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Reduction by Oral Tetracycline of Lipolysis of Triglycerides in Hair Lipid

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Abstract. Oral tetracycline hydrochloride (Amphocycline®) reduced significantly ($P < 0.05$) the mean free fatty acid/triglyceride (FFA/TG) ratio in hair lipid, from 1.56 to 0.47 after treatment for 4 weeks, and to 0.64 after treatment for 8 weeks in 15 young men and women undergoing treatment for acne vulgaris. In a second similar group of 16 subjects who received the same oral therapy with antibiotic but who also applied a 10% (w/v) solution of ethyl lactate twice daily to affected areas on the face, the mean FFA/TG ratio was also reduced significantly ($P < 0.05$) from 1.25 to 0.61 (after 4 weeks) and to 0.64 (after 8 weeks). In a third group of 16 subjects, whose only treatment was local application of ethyl lactate solution, the mean FFA/TG ratio was essentially unchanged throughout the period of treatment from 1.15, being 1.50 and 1.22 after 4 weeks and 8 weeks, respectively.

Key words: Oral tetracycline; Acne vulgaris; Hair lipid lipolysis

Skin surface lipid usually contains a substantial proportion of free fatty acids (FFA) which are formed by the action of bacterial lipases on sebum triglycerides (TG) (8). Drugs of the tetracycline group, which are widely used for the treatment of

acne vulgaris, are known to reduce this lipolysis either by direct inhibition of the lipases or by inhibition of the growth of the lipase-producing bacteria, or by a combination of both actions (2). Substantial reduction of lipolysis may be achieved after systemic therapy for 4 weeks (6).

The findings were reported recently of a clinical trial comparing the efficacy of local application of a 10% (w/v) solution of ethyl lactate with that of oral tetracycline in the therapy of acne vulgaris (5). In the course of this trial, hair samples were collected in order to assess the effects of oral tetracycline on the chemical composition of hair lipid. This report presents the findings of the study on hair lipid composition. It is shown for the first time that oral tetracycline causes a reduction of lipolysis of hair lipid TG similar to its inhibitory effect on lipolysis of TG in facial skin surface lipid.

MATERIALS AND METHODS

Clinical. 20 males and 24 females, age range 14 to 30 years (mean 19.0), out of 49 subjects completed the trial. The subjects were assigned to three treatment groups. One group received oral tetracycline (Amphocycline®, E. R. Squibb) and applied a 10% (w/v) solution of ethyl lactate in a water-ethanol-propylene glycol vehicle to acne-affected areas on the face. A second group received oral tetracycline and applied a placebo lotion (solvent-mixture vehicle). The third group used local application of the ethyl lactate solution. The daily dosage of tetracycline was 1.5 g during the first week, reducing to 0.5 g in the second and third week, and finally to 0.25 g from the fourth to the eighth week, inclusive. Throughout the trial the subjects used only the commercial shampoo supplied for washing the hair. The subjects were requested to avoid the use of other hair products or treatments for the duration of the trial.

Analytical. Three hair samples (each c. 100 mg) were collected from each subject during attendance at the clinic. The first was collected on the day the trial began, the second after 4 weeks and the third at the end of the trial (8 weeks). The hair samples were stored at room temperature until analysis, which was between 1 and 2 weeks after collection. Hair lipids were extracted with diethyl ether at room temperature and quantified by charring an aliquot part in conc. sulphuric acid at 200°C in order that suitable amounts could be taken for thin-layer chromatography on silica gel (9). FFA and TG were assayed by scanning transmission densitometry using a Chromascan Mark II (Joyce, Loeb and Co. Ltd., Gateshead-on-Tyne, England) after spraying the developed plates with 20% (w/v) aq. ammonium hydrogen sulphate and heating at 180°C for 60 min. Because the charring intensities per unit weight of FFA and TG differ considerably, appropriate corrections must be made to convert peak areas from the densitometer traces to weights of FFA and TG. Standard curves of amounts of

Table 1. Ratios of free fatty acid to triglyceride (FFA/TG) in hair lipid

Mean $\pm$ standard error (number of samples)

	Treatment		
	Topical ethyl lactate	Ethyl lactate+oral tetracycline	Oral tetracycline
Pre-treatment	1.15 $\pm$ 0.078 (15)	1.25 $\pm$ 0.049 (16)	1.56 $\pm$ 0.072 (15)
4-week treatment	1.50 $\pm$ 0.057 (16)	0.61 $\pm$ 0.062* (15)	0.47 $\pm$ 0.055* (15)
8-week treatment	1.22 $\pm$ 0.073 (16)	0.64 $\pm$ 0.064* (14)	0.64 $\pm$ 0.068* (14)

* Values significantly different from those measured pre-treatment ($P < 0.05$).

FFA and TG plotted against peak areas were kindly made available to the authors by Dr K. D. Bingham and are based on some unpublished work (K. D. Bingham, C. A. Lewis and B. Hayward). The FFA and TG mixtures used as standards had been separated from human scalp lipid by column chromatography on silica gel. The levels of significance of difference were evaluated by using the method of analysis of variance.

