor for production of eicosanoids, known virulence factors, stimulating germ tube formation and inflammation during infection and can be incorporated into the phospholipids of yeasts. It has been shown that some of NSAIDs have biofilm and viability inhibiting properties. The degree of inhibition of bacterial and fungal varies with type of drug used and the virulence of strains.

For illustrating any possible pharmaceutical activities of some NSAIDs (aspirin, sodium diclofenac, piroxicam, and ibuprofen), the antimicrobial action of these compounds was investigated in different studies against many isolated strains of pathogenic bacteria and yeasts.

Key words: NSAIDs, antibacterial, prostaglandins, yeasts

INTRODUCTION

A number of non-antibiotic drugs possess an antimicrobial effect that has generally been regarded as a side-effect, as is the case with antidiuretic, antidiabetic, psychotherapeutic and non-steroidal anti-inflammatory drugs (NSAIDs). Non-steroidal anti-inflammatory drugs are among the most widely

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prescribed drugs worldwide, being the drug of first choice in the treatment of degenerative inflammatory diseases. Arachidonic (AA) acid is subsequently converted by lipooxygenases and cyclooxygenases (COXs) to eicosanoids. Inhibition of cyclooxygenases, and therefore prostaglandins production, is the common mechanism of action of the NSAIDs. In addition, AA is a precursor for production of eicosanoids, known virulence factors, stimulating germ tube formation and inflammation during infection and can be incorporated into the phospholipids of yeasts. Non-steroidal anti-inflammatory drugs (sodium diclofenac, aspirin, piroxicam, and ibuprofen) are inhibitors of the cyclooxygenase (COX) isoenzymes. These drugs specifically block the biosynthesis of mammalian prostaglandins by inhibiting one or both of COX isoenzymes. Eicosanoids are synthesized via two pathways: the cyclooxygenase pathway and the lipooxygenase pathway. COX is the key enzyme in the synthesis of PGs, prostacyclin and TXs. Two isoforms of the COX enzyme have been characterized: cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). These two isoforms of COX are almost identical in structure but have important differences in substrate and inhibitor selectivity. COX-1 is constitutively present in almost all tissues and it produces protective PGs maintaining homeostasis in many organs (e.g. stomach, kidney), while COX-2 is induced by inflammatory stimuli and by cytokines. In addition to the induction of COX-2 in inflammatory lesions, it is present constitutively in the brain and spinal cord, where it may be involved in nerve transmission, particularly that for pain and fever. PGs made by COX-2 are also important in ovulation and in the birth process.

For illustrating any possible pharmaceutical activities of some NSAIDs (aspirin, sodium diclofenac, piroxicam, and ibuprofen), the antimicrobial action of these compounds was investigate in different studies against many isolated strains of pathogenic bacteria and yeasts.

MECHANISM OF ACTION OF NSAIDS

Metabolites of AA may serve both as mediators of inflammation and as physiologic agonists deleterious to tissues. The mode of action by which NSAIDs reduce inflammation seems to be their ability to inhibit metabolism of AA. The highly complex AA cascade is shown in Fig. 1.

The cascade begins when AA is liberated from membrane phospholipids. Arachidonic acid, a C_{20} polyunsaturated fatty acid (eicosanoid), is

