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Research Article

Topical Application of Oxifulvic Acid Suppresses the Cutaneous Immune Response in Mice

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ABSTRACT The antiinflammatory activity of topically applied coal-derived fulvic acids (called oxifulvic acid) at 4.5% and 9% was compared with that of diclofene sodium at 1% and betamethasone at 0.1% in a murine model of contact hypersensitivity. Mice were sensitised with dinitrofluorobenzene and challenged 6 days later by application to the dorsal surface of the right ear. The inflamed ears of the mice were then treated topically, and the thickness of the ears was measured daily. Oxifulvic acid at both concentrations compared favourably with both diclofene sodium and betamethasone in suppressing the cutaneous inflammatory response. Oxifulvic acid possesses antiinflammatory properties and may be of clinical benefit in the treatment of inflammatory skin conditions in humans. Drug Dev. Res. 53:29–32, 2001.

Key words: oxifuvic acid; diclofene sodium; betamethasone; topical antiinflammatory; mice

INTRODUCTION

Humic substances are ubiquitous in nature and are formed during the decay of plant and animal residues in the environment [MacCarthe et al., 1985]. These substances can be divided into humic acid, fulvic acid, and humin on the basis of the solubility in water as a function of pH. Fulvic acid is the fraction that is soluble in water under all pH conditions and is in general lower in molecular size and weight and lower in colour intensity than humic acids.

The application potential of fulvic acids in the treatment of human and animal diseases has not been investigated previously, possibly because of the difficulty in isolating fulvic acids from waters and soils in nature.

A unique process has been developed to convert bituminous coal by controlled wet oxidation with oxygen in high yield to high quality humic and fulvic acids [Bergh et al., 1997]. To distinguish them from naturally occurring humic and fulvic acids, these coal-derived products are called oxihumic and oxifulvic acid, respectively. From a thorough analysis of oxifulvic acid by means of mass spectrometry and gas chromatography–mass spectrometry techniques, Bergh [1997] identified some 50 different compounds. Most of these were carboxylic acids and ordinary physiological metabolites with no evidence of any toxic compound in the product mixture.

The antimicrobial activity of oxifulvic acid has recently been described by Van Rensburg et al. [2000]. In this study, all eight microbial pathogens tested (*Staphylococcus aureus*, *Streptococcus faecalis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *β-haemolytic streptococcus*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Candida*

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albicans) were sensitive to oxifulvic acid at concentrations ≤ 1.5%.

It has also been demonstrated in a pilot study that oxifulvic acid is effective in the topical treatment of pyotraumatic dermatitis in cats and dogs [Dekker and Medlen, 1999]. In the current study, the antiinflammatory properties of aqueous oxifulvic acid creams (4.5% and 9%) were investigated in vivo in dinitrofluorobenzene sensitised mice.

MATERIALS AND METHODS

Murine Model of Contact Hypersensitivity

Ethics Committee approval was obtained for all animal experiments that were done at the Pretoria Biomedical Research Centre. Scientific Procedures and the Code of Practice for the Housing and Care of Animals Used in Scientific Procedures (Acts 1986 and 1989, respectively) were strictly adhered to. Female, 6–7-week-old, BALB/c mice were divided into 5 groups with 10 mice per group. Animals were kept in separate cages in a 12-h light:dark cycle and had free access to standard mouse chow and water. All 5 groups were sensitised by applying 25-µl of 1% dinitrofluorobenzene (DNFB; Sigma Chemical Co., St. Louis, MO) in a carrier of acetone and olive oil (4:1) to shaved abdominal skin as described by Trueb et al. [1997] and challenged 6 days later with 20 µl of a 0.15% DNFB painted on the dorsal surface of the right ear.

Treatment

The inflamed ears of the mice in each group were treated topically 4 times per day for 2 days with one of the following.