RESULTS AND DISCUSSION

In both groups receiving oral tetracycline there were significant reductions ($P < 0.05$) in the FFA/TG ratio after 4 weeks and 8 weeks (Table 1). There was no significant difference between the ratios after 4 weeks and 8 weeks. The results of the two groups were not significantly different from each other but the reductions after both 4 weeks and 8 weeks for these groups were significantly greater ($P < 0.05$) than those for the group using topical application of ethyl lactate as sole therapy. Topical therapy with ethyl lactate was without effect on lipolysis of hair lipid TG. A systemic effect of ethyl lactate on the lipolysis of hair lipid TG by scalp microflora would have been unexpected. It was shown previously that there was no significant difference between the effects of the treatments on the composition of forehead skin surface lipids when analysed by infra-red spectrometry and only the treatment with oral tetracycline and placebo lotion effected a significant reduction of lipolysis (5). All

treatments were equally effective on comedones and microcysts but only treatments with antibiotic reduced the number of inflamed lesions (5).

Unfortunately, the extraction and analysis of hair lipid could not be carried out promptly after collection of the hair samples. It is known that skin surface lipid TG may undergo lipolysis during storage after the lipid has been extracted with organic solvent (3). Therefore the lipolysis of TG in lipid of hair stored at room temperature for 14 days was investigated. It was found that the mean ratio FFA/TG in hair lipid extracted and analysed within 24 hours after collection of hair samples from 10 normal subjects not using hair or scalp products was 0.79 ± 0.032 . On storage of the hair from these subjects for 14 days before extraction and analysis of the lipid, the ratio FFA/TG was increased to 2.14 ± 0.065 ($P < 0.01$). Nonetheless, the results in Table 1 clearly show that systemic tetracycline reduces lipolysis of TG in hair lipid, even when measured in hair that has been stored for a week or more. The magnitude of the reduction will depend on both the length of storage of the hair before analysis of its lipid and the time elapsed since previous washing of the hair. The period between washing of the hair and collection of samples from the clinical trial was also a factor over which we could not exercise complete control, although all the hair samples were taken within one week after previous washing. It is possible that there was some inhibition of lipolysis *in vitro* by tetracycline so that its effect *in vivo* was enhanced.

Although inhibition of the lipolysis of sebum TG has been considered an indicator of the efficacy of acne therapy, acne is not characterised by high FFA/TG ratios of skin surface lipid (2). Also, there is no correlation between the decrease in the severity of the disease and the reduction in FFA/TG ratios during tetracycline therapy (1). Furthermore, the synthetic compound O,O-dimethyl-O-(3,5,6-trichloro-2-pyridyl) phosphate (fospirate), which dramatically reduces lipolysis of skin surface lipid within 12 hours after local application, is without significant therapeutic action on acne (10). Other evidence suggests that FFA derived from sebum TG are not primarily responsible for the inflammatory changes in acne (7). However, the anti-inflammatory effects of tetracyclines in skin through their inhibition of leukocyte chemotaxis (4) may account for part of their anti-acne activity.

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Lysine Prophylaxis in Recurrent Herpes Simplex Labialis: A Double-blind, Controlled Crossover Study

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Abstract. L-lysine has an inhibitory effect on the multiplication of herpes simplex virus in cell cultures. We have evaluated the prophylactic effect of L-lysine monohydrochloride 1000 mg daily on recurrent herpes simplex labialis in 65 patients in a double-blind, placebo-controlled, crossover study. After 12 weeks of lysine treatment the patients shifted to placebo treatment for a similar period. On the whole, lysine prophylaxis had no effect on the recurrence rate of herpes simplex. However, significantly more patients were recurrence-free during lysine

than during placebo treatment ($p=0.05$), suggesting that certain patients may benefit from prophylactic lysine administration. In the herpes lesions described, lysine had no effect on the rate of healing or on the appearance of the lesions at their worst.

Key words: Herpes labialis, drug therapy: Lysine

The multiplication of herpes simplex virus (H.S.V.) in cell cultures is inhibited by high concentrations of the amino acid L-lysine in the culture medium (6). In a previous therapeutic study (3) we demonstrated that lysine had no effect on the healing rate of recurrent herpes simplex labialis. In the recurrent herpetic lesion, virus multiplication begins in the prodromal stage and is maximal during the following 24 hours, whereafter it declines rapidly (5). Therefore, any treatment must be initiated immediately at the onset of the first symptoms. These circumstances motivated an evaluation of the prophylactic effect of lysine in recurrent H.S.V. infections.

PATIENTS AND METHODS

All subjects were otherwise healthy volunteers with at least three perioral and/or prolabbial herpes simplex episodes in the preceding 12 months. The diagnosis was in some cases based upon the observation of lesions, and in the remaining cases upon a thorough history.

A total of 79 patients were initially admitted to the study; 14 did not complete the investigation and were excluded. The final material comprised 65 patients (52 females, 13 males) aged 16–73 years (median 36 years).

The trial was conducted as a double-blind, placebo-controlled, crossover study. At the first visit the patient was given a questionnaire and tablets containing 500 mg L-lysine monohydrochloride or starch powder (placebo). The patients were instructed to take one tablet twice daily during the entire study. Every second patient started with lysine and alternate patients with placebo. Initial treatment continued for 12 weeks, whereafter the patients shifted without interruption to the alternative treatment for the following 12 weeks. During treatment the patients recorded the duration and course of their herpes simplex recurrences on the questionnaires which, together with residual tablets, were returned by mail every 4th week. In due course a fresh issue of tablets and a new questionnaire were dispatched to the patients.

The start of the episode was defined as the appearance of burning, itching, tingling (prodrome) and/or erythema. The lesion was considered healed when the crust had been discharged and all discomfort and swelling had disappeared; residual erythema could still be present. A new lesion at a non-contiguous site was considered part of the episode when appearing before the initial lesion had healed.