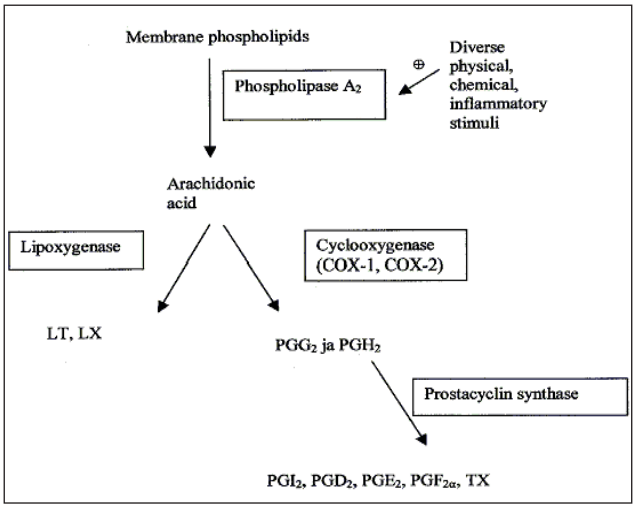

FIGURE 1. *Synthesis of PG, TX and LT*

incorporated into cell membrane phospholipids. To be an utilizable substrate for eicosanoids synthesis, the AA must be available as a free acid. Two pathways have been demonstrated for the liberation of AA from cellular phospholipids: a direct pathway involving stimulation of phospholipase A_2, and an indirect pathway involving a phosphatidy linositol-specific phospholipase C followed either by a diacylglycerol lipase or a phosphatidic acid specific phospholipase A_2 (1). Sources from which humans obtain AA include red meat, certain green leafy vegetable, and vegetable oils containing linoleic acid. In the body the linoleic acid is desaturated and elongated to form AA. Stimul activating the arachidonic acid cascade can by physical (ultraviolet B irradiation and trauma), specific immunologic reactions (IgE), or nonspecific reactions.

AA is metabolized by different pathways. The cyclooxygenase pathway leads to the formation of prostaglandin E_2 (PGE_2), prostaglandin $F_{2\alpha}$ (PG $F_{2\alpha}$), prostaglandin D_2 (PGD_2), prostacyclin (PGI_2), and thromboxane (TXA_2). At this step, oxygen-derived free radicals are liberated and may be responsible for the inflammatory response and the accompanying tissue damage. TXA_2 induces platelet aggregation and vasoconstriction. All NSAIDs inhibit prostaglandin synthesis by inhibition of cyclooxygenase enzyme. The 5-lipoxygenase pathway leads to the formation of 5-hydroxyeicosatetraenoic acid (5-HETE) and the leukotriens (LK). LKs are important in the inflammatory response. The 12-lipoxygenase pathway leads to the formation of 12-hydroxyeicosatetraenoic acid (12-HETE).

Cyclooxygenase exist in at least two isoforms designated as COX-1 and COX-2. The two isoenzymes are encoded by different genes and have

unique patterns of expression. COX-1 and COX-2 are responsible for the production of prostaglandin H_2, the first step in prostanoid biosynthesis. The COX-1 isoenzyme is essential for the maintenance of normal physiologic states in many tissues including the kidney, gastrointestinal tract, and platelets. For example, COX-1 activation in the gastric mucosa leads to prostacyclin production, which is cytoprotective. COX-2, a second cyclooxygenase isoenzyme primarily responsible for synthesis of the platelet inhibitors PGI_2 by endothelial cells (2) is induced in response to inflammatory stimuli, and is less sensitive to the effects of aspirin. The inducing stimuli include proinflammatory cytokines and growth factors, implying COX-2 in both inflammation and control of cells growth (3). The COX enzymes play an important role in cardiovascular homeostasis (4). COX-1 and COX-2 are membrane-bound proteins that reside, after synthesis and transport, primarily in the endoplasmic reticulum. Although, the genes for COX-1 and COX-2 are clearly different, the proteins actually share 60% homology at the amino acid level. Studies of the tertiary structures of COX-1 and COX-2 have demonstrated that the amino acid conformation for the substrate binding site and catalytic regions are almost identical. However, there are important differences in these regions, particularly the exchanges of Ile in COX-1 for Val in COX-2 at position 434 and 523. These substitutions results in a larger and more flexible substrate channel in COX-2 than in COX-1 and in the inhibitor binding site in COX-2, being 25% larger than that in COX-1. Although COX-1 and COX-2 have nearly identical kinetics properties, COX-1 shows negative allosterism at low concentrations of arachidonic acid. This suggests that COX-2 may be a better competitor than COX-1 for arachidonic acid released within the cell (5).

Anti-inflammatory non-steroidal agents could be divided into four main groups: *(1)* compounds capable of producing full inhibition of both COX-1 and COX-2 with poor selectivity; *(2)* compounds capable of producing full inhibition of both COX-1 and COX-2 with preference toward COX-2; *(3)* compounds that strongly inhibited COX-2 with only weak activity against COX-1; and *(4)* compounds that appeared to be only weak inhibitors of COX-1 and COX-2 (6). The relationship between NSAID use and serious gastrointestinal toxicity complications has been examined in a number of studies. One of the most complete studies is a meta-analysis of reports between 1985 and 1994 in which 11 NSAIDs were ordered for their association with serious complications. The order of the NSAIDs, from the least to most damaging, was 1-ibuprofen, 2-diclofenac, 3-diflunisal, 4-fenoprofen, 5-aspirin, 6-sulidac, 7-naproxen, 8-indomethacin, 9-piroxicam, 10-ketoprofen, and 11-tolmetin. Comparison of the COX-1 selectivity of these compounds demonstrates that compounds associated with the greatest gastrointestinal toxicity have the greatest COX-1 selectivity. NSAIDs vary in their relative inhibitory effects on COX-1 and COX-2. Aspirin is approximately 166 times more potent an inhibitor of COX-1 as compared with COX-2 (4).

