The Role of Tetracycline in Chronic Blepharitis

Inhibition of Lipase Production in Staphylococci

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Tetracycline administered in low doses can be effective in the long-term management of patients with meibomian keratoconjunctivitis (MKC). However, the mechanism of action does not appear to be a reduction of bacteria. Seventy-five percent of the ocular staphylococci in such patients are resistant to tetracycline. An alternative mechanism of action could be the inhibition of production of extracellular enzymes by the ocular flora. Inhibition of lipase production could result in lowered levels of toxic hydrolysis products (free fatty acids), which may exacerbate the disease process. The authors tested this hypothesis by examining the differential effect of tetracycline on growth and lipase production in a tetracycline-resistant and tetracycline-sensitive strain of Staphylococcus epidermidis and S. aureus isolated from patients with MKC and Staphylococcal blepharitis. Tetracycline caused significant decreases in the production of lipase in the sensitive and resistant strains of S. epidermidis without concomitant decreases in growth. In contrast, S. aureus strains showed parallel decreases in both lipase production and inhibition of growth. The authors propose that the sensitivity of lipase production to tetracycline, in tetracycline-resistant S. epidermidis, may partially explain the clinical improvement observed in MKC patients. Invest Ophthalmol Vis Sci 32:2970–2975, 1991

Chronic blepharitis is a complex, frustrating disease that can manifest several different, sometimes overlapping, arrays of signs and symptoms.1-5 We recognize six clinically distinct groups of chronic blepharitis.1,2 Of these groups, meibomian keratoconjunctivitis (MKC) generally presents the most severe array of signs and symptoms. There is a frequent and significant association with sebaceous gland dysfunction, plugging and inflammation of the meibomian glands, and an unstable tear film.1,2,5 A causative bacterial pathogen cannot be cultured, and the ocular flora appear normal in every respect.8,9 However, 75% of all Staphylococcus epidermidis isolates from MKC patients are resistant to tetracycline. These patients also have significantly increased numbers of S. epidermidis isolates that hydrolyze wax and sterol esters, the principal components of meibomian gland secretions.10

Comparative studies of meibomian secretions among MKC patients and normal subjects have revealed significant differences in the intact wax/sterol esters and the free fatty acids that make up these esters.11-13 Free fatty acids are extremely potent substances.14-16 Even at low concentrations, they can be toxic to ocular tissues and may affect the solubility of the other lipid classes or the way the meibomian secretions interact with the tear film. Bacterial release of free fatty acids from wax and sterol esters through production of lipase could alter the characteristics of the tear film or contribute to the inflammation of ocular tissues, both of which are characteristics of MKC.6

Because MKC presents severe signs and generally causes major discomfort to the patient, we favor combined therapy, including oral tetracycline, for control.7,17 Whereas most clinicians use oral tetracycline therapy for the treatment of MKC, there has not been a well-controlled study to demonstrate its effectiveness in this condition. Oral tetracycline therapy has, through common usage, become the treatment of choice for MKC. It initially is administered at 1–2 g/day. Once the condition is controlled, months of
therapy with low doses (250 mg/day) often is necessary to prevent recurrence. One of the puzzling aspects of long-term tetracycline therapy in MKC is the ability of this drug to ameliorate the symptoms of the disease processes at doses that are obviously below the minimum inhibitory concentration (MIC) for most of the S. epidermidis strains isolated from patients suffering from MKC. If S. epidermidis were primary in the pathophysiology of MKC, subinhibitory doses of tetracycline should have no therapeutic value.