1. Aqueous oxifulvic acid creams (4.5% and 9%) produced by blending appropriate quantities of oxifulvic acid and additional water into emulsifying ointment BP.
2. Diclofene sodium 1% (Voltaren Emulgel).
3. Betamethasone 0.1% (Betnovate).
4. Emulsifying ointment BP as control.

The thickness of the ear was measured with engineering calipers 1 mm from the tip of the ear before challenge and 24 h and 48 h later. Mice were killed after the last measurement, and the affected ears of the control group as well as the oxifulvic acid groups were embedded in paraffin wax for microscopic evaluation. The thickness of the ears were measured microscopically 5 mm from the tip.

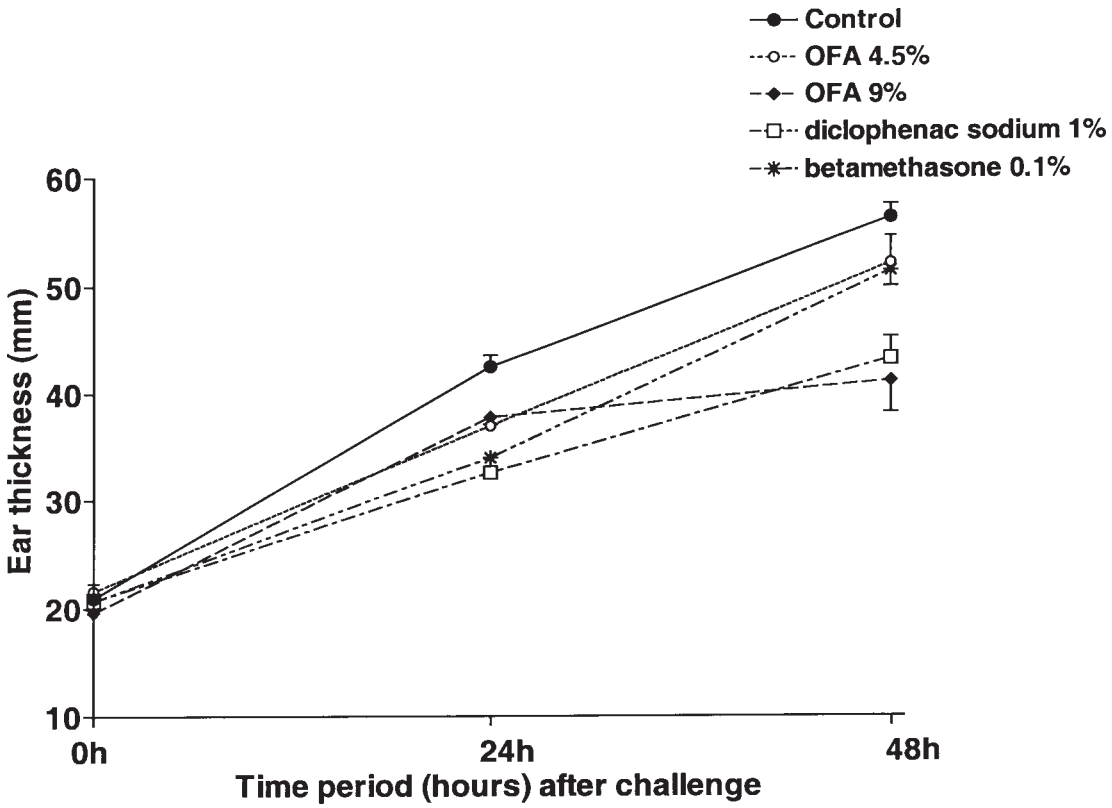

Fig. 1. The antiinflammatory properties of oxifulvic acid (OFA) (4.5% and 9%) compared with that of 1% diclofene sodium and 0.1% betamethasone applied topically in dinitrofluorobenzene-sensitised mice. Results are expressed as the mean ± SEM thickness of the ears measured with engineering calipers before challenge and 24 h and 48 h after challenge.