EFFECTS OF NSAIDS ON SOME PATHOGEN BACTERIA

Diclofenac sodium has remarkable inhibitory action both against drug-sensitive and drug-resistant clinical isolates of various Gram-positive and Gram-negative bacteria. Dutta N.K. and his colleagues had determinate the ability of diclofenac to protect mice from a virulent *Salmonella* infection. Their study had demonstrated that diclofenac (1.5-3 microg/g) protected animals from the lethality of *Salmonella* (7). The time-kill curve study indicates of diclofenac comes in part, from its ability to inhibit the DNA synthesis of *E. coli* and *L. monocytogenes*. Diclofenac could protect murine listeriosis, salmonellosis, and tuberculosis at doses ranged within its maximum recommended human or non-toxic *ex-vivo* doses (8).

Although few studies found that ibuprofen and acetaminophen has significant effects to reduce some of body disorders after bacterial infection, antibacterial action of these agents are not clear for many species of pathogenic bacteria. Ibuprofen and acetaminophen were tested for antibacterial activity against seven isolates of bacteria including gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtillis*) and gram negative bacteria *(E. coli*, *Enterobacter aerogene, E. cloacae, Salmonella typhi* and *Paracoccus yeei*) (9). The *Staphylococcus aureus* and *Paracoccus yeei* strains were susceptible to lower concentration of ibuprofen and acetaminophen (MIC=1.25 mg/ml) and *Enterobacter* were resistant. The same strains were tested with diclofenac sodium, indomethacin and mefenamic acid. Diclofenac seems to be effective to inhibit the growth of bacteria in lower concentration (2.5-5 mg/ml). *Staphylococcus aureus* could be considered the most susceptible bacteria to diclofenac than other strains (according to disc diffusion method). Spectrophotometer assay gave much more valuable value about the inhibitory action of tested chemical

agents. Diclofenac sodium also considered the powerful compound on tested bacteria. Comparing with control, the growth of all isolates was significantly reduced by 2.5mg/ml (MIC) of diclofenac sodium. Meanwhile, *Paracoccus yeei* tend to be the most susceptible strain to lower level of diclofenac (0.15-0.3mg/ml) followed by *B. subtillis* and *S. aureus* (0.6-1.25mg/ml) (10).

In another study, aspirin or ibuprofen was administered to mice undergoing treatment of tuberculosis infection (*Mycobacterium tuberculosis*) to determine if these non-steroidal anti-inflammatory drugs enhance pyrazinamide activity *in vivo* (11). Simultaneous administration of either aspirin or ibuprofen with pyrazinamide resulted in a further decrease of about 0.4 $\log_{10}$ CFU in the lung and more than 1 $\log_{10}$ CFU in the spleen compared with mice receiving pyrazinamide alone. Aspirin and ibuprofen enhance the effect of pyrazinamide during the initial phase of tuberculosis treatment in the mouse model.

The antimicrobial ability of diclofenac sodium, indomethacin and mefenamic acid to eliminate pathogenic organisms is not limited with direct inhibitory action of those organisms, but also includes indirect effects by using the main function of such compounds as anti-inflammatory to facility the destruction of affected organisms. In meningitis patients, diclofenac sodium and indomethacim reduce the inflammation resulted from infection with bacterial meningitis (12).

EFFECTS OF NSAIDS ON *CANDIDA ALBICANS*

Candida albicans is the commonest causative agent of human fungal infection and an opportunistic dimorphic fungus that inhabits various host mucosal sites. *Candida* species are found in the human gastrointestinal tract, from oropharynx to anus, in the female gynecological tract and on the skin. Small number of yeast colonies is normally present, increasing in number when the normal microbial flora is altered by antibiotics or when there is a defect in immune competence. *Candida albicans* can cause both superficial and serious systemic disease. Conversion from the yeast to the hyphal form has been associated with increased virulence and mucosal invasiveness. The prophylactic and curative treatment with antifungal drugs can cause the appearance of *Candida* resistant-strains to these antifungal drugs. During *Candida albicans* infection, arachidonic acid (AA) is released from phospholipids of infected host cells by fungal phospholipases (13). *C. albicans* utilizes the released AA as the sole carbon source for cell growth and morphogenesis.