A somewhat parallel relationship to MKC has been presented in the dermatologic literature for acne vulgaris. Systemic therapy with tetracycline for acne vulgaris results in a decrease in the amount of free fatty acids on the skin surface. It is believed that tetracycline inhibits the hydrolysis of sebum lipids, carried out mainly by Propionibacterium acnes (a normal inhabitant of the skin surface), and that this occurs before there is a detectable decrease in the bacterial count of these organisms. Extremely high concentrations of tetracycline, well in excess of the MIC’s for most resistant organisms, can directly inhibit the activity of lipase enzymes. However, this would not explain the efficacy of low-dose tetracycline therapy. Because of tetracycline’s inhibitory effect on protein synthesis in bacteria, successful treatment of MKC with low doses of tetracycline may lie in the ability of this drug to inhibit lipase production without significantly diminishing the number of recoverable organisms. This was, in effect, demonstrated in vitro with one sensitive and one resistant strain of S. aureus, in which synthesis of lipase was inhibited with concentrations of tetracycline below the MIC for the organisms.

The following research was initiated to examine the effect of tetracycline on the production of lipase by both tetracycline-sensitive and -resistant strains of S. epidermidis and S. aureus isolated from patients with MKC and staphylococcal blepharitis, respectively. We report herein that lipase production can be inhibited by concentrations of tetracycline many times lower than the MIC’s for S. epidermidis, but not S. aureus.

Materials and Methods

Culture and lipase/esterase assay methodology have been described in detail elsewhere. Isolates of staphylococci were maintained on brain/heart infusion slants until needed. Isolates were transferred to Brucella blood agar for affirmation of purity before use. Isolates were identified by standard methods. In addition, coagulase-negative staphylococci (C-NS) were further speciated using API Staph-Ident (Analytab Products, Plainview, NY). Initial antimicrobial susceptibilities were determined by the Barry agar overlay method, using commercially available antimicrobial discs and Mueller Hinton agar (BBL, Cockeysville, MD). Determination of MICs of tetracycline were determined by standard methods, modified as described here.

For this study, two isolates of two different species were chosen from two different groups of chronic blepharitis. One tetracycline-resistant and one tetracycline-sensitive strain of S. epidermidis were selected from C-NS isolates obtained from MKC patients. For the sake of interspecies comparison, one tetracycline-resistant and one tetracycline-sensitive strain of S. aureus were selected from a collection of this species isolated from patients with staphylococcal blepharitis.

The medium used for MIC and lipase inhibition determinations consisted of peptone, 10 g; NaCl, 9.0 g; yeast extract, 2.4 g; 1 l distilled H2O. The pH was adjusted to 7.5, and the medium was autoclaved for 15 min at 121°C. Tetracycline hydrochloride (Sigma Chemical Company, St. Louis, MO) was added to the peptone broth to give twofold serial dilutions. For sensitive strains, this ranged from 2.5 μg/ml to 0.01 μg/ml. The range for resistant strains was 100 μg/ml to 0.39 μg/ml.

Growth inhibition and MIC determinations were made as follows. A bacterial suspension was prepared in normal saline from a 24-hr Brucella blood agar culture. The suspension was adjusted to match a McFarland number 2 nephelometer standard; 0.1 ml inocula (approximately 6 × 10^8 cells/ml) was transferred to screw-capped glass culture tubes containing 5.0 ml of the appropriate dilution of tetracycline in peptone broth. The tubes were incubated with the caps loose for 18 hr at 35°C on a rotating drum (Belco Glass Co., Vineland, NJ) at 8 rpm. Growth was determined spectrophotometrically on a Spectronic 20 set to record absorbance at a wavelength of 660 nm. The MIC was defined as the lowest concentration that inhibited all growth.

Inhibition of lipase production was determined as follows. The substrate, p-nitrophenyl palmitate (p-NPP, Sigma Chemical Co.), was dissolved in acetone at a concentration of 10 mg/ml. Aliquots of 50 μl were dispensed to screw-capped glass culture tubes. The acetone was evaporated to dryness with a stream of nitrogen. The caps were tightened, and the tubes were autoclaved for 15 min at 121°C. Tubes were allowed to cool to room temperature before 5.0 ml of serially diluted tetracycline in peptone broth was added. Tubes were inoculated and incubated as described for MIC determinations. Lipase activity, used as an indi-
rect measure of lipase production, was determined by assaying for the product of the hydrolysis of p-NPP, p-nitrophenol. The contents of each tube in a series were filtered through a 0.45-μm filter (in a 25-mm filter unit attached to a 10-ml plastic syringe). The bacteria-free filtrate was analyzed spectrophotometrically at a wavelength of 420 nm. Tetracycline produced a competing absorbance at this wavelength. Therefore, an identical series of tetracycline dilutions in peptone broth but without p-NPP (or bacteria) was used as a set of blanks for the series of dilutions containing both tetracycline and p-NPP.

