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Flurbiprofen, a unique non-steroidal anti-inflammatory drug with antimicrobial activity against *Trichophyton*, *Microsporum* and *Epidermophyton* species

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ABSTRACT

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Aims: The objective of this study was to determine the antifungal activity of flurbiprofen against dermatophytes like *Trichophyton*, *Microsporum* and *Epidermophyton* species.

Methods and Results: Susceptibility tests were performed against dermatophytes like *Trichophyton*, *Microsporum* and *Epidermophyton* species by the microbroth dilution method. Among the dermatophytes tested, *Trichophyton*, *Microsporum* and *Epidermophyton* species are remarkably susceptible to this compound (MIC_{50}: 8–16 μg ml^{-1}). A yeast pathogen, *Candida albicans*, and a bacterium, *Staphylococcus aureus*, are also susceptible to flurbiprofen.

Conclusions: Flurbiprofen is a non-steroidal anti-inflammatory compound with strong antifungal activity, which is not found in two well known and medically used antifungal organic acids like benzoic and salicylic acids.

Significance and Impact of the Study: The present action of flurbiprofen on microbes indicates its future prospects as an antimicrobial agent against dermatophytes and yeast pathogens. However, in view of the anti-inflammatory property of flurbiprofen, its antifungal action may provide an additional advantage for use as a skin ointment.

Keywords: antifungal activity, *Candida albicans*, dermatophytes, flurbiprofen, minimum inhibitory concentration.

INTRODUCTION

Flurbiprofen, an inhibitor of prostaglandin biosynthesis (Reynolds 1989) has marked analgesic, anti-inflammatory, and antipyretic properties and is widely used orally for the relief of pain. In order to achieve better local anti-inflammatory effect, the drug is also currently under investigation for development as an efficient vehicle for topical administration to treat the inflammatory symptoms of arthritis and to relieve soft tissue pain (Singh *et al.* 1993; Suresh *et al.* 2001).

In this report we have shown that flurbiprofen also has strong antifungal activity against dermatophytes like *Trichophyton*, *Microsporum* and *Epidermophyton* species and may inhibit the growth of various pathogenic organisms.

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MATERIALS AND METHODS

Dermatophytes and other organisms used for determining the spectrum of antimycotic activity of flurbiprofen were obtained from our laboratory culture collection during our previous works (Sanyal *et al.* 1993; Chowdhury and Mukhopadhyay 1996; Chowdhury *et al.* 1996b). Serial drug dilutions were performed as described for the National Committee for Clinical Laboratory Standards (NCCLS) reference method (NCCLS 1998). Prior to MIC determination, sensitivity tests of flurbiprofen were performed by a broth macro-dilution method following the recommendations of the NCCLS (2000) protocol.

The minimum inhibitory concentration (MIC) was determined by exposure to variable amounts of stock solutions of test compounds (prepared in methanol) to obtain final concentrations of these drugs between 2 and 1024 μg ml^{-1}. The compounds were added directly to the growth medium adjusted to pH 5·0 as follows: Sabouraud's medium for mycelial fungi [yeast medium, containing 0·3% yeast extract (Difco Laboratories, Detroit, MI, USA), 0·5% peptone (Oxoid, Unipath Ltd, Basingstoke, UK), 0·3% malt extract (Oxoid), and 1% dextrose for *Candida albicans*] and Mueller Hinton broth for *Staphylococcus aureus* (5 ml in glass tubes).

The media in the tubes were inoculated with 0·05 ml of inoculum of the test organism. Spore suspensions (for mycelial fungi) or cell suspensions (24 h liquid culture of *C. albicans* and *S. aureus*) of appropriate Klett readings at 660 nm (25 and 15 as Klett readings for the spore and cell suspensions, respectively) were prepared as described earlier (Sanyal *et al.* 1992; Chowdhury *et al.* 1996a; Chowdhury *et al.* 1998) and used as inocula. The tubes were incubated under stationary conditions at 35°C for 48 h for the mycelial fungi and at 37°C for 24 h for *C. albicans* and *S. aureus*.

The MIC of a drug was defined as the lowest concentration at which no visible growth was detected at the appropriate time interval. MICs determined were stable at least up to 96 h. As flurbiprofen, benzoic acid and salicylic acid have very low solubility in water, these drugs were dissolved in methanol prior to their incorporation into the assay system during MIC determinations. The presence of methanol in the assay tubes did not have any effect on the MIC of the drugs at a concentration of 5·6% (v/v), the maximum methanol concentration used in the assay.

RESULTS AND DISCUSSION

Flurbiprofen, a bi-phenyl acetic acid derivative (2-fluro-α methyl 4-biphenyl acetic acid) inhibited growth *in vitro* of various organisms at a concentration below 64 μg ml^{-1}. Three different types of micro-organisms – dermatophytes, yeast pathogen and saphrophyte – were used to study the anti-microbial activity of flurbiprofen (Table 1). They were *Trichophyton mentagrophytes*, *T. rubrum*, *T. tonsurans*, *T. interdigitale*, *Microsporum fulva*, *M. gypseum*, *M. canis*, *Epidermophyton floccosum* (dermatophytes), *C. albicans* (a yeast pathogen) and *Aspergillus niger* (a saphrophyte).