There are studies which evidence the capacity of sodium diclofenac to inhibit the yeast to hypha transition. *C. albicans* is a fungus that can exist in three morphotypes: budding yeast, pseudohypha and true hypha. Fungal yeast-mycelium dimorphism is of interest because of the economic and medical importance of dimorphic fungi and because these organisms may serve as model for studying differentiation. The presence of the filamentous form and budding is associated with virulence and pathogenicity, but both forms may be involved in the development and progress of disease. *C. albicans* is able to develop single spherical cells including typical yeast cells and chlamydospores, as well as elongated cells developing into multicellular true hypha or pseudohypha. Thus, the term dimorphism, which traditionally is reserved for the yeast-true hypha inter-conversion, in a more general sense designates the main theme of *C. albicans* and possibly fungal morphogenesis in general. Budding-yeast cells can be induced to form true hypha, which grow by continuous apical extension followed by septation. Pseudohypha grew differently from true hypha, by unipolar budding: buds develop into elongated cells, which remain attached to mother cells, stop growth and resume budding. Some environmental factors have been reported as determinants of morphological regulation, particularly in *C. albicans*. Exogenous PGE_2 from either host or fungal sources enhances germ tube formation in *C. albicans*, implicating fungal eicosanoids as a morphogenic factor (14). Prostaglandins production could be inhibited by diclofenac or aspirin, which also suppresses the growth of the yeast form and prevents the yeast to hypha transition of *Candida albicans*. For the production of germ tubes, diclofenac registered an important inhibition effect on *C. albicans* cells (15). In another study, Ghalehnoo Z.R. and his colleagues (16) presented the role of sodium diclofenac in the dimorphic transition in *Candida albicans*. The results indicated that effect of diclofenac was dependent on the concentration of this compound and preincubation with 500 microg/ml diclofenac completely inhibited hypha formation in liquid and solid media. There is some evidence that diclofenac inhibits the lipooxygenase pathways, thus reducing formation of the leukotrienes. Also, diclofenac may inhibit phospholipase A_2 as part of its mechanism of action. Phospholipase A_2 and phospholipase B have been identified in a large number of eukaryotic microbes, including *Candida albicans*, *Cryptococcus neoformans* and

Aspergillus fumigatus. Phospholipases A and B cleave the fatty acid side chains of phospholipids have been implicated in virulence in a number of parasitic and antifungal species, presumably via destruction of host cell membranes and subsequent lysis (14). These additional actions may explain the high potency of diclofenac. Another non-steroidal anti-inflammatory drug, ibuprofen, was described as being able to reverts resistance related to efflux activity in *C. albicans* (17). Fluconazole resistant isolates revering to susceptibility after incubation with ibuprofen showed *CDR1* and *CDR2* genes overexpresion especially of the latter (18).

Diclofenac has recently been discovered to inhibit microbial biofilms. A biofilm is a population of cells growing on a surface and enclosed in an exopolysaccharides matrix. Biofilms confer resistance on micro-organisms to antibiotic treatment. The development of resistance by microorganisms to antimicrobial drugs has been one of the greatest problems hampering antimicrobial therapy. Bacterial biofilms show enormous levels of antibiotic resistance, which is a general feature of all biofilms.

Another study explored for the first time the possible effect of aspirin on *Candida* spp. biofilm-producing capacity (19). Two strains of *C. guilliermondii*, and one strain per species of *C. kefyr, C. glabrata, C. albicans*, and *C. parapsilosis* were included in the study. The significant effects of aspirin on growth and biofilm formation of *Candida* species were achieved only with supratherapharmacological concentration of the drug. Effect of aspirin and piroxicam of cell viability on *Candida* species was also studied (20). Piroxicam drastically reduced the viability of planktonic cells to 0.94% and aspirin had reduced to the 4.45% of that of untreated control cells.

CONCLUSION

The increasing frequency of invasive fungal infections and the high mortality rate associated with disseminated fungal diseases have underscored the importance of finding new therapy or improving for fungal infections. Inhibitors of cyclooxygenase isoensymes (aspirin and diclofenac) are effective in decreasing germ tube formation of *Candida albicans*. Nonsteroidal anti-inflammatory drugs specifically block the biosynthesis of fungal prostaglandins may be one strategy to combat fungal colonization and infection. The degree of inhibition of bacterial and fungal varies with type of drug used and the virulence of strains. The analgesic and anti-inflammatory properties of anti-inflammatory drugs might represent an additional advantage for their use in the management of bacterial and fungal infections.

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