

CLINICAL REPORT

Clinical and Microbiological Comparisons of Isotretinoin vs. Tetracycline in Acne Vulgaris

Cristina OPRICA^{1,2}, Lennart EMTESTAM¹, Lena HAGSTRÖMER¹ and Carl Erik NORD²

¹Department of Medicine, Division of Dermatology and Venereology, and ²Department of Laboratory Medicine, Division of Clinical Bacteriology, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, Sweden

The aim of this study was to compare the clinical and microbiological effect on *Propionibacterium acnes* of oral tetracycline plus topical adapalene vs. oral isotretinoin in moderate to severe acne vulgaris. Male and female acne patients with moderate or severe inflammatory disease were enrolled and assigned randomly to 6 months of treatment with oral tetracycline hydrochloride plus topical adapalene, or oral isotretinoin, in a controlled, open study. After cessation of oral treatment the antibiotic-treated group received topical adapalene for the 2-month follow-up period. Clinical and microbiological assessments were performed. Skin samples for microbial identification and quantification were taken at baseline, after 2, 4 and 6 months of treatment, and 2 months after cessation of treatment. Patients treated with isotretinoin showed prolonged significant remission compared with the other group. The density of resistant propionibacteria did not change significantly in any of the groups and there was no correlation between resistant *P. acnes* and the clinical response in any of the regions investigated. Antibiotic treatment was found to be a good alternative to isotretinoin, regardless of the presence of antibiotic-resistant *P. acnes*, although isotretinoin had a better effect, with prolonged remission after treatment. **Key words:** acne; antibiotics; oral retinoids; resistance; *Propionibacterium acnes*.

(Accepted October 12, 2006.)

Acta Derm Venereol 2007; 87: 246–254.

Lennart Emtestam, Division of Dermatology and Venereology, 143, Karolinska University Hospital Huddinge, SE-141 86 Stockholm, Sweden.
E-mail: lennart.emtestam@ki.se

Acne is an extremely common skin disease affecting almost all adolescents (1). Treatments for acne are aimed at the main pathogenic factors: follicular hyperkeratosis, hyper-seborrhoea, colonization with *Propionibacterium acnes*, inflammation and immunological reactions (2). *P. acnes* is an important factor in the development of inflammatory reactions in acne patients and, even though its precise role is still unclear, it seems to be connected to the local microenvironment and the production of cytokine-like substances (3).

Cyclines are broad-spectrum bacteriostatic antibiotics and are the most commonly prescribed oral antibiotics for moderate to severe inflammatory acne (4). According to recent guidelines regarding acne therapy, oral antibiotics should not be used alone, and combination with a topical retinoid is highly recommended in cases of inflammatory disease (4, 5). Topical retinoids target the microcomedo, which is the precursor of acne lesions, and are the preferred drugs for maintenance therapy (5). Adapalene (ADA) is a third-generation topical retinoid, which is safe, highly efficient and very well tolerated (5).

Isotretinoin (ISO) is a synthetic retinoid (13-cis-retinoic acid) and is a very effective agent for the treatment of severe inflammatory acne, and for the treatment of moderate acne in the presence of scars or psycho-social problems. ISO affects all factors involved in the pathogenicity of acne: sebum production, follicular hyperkeratinization, microbial colonization by *P. acnes* and release of inflammatory mediators into the dermis (6).

The resistance of *P. acnes* strains has risen recently (7–9), but an association between carriage of resistant propionibacteria and treatment failure has yet to be established. Acne is no longer considered a simple infectious disease, and the production of inflammatory substances seems to be an important part of the disease pathophysiology (10).

The aim of this study was to compare the clinical effect of oral tetracycline (TET) plus topical ADA to oral ISO in patients with moderate to severe acne and to investigate the density and antimicrobial susceptibility of *P. acnes* skin strains isolated before, during and after treatment, in correlation with the clinical response.

MATERIALS AND METHODS

Study design

This single-centre, randomized, parallel-group study was conducted among patients with moderate to severe acne attending for treatment at the Division of Dermatology, Karolinska University Hospital Huddinge, Stockholm. Approval for the study was obtained from the ethics committee of the hospital. Before initiation of the study, each patient or guardian (in the case of minor patients) was fully informed about the study and written informed consent was obtained from patients or guardians.

Study population

Male and female patients who had moderate or severe inflammatory acne vulgaris (at least grade 3 on the face, back or chest according to the Leeds technique) and were in the age range 15–35 years were enrolled in the study. The clinical diagnosis of moderate or severe acne was based on the presence of papulo-pustular acne with nodular lesions or nodular/conglobated acne.