Each experiment was repeated nine times. The values for each dilution were normalized to give percentage inhibition of either growth or lipase production for each isolate. These data were collated and plotted as mean values plus or minus the 95% confidence limits. Significant differences between inhibition of growth and lipase production for each concentration of tetracycline were determined by t-test.28

Results

The results are shown graphically for S. epidermidis in Figures 1 and 2; S. aureus results are shown in Figures 3 and 4. The MICs were as follows: sensitive S. epidermidis, 1.25 μg/ml; resistant S. epidermidis, 50 μg/ml; sensitive S. aureus, 2.5 μg/ml; resistant S. aureus, 50 μg/ml. It is interesting to note the differences between the two species. Tetracycline inhibited lipase production at much lower concentrations than it inhibited growth in the two S. epidermidis strains.

This phenomenon was not observed in the two S. aureus strains. For ease of comparison it is worthwhile to look at several key points on the figures.

One such point is the first significant decrease in lipase production. For the sensitive strain of S. epidermidis (Fig. 1), the first significant decrease occurred at a tetracycline concentration 62.5 times below the
Growth

Fig. 4. Tetracycline inhibition of growth and lipase production in tetracycline-resistant S. aureus. (Each point represents the mean ± 95% confidence limits of nine replicates.)

MIC (0.02 µg/ml). At this concentration, growth was not significantly inhibited (98% of maximum), but lipase production had dropped to 70% of maximum. For the resistant strain of S. epidermidis (Fig. 2), the first significant inhibition of lipase production occurred at 0.78 µg/ml, a concentration 64 times lower than the MIC. Again growth was not inhibited at this concentration, whereas lipase production had declined to 82% of maximum. In contrast, the S. aureus strains showed much less sensitivity to tetracycline inhibition of lipase production. The sensitive strain (Fig. 3) first showed significant reduction in lipase synthesis (40% of maximum) at a concentration eight times below the MIC (0.31 µg/ml). However, growth was significantly inhibited at this concentration (55% of maximum). In fact, the difference between inhibition of growth and lipase production at this concentration was not significant. The resistant strain (Fig. 4) showed significant reduction in lipase synthesis (60% of maximum) at a concentration eight times below the MIC (6.25 µg/ml). Growth was not significantly inhibited at this concentration (90% of maximum).

The point at which lipase production ceases (less than 5% of maximum) is another useful point for comparison. For the sensitive strain of S. epidermidis, the cessation occurred at a tetracycline concentration eight times lower than the MIC (0.16 µg/ml). Growth also was significantly reduced at this concentration but was 75% of maximum. In the resistant strain, lipase production ceased at a tetracycline concentration four times lower than the MIC (12.5 µg/ml). Growth inhibition was 82% of maximum. In the sensitive strain of S. aureus, lipase production did not cease until growth had been totally inhibited (MIC of 1.25 µg/ml). The resistant strain was similarly affected. Lipase production ceased at a concentration of tetracycline one dilution removed from the MIC (25 µg/ml). Growth was inhibited to 20% of maximum at this concentration.

To summarize the results: tetracycline caused significant decreases in the production of lipase in both sensitive and resistant strains of S. epidermidis, without concomitant decreases in growth. In contrast, S. aureus strains showed parallel decreases in both lipase production and inhibition of growth.