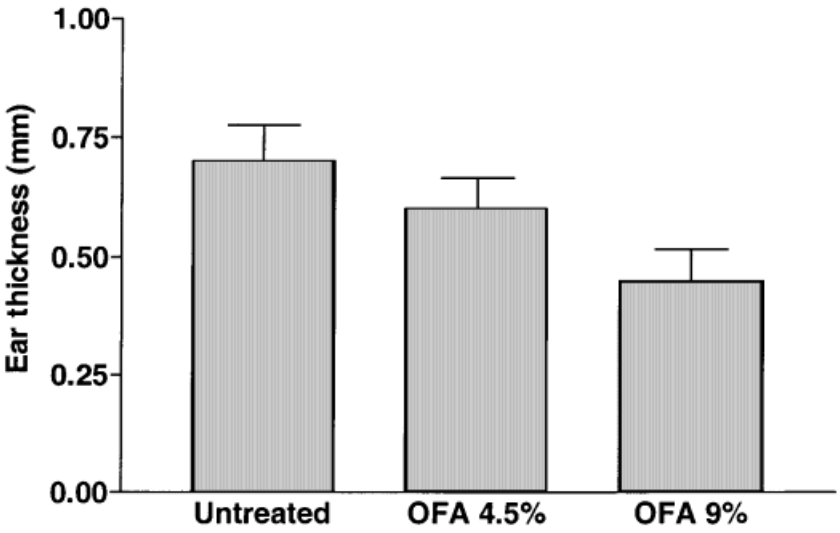

Fig. 2. The antiinflammatory properties of oxifulvic acid (OFA) (4.5% and 9%) in dinitrofluorobenzene sensitised mice. Results are expressed as the mean ± SEM thickness of the ears imbedded in paraffin wax and measured microscopically 5 mm from the tip of the ear 48 h after challenge.

Statistical Analysis

Results are expressed as the mean ± SEM. Statistical comparisons were made using the Student's t-test for paired values.

RESULTS

All four treatments caused a significant ($P < 0.05$) decrease in ear swelling on both days (Fig. 1). Diclofene sodium and betamethasone proved to be better than both concentrations of oxifulvic acid on day 1, whereas the 9% oxifulvic acid cream and diclofene sodium were superior to betamethasone and the 4.5% oxifulvic acid on day 2. The results obtained with the oxifulvic acid treatments on day 2 were confirmed microscopically (Fig. 2). In this case, only the 9% oxifulvic acid treatment caused a significant decrease in ear swelling ($P < 0.05$). Histologically, there was no difference seen in the numbers of neutrophils, lymphocytes, and macrophages at the infected areas between the control and the oxifulvic acid treatment groups.

No signs of toxicity were observed during the 2 days of treatment with the two oxifulvic acid creams, as well as the betamethasone treatment. However, the mice in the diclofene sodium treatment group showed severe signs of stress, apathy, and loss of appetite.

DISCUSSION

The role of free radicals in inflammation and tissue destruction has been well documented [Winrow et al., 1993]. Although fulvic acid has been shown to possess superoxide and hydroxyl radical scavenging properties [Wang et al., 1996], there are no reports on its antiinflammatory activity in vivo.

In this study, we compared the antiinflammatory effects of topical fulvic acids derived from coal, with diclofene sodium and betamethasone on contact hypersensitivity in vivo. Oxifulvic acid compared favourably with the two generally used antiinflammatory agents as an inhibitor of contact hypersensitivity reactions. These two agents are, however, associated with serious adverse side effects [Grace et al., 1999; Lebrun-Vignes et al., 2000]. Oxifulvic acid, on the other hand, did not produce any measurable toxicity in experimental animals during either acute or subchronic oral or dermal exposure to 1,000 mg/kg/d for a period of 90 days (report by Biocon Pty Ltd.).

These antiinflammatory properties, together with its antimicrobial properties, suggest that oxifulvic acid, applied topically, might be an effective and safe treatment for inflammatory conditions of the skin.

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