SHORT COMMUNICATION

Effects of a mixture of fatty acids from sugar cane (Saccharum officinarum L.) wax oil in two models of inflammation: Zymosan-induced arthritis and mice tail test of psoriasis


Abstract

A mixture of fatty acids obtained from sugar cane (Saccharum officinarum L.) wax oil (FAM), in which the main constituents are palmitic, oleic, linoleic, and linolenic acids, was evaluated in two models of inflammation: zymosan-induced arthritis and in the tail test for psoriasis, both on mice. In the first model, FAM significantly reduced zymosan-induced increase of β-glucuronidase (DE50 90.77 mg/kg). Histopathological studies showed inhibition in cellular infiltration and reduction of synovial hyperplasia and synovitis, whereas in the second test, histopathological and ultrastructural studies showed that topical application of FAM induced orthokeratosis with the presence of keratohyalin granules in the previously parakeratotic adult mouse tail, and without effects on epidermal thickness. The ED50 of FAM in this model was 155.10 mg.

The results of our studies showed that topical application of FAM exerts an important anti-inflammatory activity in both tests without evidence of irritant effects. The anti-inflammatory effects exerted by FAM may be due to its inhibitory effects on arachidonic acid metabolism. To our knowledge, this is the first report on the anti-inflammatory effect of sugar cane by-products in experimental models of arthritis and psoriasis.

Keywords: Anti-inflammatory agent; Fatty acids; Sugar cane; Arthritis; Zymosan; Psoriasis

Introduction

Recently, it was reported that a mixture of fatty acids from sugar cane (Saccharum officinarum L.) wax oil (FAM), which mainly contains palmitic, oleic, linoleic, and linolenic acids, exerts anti-inflammatory action in various in vitro and in vivo experimental models (Ledon et al., 2003). Particular composition of the fatty acids and ratio are unique in sugar cane.

Rheumatoid arthritis and psoriasis are inflammatory diseases of unknown etiology. Several inflammatory mediators are involved in these illnesses (Szekanecz et al., 1998) The effective therapies for both diseases are limited. Glucocorticosteroids, for example, have been used for many years, but they have a lot of side effects (Travers, 2000).

The interest in some kind of oils for treating psoriasis and arthritis increased with the epidemiological studies.
carried out in the 1960s and 1970s that showed that Eskimos, compared to Danes, have low incidence of inflammatory diseases (Okuyama et al., 1997). Studies on essential fatty acids in plasma consistently showed low levels of linoleic acid in Danes. It has been shown that essential fatty acid deficiency induces inflammatory processes in rats and humans, which are reversed by the cutaneous application of linoleic acid in various forms (Ziboh, 1996). Therefore, taking into account these findings, we decided to test FAM in arthritis induced by zymosan and to determine the potential antipsoriatic effect of local application of this mixture, in animal models that mimic some of acute inflammatory responses seen in both diseases (Gegout et al., 1995; Bosman et al., 1992).

Materials and methods

Chemicals, materials, and animals

Sugar cane wax oil was obtained from “Amancio Rodriguez” Cuban sugar factory.

All reagents were purchased from Sigma Chemical (St. Louis, MO). Animals from the National Center for Production of Laboratory Animals (CENPALAB, Havana, Cuba) were used in this study. The experiments were carried out in accordance with the ethical guidelines for investigations in laboratory animals (EEC Directive of 1886 (86/609/EEC)).

Chemical composition of mixture of fatty acids from sugar cane wax oil (FAM)

FAM was obtained from the saponification of sugar cane wax oil, which was derived from the fraction present in the cuticular wax of the sugar cane stalk. This wax was extracted as described in Pomunski and Kopacs (1960), and the composition obtaining by gas chromatographic analysis of fatty acids was reported previously (Ledon et al., 2003).