Discussion

The effect of tetracycline on lipase production in S. epidermidis is striking, especially in contrast to the effect seen in S. aureus. It is easy to see how subinhibitory doses of tetracycline could dramatically influence the physiological capabilities of these organisms without necessarily causing significant changes in population size. If lipase is playing a role in the disease processes of chronic blepharitis (in particular MKC), the therapeutic value of tetracycline may lie not so much in its ability to eliminate staphylococci but in its ability to produce substantial decreases in the lipolytic activity of these organisms.

It appears that low oral doses of tetracycline may produce in vivo concentrations capable of initiating the kind of changes seen in vitro. Literature values show that after a single oral tetracycline dose of 250 mg, serum concentrations can reach a maximum of about 2 µg/ml in 2–4 hr.24 The level decreases during the next 12–24 hr, falling to 1.5 µg/ml within the first 6 hr, and to 0.45 µg/ml within 24 hr. Dosages of 250 mg every 6 hr (4 times/day) are required to maintain serum concentrations at about 2 µg/ml.

There are no data available that show achievable concentrations in the various external ocular tissues at these dosages. However, because tetracycline binds more readily to lipoid media, greater concentrations tend to appear in tissues than in aqueous body fluids. Concentrations within the meibomian glands would be expected to be much higher than concentrations in tears. Tetracycline cannot be detected in healthy skin, even at serum levels of 6.0–9.5 µg/ml.29 However, tetracycline has been shown to reach higher levels in ischemic tissue than surrounding normal tissue.24 Relatively high levels have been recorded in the skin damaged by sun, chemicals, or dermatoses (0.5–6 times greater than serum levels).29 However, most of the drug was contained in the dermis. Skin surface lipids recovered from 23 acne patients taking 250 mg/day of
tetracycline, showed levels of from 0 (in 10 subjects) to 1.85 μg/100 mg lipid (mean approximately 0.60 μg/100 mg lipid).\textsuperscript{20}

Because a tetracycline concentration of only 0.16 μg/ml can cause cessation of all lipase production in sensitive strains of \textit{S. epidermidis}, it probably is safe to assume that effective levels of the drug are achievable in all ocular surface tissues, even at low oral dosages. Levels of 12.5 μg/ml, necessary to completely inhibit lipase production in resistant strains of \textit{S. epidermidis}, may or may not be achievable at these dosages. Given a high level of inflammation and assuming concentrations in meibomian secretions similar to skin surface lipid, it may be possible to approach effective levels. However, even a concentration of half this (6.25 μg/ml), will produce a significant and dramatic reduction in the synthesis of lipase (29% of maximum). By comparison, it probably is impossible to achieve \textit{in vivo} levels of tetracycline that will affect lipase production in resistant strains of \textit{S. aureus}. However, this is not a practical problem because the organism does not seem to be greatly involved in MKC.

The most recent and tenable theory of what might be called an initiating event in meibomian gland dysfunction has come from the research of Jester and colleagues\textsuperscript{31-33} at the University of Southern California. They have shown convincingly that keratinization of epithelial cells results in plugging of the meibomian gland duct. Trapped secretions and cell debris could provide a rich substrate for the normal bacterial flora. Production of lipases and other hydrolytic enzymes could cause further irritation and stimulate more keratinization such that the process could escalate to the severe stages often seen in MKC. This process also lends itself to the waxing and waning that are typical of this disorder and could explain why both simple physical maneuvers (lid scrubs and hot compresses), as well as chemotherapy, are necessary to bring about relief (ie, dissolving the plugs, releasing fresh secretions, and inhibiting production of hydrolytic enzymes).

In conclusion, we have shown that tetracycline can significantly impair the production of extracellular lipase in \textit{S. epidermidis} without significantly affecting the growth of the organism. This effect is different from what is seen with \textit{S. aureus}. We propose that this mechanism may partially explain the clinical improvement seen in MKC patients who are taking low doses of oral tetracycline.

**Key words:** chronic blepharitis, staphylococci, tetracycline, lipase, meibum, meibomian keratoconjunctivitis, esterase

**References**