Patients were excluded if they had only comedonal or papulo-pustular acne with no nodules or very severe forms (acne fulminans), or if they had used oral/topical acne treatments within 8 weeks of the start of the study, or systemic retinoids within 12 months of the start of treatment (2). Patients receiving drugs that may interfere with tetracycline (i.e. retinoids, anticoagulants, antacids, iron preparations or hepatic enzyme inducers) were excluded. Also excluded were: pregnant women or those who wanted to become pregnant, breastfeeding mothers, patients with systemic or psychiatric diseases (including drug or alcohol abuse), patients with any dermatological condition that might interfere with the evaluation of acne or with acne due to secondary causes, patients participating in any other clinical trial, and those with hypersensitivity or allergy to the study medication.

Treatment and study procedures

Patients were assigned randomly to one of 2 treatment groups, using a computer-generated randomization code known only to a person not involved in the trial. The first group (TET/ADA group) received a 24-week treatment with TET hydrochloride (500 mg twice daily, one h before meals) and topical ADA once a day in a thin film on the affected area (Differin® gel, 0.1%, Galderma Nordic AB, Stockholm, Sweden). The second group (ISO group) received oral ISO (Roaccutane®, Roche), 1 mg/kg/day, in two divided doses (11). After cessation of the oral treatment, the patients in the TET/ADA group used topical ADA once a day as maintenance therapy during the 2-month follow-up period (4), while the patients in the ISO group did not use any maintenance therapy in the follow-up period. ADA was applied once a day at night time and skin sampling was performed for all the patients in the morning. The face was washed at least one h before sampling.

ISO is a potent teratogen, and for women treated with this drug a pregnancy test was performed. The ISO-treated females were independently given low-dose combination hormones as oral contraceptives according to Swedish recommendations, either Diane® (Schering), Cilest® (Jansen-Cilag) or Trinovum® (Jansen-Cilag). Oral contraception was started before and continued during the treatment period and 6 weeks' post-therapy (5, 11). TET is also contraindicated during pregnancy and female patients receiving this drug were informed about possible modifications of bones and teeth structure in developing fetuses (12). Patients were instructed to avoid extensive exposure to the sun or ultraviolet rays during treatment in order to prevent photosensitization, and not to use cosmetics with antiseptic properties. No other anti-acne drugs, antibiotics or corticosteroids were permitted during the study. Patient compliance was assessed by counting the capsules returned to the dermatologist.

Clinical assessments

The clinical efficacy assessments were based on the lesion-counting and acne-grading system described by Burke & Cunliffe (13). At each visit (baseline, follow-up visits at 2, 4, 6 months of treatment, and 2 months after cessation of treatment) lesions were counted on the face, back and chest and categorized into non-inflammatory (open and closed comedones), superficial inflammatory (papules, pustules) and deep inflammatory (no-

dules). The sum of all these lesions was the total lesion count at each visit. Acne grading was carried out independently by two dermatologists at each visit.

In order to evaluate patients' perception and assessment regarding the two treatments, the Dermatology Life Quality Index (DLQI) (14), a self-administered questionnaire designed to measure the impact of skin diseases on patients' quality of life, was completed by all patients before the treatment started and after the treatment had stopped.

For patients receiving ISO, co-medication with vitamin A (increased toxicity), tetracycline (cranial hypertension) and high doses of aspirin (potentiation of mucosal damage) were prohibited (15).

Microbiological assessments

Skin samples were taken at baseline, after 2, 4, 6 months of treatment and 2 months after cessation of treatment. Samples were taken from five areas (forehead, right cheek, left cheek, back and chest) using a soft agar gel with a cross-sectional area of approximately 2 cm², which was pressed against the skin surface for 10 seconds without rotation. A slice of the soft agar with the bacterial-bearing surface was cut off, placed in a sterile glass tube with pre-reduced peptone-yeast extract medium and immediately transported to the laboratory for microbial cultivation, identification and quantification, as previously described (9). The determination of resistant strains consisted of plating each strain on a non-selective medium containing antibiotics at breakpoint value for the isolation of resistant bacteria using the agar dilution method (16). The strains were considered to be resistant if they grew on the agar plate containing antibiotic concentration at the breakpoint value and above the breakpoint value. Minimum inhibitory concentration (MIC) was defined as the lowest concentration of antimicrobial agent resulting in a marked change in the appearance of growth compared with the control plate, as described in the NCCLS protocol (16).

The strains were tested for TET, clindamycin (CL), erythromycin (EM) and linezolid (LIN) and the breakpoints were according to the European Committee on Antimicrobial Susceptibility Testing recommendations (9).

Safety measures

Laboratory monitoring of patients was performed before the treatment started and after 2, 4 and 6 months of therapy. Monitoring of the patients who received ISO included blood counts, liver enzymes, triglycerides, fasting total cholesterol, low-density lipoproteins and high-density lipoproteins. The patients were instructed how to minimize the drying effect of ISO by using moisturizers and a prophylactic eye lubricant. Laboratory monitoring in the group of patients receiving TET included a complete blood count, and a 12-h fasting blood chemistry panel.

At each visit the patients were questioned about possible side-effects of the drugs.