Zymosan-induced arthritis in mice

Female 6-week-old OF1 mice (20–25 g, 10 animals/group) were injected intra-articularly with 10 μl of a 15 mg/ml sterile suspension of zymosan. Four days after zymosan injection, each group was treated either with FAM (53, 106, 212, and 425 mg/kg), or vehicle or triamcinolone (9-fluoro-11β,16α,17,21-tetrahydroxy-pregna-1,4-diene-3,20-dione) (10 mg/kg), which was used as a reference drug. They were administered orally on a daily basis, from days 4 to 12. Thereafter, mice were then killed by cervical dislocation and the synovial fluid of knee joints was sampled in order to measure the level of β glucuronidase enzyme. The knee joints were then totally removed for histological studies (Remirez et al., 1999).

Determination of β glucuronidase activity

The patellar ligament was cut and the synovial cavity incised, the total fluid was then absorbed by means of small pieces of filter paper (No. 1575-Prolabo). The paper tips were cut and deposited at the bottom of tubes containing 0.9 ml of 50 mM acetate buffer, pH 4.5. The enzyme was measured in the presence of its substrate (phenolphthalein mono β glucuronic acid), 20 mM, after incubation for 17 h at 37 °C. Then 2.5 vol. of 200 mM glycine buffer pH 10.45 was added in order to induce the coloration of the phenolphthalein produced by the enzymatic cleavage of the substrate. Samples were read at 540 nm, and the coloration is stable for at least 1 h. Titers were based on comparisons with standard curves obtained with β glucuronidase (type B-1 from bovine liver). The values are expressed in units of enzymatic activity (Folliard and Terlain, 1992).

Histological processing

Knee joints were removed and fixed in 10% phosphate buffer formalin. The tissues were decalcified with 5% formic acid solution and then processed and embedded in paraffin. Total joint sections (6 μm) were prepared and stained with hematoxylin and eosin. Arthritis was assessed semiquantitatively in standardized frontal section of the knee joint, which included the presence of subsynovial inflammation, destruction of articular cartilage, and general destruction of the joint with pannus formation and bone erosion (Beckman et al., 1998). These parameters were scored on a scale of 0–4 as described previously by Remirez et al. (1999).

Mouse tail test for psoriasis

The modified mouse tail test established by Bosman et al. (1994) was used. Tails of mice (10 animals/group) were treated locally on its proximal part with 0.1 ml of FAM at doses of 27, 53, 106, 212, or 425 mg or with vehicles (saline or paraffin) or non-treated. Animals were treated weekly, twice daily, for 3 weeks. Another group of animals was orally treated with 0.1 mg/kg/day, with a suspension of retinoic acid in water and used as positive control group. At the end of the treatment, animals were killed by cervical dislocation and longitudinal sections of tails of about 5 mm thickness were prepared and stained with hematoxylin–eosin for histological examination. Smaller pieces were taken for ultrastructural studies.
Histological examination

Ten sequential scales were examined for the presence of a granular layer induced in the previously parakeratotic skin areas. The induction of orthokeratosis in those parts of the adult mouse tail, which have normally a parakeratotic differentiation, was quantified measuring the length of the granular layer (A) and the length of the scale (B). The proportion \( \frac{A}{B} \times 100 \) represents the % orthokeratosis per scale, and the drug activity (DA) was calculated as follows:

\[
DA = \frac{\text{mean OK of treated group} - \text{mean OK of control group}}{100 - \text{mean OK of control group}} \times 100 \quad \text{where OK = orthokeratosis.}
\]

The measurements were carried out at the border of the scale with a semiautomatic image evaluation unit (Casaco´et al., 1999).

Measurement of epidermal thickness

It was obtained by measuring the distance between the dermoepidermal borderline and the beginning of the horny layer. Five measurements per animal were made in every 10 scales and the mean of different animals was calculated.

Ultrastructural processing

Skin tail pieces were also fixed in 5% glutaraldehyde for 24 h and postfixed in 1% osmium tetroxide for about 8 h, buffered in 1 mol/l sodium cacodylate buffer (pH 7.4). The pieces were dehydrated in graded concentrations of acetone and embedded in Spurr resin. It was necessary to prepare semithin sections for recognizing interfollicular regions and then prepare ultrathin sections in these areas. Sections were contrasted with uranyl acetate and lead citrate and a JEOL JEM 100S Transmission Electron Microscope was used for the observation of sections (Casacó et al., 1999).