Among the dermatophytes tested, *Trichophyton*, *Microsporum* and *Epidermophyton* species are remarkably susceptible to this compound (MIC_{50}: 8–16 μg ml^{-1}). A yeast pathogen, *C. albicans* (MIC_{50}: 32 μg ml^{-1}) and a bacterium, *S. aureus* (MIC_{50}: 16 μg ml^{-1}), as a control organism are also susceptible to flurbiprofen.

The results of the experiment (Table 2) indicate that the concentration of flurbiprofen required for growth inhibition of the micro-organisms depends on the pH of the growth media. Antifungal activity of flurbiprofen is more effective below pH 7·0 with the maximum effect around pH 5·0 (Table 2), like other antifungal organic acids. However, its growth inhibitory activity against *T. mentagrophytes* is unique and remarkably high (8 μg ml^{-1}) even at pH 7·0 (32 μg ml^{-1}) compared with other organisms tested. *Aspergillus niger*, a pathogenic organism, was not susceptible to this compound.

Superficial fungal infections of skin are caused by dermatophytes belonging to three genera – *Trichophyton*, *Microsporum* and *Epidermophyton* species (Conant *et al.*

Table 1 Comparison of antimicrobial activities of flurbiprofen, benzoic acid and salicylic acid

	MIC* (μg ml^{-1}) at pH 5·0					
	Flurbiprofen		Benzoic acid		Salicylic acid	
Organisms (no. of isolates)	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Trichophyton mentagrophytes (5)	8	16	128	256	128	256
Trichophyton rubrum (5)	16	32	128	256	128	256
Trichophyton tonsurans (4)	16	32	128	128	128	128
Trichophyton interdigitale (5)	16	32	128	256	128	256
Trichophyton violaceum (4)	16	32	128	256	128	256
Microsporum fulva (4)	16	64	128	256	128	256
Microsporum gypseum (3)	16	32	128	128	128	256
Microsporum canis (3)	16	32	128	256	256	512
Epidermophyton fluccosum (5)	16	32	128	128	128	256
Candida albicans (4)	32	64	128	256	>256	>512
Aspergillus niger (5)	256	>512	>512	>512	>512	>512

*Each MIC determination was repeated three times to ensure the reproducibility of the results. MICs determined were stable at least up to 96 h. MIC_{50} and MIC_{90} are the MICs at which 50 and 90% of the isolates are inhibited, respectively.

Table 2 Effect of pH on antimicrobial potency of flurbiprofen

Organisms	MIC_{50} ($\mu g\ ml^{-1}$) at pH*			
	4	5	6	7
Trichophyton mentagrophytes	4	8	16	32
Epidermophyton fluccosum	8	16	32	64
Microsporum fulva	8	16	32	64
Candida albicans	16	32	64	128

*MIC_{50} was determined as described in the text with appropriate growth media adjusted to different pH values.

1971). Management of these infections depends upon a random selection of an antifungicide out of a large number of topical agents belonging to different groups, e.g. organic acids with derivative, polyene antifungal antibiotics, and other antifungal agents (Banerjee and Bose 1963; Jones 1982). Antifungal sensitivity testing with these agents may help in selection of a proper medication particularly in chronic infections.

Flurbiprofen inhibited growth *in vitro* of various pathogenic micro-organisms such as *Trichophyton*, *Microsporum* and *Epidermophyton* species (dermatophytes). The yeast pathogen, *C. albicans* and a bacterium, *S. aureus* are also responsible for skin infections and are susceptible to flurbiprofen. Thus, this compound may find efficient application as a topical skin ointment for the treatment of superficial fungal, yeast and bacterial infections, particularly, in cutaneous mycoses. Interestingly, the action of flurbiprofen against *Trichophyton*, *Microsporum* and *Epidermophyton* species and *C. albicans* was fungistatic and not fungicidal (data not shown).

Flurbiprofen is eight- to 16-fold more active (Table 1) against a range of pathogens than benzoic and salicylic acids, the commonly used antifungal constituents of Whitfield's ointment, the formulation of which is still considered effective for the treatment of superficial mycoses (Munro 1983). In antifungal ointment, flurbiprofen should provide additional advantage by virtue of its anti-inflammatory activity, which does not exist in other antifungal organic acids like benzoic, salicylic or unsaturated long chain fatty acids. The anti-inflammatory property should help in relieving the skin inflammation associated with the infection (Goto *et al.* 1993). The local anti-inflammatory efficacy of the drug is well documented and flurbiprofen is now being used in topical formulations for such activity (Singh *et al.* 1993). Very recently, it has reported that 0·3% flurbiprofen and 0·3% triclosan gel can be used alone as anti-inflammatory agents or as an adjunct to scaling in periodontal therapy (Suresh *et al.* 2001).

The present action of flurbiprofen on microbes indicates it can be used as an antimicrobial agent against dermatophytes and yeast pathogens. However, in view of its anti-inflammatory property, flurbiprofen provides an additional benefit for use as a skin ointment. Various clinical application of flurbiprofen directed toward dermatophytes are now being investigated using an animal model in our laboratory.

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