Statistical analysis

Data are presented as means ± standard deviation. Mean differences between groups were compared by one-way analysis of variance (ANOVA) and a Duncan’s multiple comparison test. Due to a non-gaussian distribution of orthokeratosis values (100% is the maximal effect) the Kruskal–Wallis test was used on psoriasis tail test. Values of \( p < 0.05 \) were considered to be significant. The ED50 value was determined from the best-fit regression line of a dose–response curve.

Results and discussion

In zymosan-induced arthritis, complement is activated via alternative pathway and the secretion of lysosomal enzymes into the knee joint synovial fluids is induced. This activity correlates with histomorphological changes observed in the joint, such as vasculitis, synovitis, and sometimes pannus formation. Arthritis induced by zymosan resulted in a significant increase of \( \beta \) glucuronidase levels, which were decreased by FAM with a DE50 = 90 ± 7 mg/kg. This effect was dose-dependent (Fig. 1). Triamcinolone almost completely abolished enhanced \( \beta \) glucuronidase activity in the synovial fluid of zymosan-treated animals.

In agreement with these findings, histological evaluation revealed that the group treated with zymosan showed severe destruction of cartilage with loss of the general architecture and pannus formation. There was erosion of bone structure accompanied by severe inflammation of articular tissues (grade 4) (Fig. 2A). FAM and triamcinolone treatment revealed a marked and dose-dependent decrease of histology score (grade 2 and 1), the inflammatory response was less severe, and there was no destruction of general joint architecture or pannus formation. Also, after treatment the reduction of bone erosion was pronounced (Fig. 2B, FAM 425 mg/kg).

In psoriasis’ model, the induction of a granular layer by topically administered drugs was measured in previously parakeratotic scale regions in the mouse tail. In the tail skin samples of negative control group, lack of granular layer in epidermal stratum was observed, as occurred normally in scale regions of adult mouse tail (Figs. 3A and 4A). FAM and retinoic acid induced a significant and dose-dependent increase in orthokeratosis in the mouse tail epidermis (DE50 = 145 ± 10 mg) (Fig. 3B, Table 1) with a percentage of orthokeratosis induction of FAM ranging from 23.8% to 60.1%. In the interfollicular regions of tail skin specimen removed
Fig. 2. (A) Histological section of knee joint injected with zymosan. Acute inflammation is present. The surface of the cartilage is eroded and there is pannus formation (p) (grade 4). Hematoxilin–eosin, 10 × . (B) Mice treated with zymosan and FAM (425 mg/kg). Histology of arthritic knee joints shows restoration of articular cartilage and absence of pannus formation. Hematoxilin–eosin, 10 × .

Fig. 3. (A) Histopathology of a mouse tail scale after treatment with saline for 3 weeks (negative control). The granular layer is in the neighboring of the hair follicle. Hematoxilin–eosin staining, 50 × . (B) Histopathology of a mouse tail scale after topical treatment with the fatty acid mixture from sugar cane wax oil for 3 weeks. The granular layer covers the whole scale (g). Hematoxilin–eosin staining, 50 × .

Fig. 4. (A) Control animal. Ultrastructure of interfollicular region of mouse tail skin. Observe lack of granular layer in the epidermal stratum. Bar: 1 μm. (B) Animal treated with the fatty acid mixture. Ultrastructure of interfollicular region showing nucleus (N) and keratohyalin granules (arrow) of granular cells. Bar: 1 μm.

Table 1. Effect of the fatty acids mixture (FAM) from sugar cane wax oil on epidermal differentiation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Orthokeratosis (%)</th>
<th>Activity (%)</th>
<th>Change in epidermal thickness (%)</th>
</tr>
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<tbody>
<tr>
<td>Non-treated</td>
<td>27.0 ± 2.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Saline</td>
<td>27.1 ± 2.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Paraffin</td>
<td>29.3 ± 2.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Retinoic acid (0.1 mg/kg)</td>
<td>75.6 ± 3.1*</td>
<td>66.5</td>
<td>5</td>
</tr>
<tr>
<td>FAM 27 mg</td>
<td>44.4 ± 3.7*</td>
<td>23.8</td>
<td>0</td>
</tr>
<tr>
<td>FAM 53 mg</td>
<td>53.2 ± 3.3*</td>
<td>35.8</td>
<td>3</td>
</tr>
<tr>
<td>FAM 106 mg</td>
<td>60.0 ± 2.4*</td>
<td>45.2</td>
<td>6</td>
</tr>
<tr>
<td>FAM 212 mg</td>
<td>67.0 ± 3.4*</td>
<td>54.7</td>
<td>7</td>
</tr>
<tr>
<td>FAM 425 mg</td>
<td>70.9 ± 4.5*</td>
<td>60.1</td>
<td>7</td>
</tr>
</tbody>
</table>

Values are means ± SD.

*p < 0.05 in regard to non-treated (n = 10 animals/group). ED50 155 ± 10 mg.
from fatty acid mixture-treated mice, granular cells with prominent nucleus and cytoplasm with the typical keratohyalin granules were found in the epidermal stratum (Fig. 4B). FAM did not produce significant changes on the epidermal thickness (Table 1).

These results are in agreement with our previous work where we showed that FAM exerts anti-inflammatory action in some in vivo and in vitro experimental models (Ledon et al., 2005).

At present, rheumatoid arthritis and psoriasis remain pathologies for which no complete cure is available. Corticosteroid treatment, which leads to a quick remission of symptoms, must be used for a short term because of its serious adverse effects. Therefore, it thus remains as a valuable therapeutic objective to find out a compound with some of the beneficial properties of the anti-inflammatory drugs but without their deleterious effects.

Zymosan is well known as a powerful releaser of arachidonic acid metabolites, which are very important for vasodilation and swelling as well as the release of chemotactic factors that recruit polymorphonuclear cells to the affected zone and that contribute actively to the early phase of inflammatory reaction (Remirez et al., 1999).

TNFα and other cytokines are well-known mediators of zymosan-induced arthritis. The presence of increased amounts of arachidonic acid derivatives in psoriatic lesions (Bosman, 1994) confirm that arachidonic acid metabolites have a mediator role in psoriatic disease.

For the treatment of these illnesses, inhibitors of arachidonic acid metabolism such as fatty acids are commonly used (Joe et al., 1999). Fatty acids are important for cutaneous eicosanoids metabolism since they can exert remarkable effects on epidermal phospholipid fatty acid composition, as well as on the release and metabolism of arachidonic acid. The incorporation of other fatty acids in the tissue is paralleled by elevated levels of biosynthesis of metabolites with potent anti-inflammatory effects or with minor inflammatory effects than those derived from arachidonic acid (Belch and Muir, 1998).

In this context, FAM inhibited degranulation of cells in hypersensitivity models such as ovalbumin-induced sensitization and oxazolone-induced sensitization in mice. Also, FAM inhibited the chemotaxis of neutrophils (Ledon et al., 2003).

Sadeghi et al. (1999) reported that diets rich in unsaturated fatty acids diminish production of pro-inflammatory cytokines in vivo, whereas Pompeia et al. (2000) showed that activation of T lymphocytes was strongly inhibited by unbound fatty acids. The effect could be related to the presence of n-3 and n-6 essential fatty acids in the mixture, because some of them are effective in the induction of orthokeratotic differentiation in the mouse tail test and in some animal models of inflammatory diseases such as psoriasis and arthritis (Bosman, 1994; Joe et al., 1999).

On the other hand, the fact that FAM did not affect on epidermal thickness is an indicator of its negligible irritant effect.

Our results suggest that FAM may be a valuable approach in order to control inflammation in arthritis and psoriasis. However, further studies will be needed to elucidate the mechanism(s) involved in this effect of FAM of *S. officinarum* L.) wax oil.

References