Statistical analysis

Analysis of clinical efficacy parameters was performed on the intention-to-treat population, which included all patients who had at least one post-baseline evaluation. For patients who prematurely discontinued the treatment, the last observations were carried forward.

Lesion counts were log natural transformed in order to normalize them, and their reduction from baseline was analysed using two-way analysis of variance (ANOVA), followed by a *post hoc* test.

The response variables according to the Leeds grading score were divided into three categories of severity: I = grade 0–1;

II = grade 2–3 and III = grade ≥ 4 and the PROC GENMOD from Statistical Analysis Software (SAS) 6.12 software was used for further analysis (17).

DLQI scale values were analysed using non-parametrical tests (Friedman's test and Mann-Whitney *U* test).

Bacterial counts were expressed as $\log_{10}$ colony-forming units (CFU)/cm² skin. Changes in total bacterial counts relative to baseline were analysed by repeated-measures ANOVA followed by *post hoc* tests.

The probability of the patients belonging to one of the two groups carrying resistant strains was analysed using a logistic regression analysis with baseline variable as a covariate. The detection accuracy of the method was described by the area under the receiver operating characteristic (ROC) curve. Correlations between clinical and microbiological data were analysed using the Spearman rank correlation coefficient.

All statistical tests were two-sided and significance was declared at the 0.05 level. Statistical analyses were performed using Statistica version 7.0 software.

RESULTS

Patients

Recruitment of patients began in December 2001 and the last patient was recruited in October 2003. A total of 52 patients were randomized; 26 to receive TET/ADA and 26 to receive ISO. Of these, 3 patients abandoned the study after randomization and were not included in further analyses. Overall, 7 patients in the ISO group and 6 patients in the TET/ADA group abandoned the study at different times (Fig. 1). The therapy was regarded as completed due to a very good clinical response before the end of 6 months in one patient from the ISO group.

The demographic and baseline data were well matched and are presented in Table I.

Efficacy evaluation

Both medications resulted in a reduction in the number of superficial inflammatory (Fig. 2a), deep inflammatory (Fig. 2b) and non-inflammatory lesions (Fig. 2c) ($p < 0.001$), but ISO demonstrated an advantage over

Table I. Demographic and baseline characteristics of the intention-to-treat population

Criterion	TET/ADA <i>n</i> = 25	ISO <i>n</i> = 24
Age (years) (median $\pm$ SD)	19 $\pm$ 5.5	18 $\pm$ 6
Age range (years)	15–34	15–35
Gender, <i>n</i> (%)		
Male	15 (60)	17 (70.8)
Female	10 (40)	7 (29.1)
Body-mass index (median $\pm$ SD)	22 $\pm$ 3.4	22.7 $\pm$ 2.4
Race, <i>n</i> (%)		
White	23 (92)	23 (95.8)
Black	1 (4)	–
Oriental	1 (4)	1 (4.1)
Disease duration (years)	4 $\pm$ 4.2	3 $\pm$ 5.4
Previous antibiotic treatment ^a (%)	12 (48)	9 (37.5)
Acne grading (median $\pm$ SD)		
Face	5 $\pm$ 2.9	5 $\pm$ 2.1
Back	3 $\pm$ 2.4	3 $\pm$ 2.3
Chest	2 $\pm$ 1.7	1 $\pm$ 1.8
Lesion type (median $\pm$ SD)		
Open/closed comedones	110 $\pm$ 111.7	165 $\pm$ 106
Papules/pustules	50 $\pm$ 64.5	46 $\pm$ 53.1
Nodules	5 $\pm$ 6.5	7 $\pm$ 6
Presence of facial scars (%)	68	66
$\log_{10}$ <i>P. acnes</i> counts (mean $\pm$ SD)	3.1 $\pm$ 1	2.6 $\pm$ 1.2

^aMore than 8 weeks before inclusion in the study.

TET: tetracycline; ADA: adapalene; ISO: isotretinoin.

TET/AD. There was a significant difference between ISO and TET/ADA treatment results, which began after 2 months of treatment (for non-inflammatory lesions) and after 4 months (for superficial inflammatory lesions). There was no difference between the treatment groups with regard to deep inflammatory lesions after 6 months of therapy. After the treatment had stopped, patients in the ISO group had fewer lesions than those in the antibiotic group. There was no correlation between patients' age or acne duration and the clinical response in either group.

Four percent of patients in the TET/ADA group and 16.6% in the ISO group were found to have no inflammatory lesions and less than 10 non-inflammatory lesions after 6 months of treatment ($p > 0.05$). After the follow-up period, no patients in the TET/ADA group and 20.8% in the ISO group were found to have no inflammatory lesions and less than 10 non-inflammatory lesions ($p = 0.02$).

Total lesion counts were summarized and analysed by sub-group for gender. Both male and female patients in the ISO group had greater reductions after 6 months of treatment (87.3% and 90.6%, respectively) vs. similar gender sub-groups treated with TET/ADA (43.3% and 62.5%), $p < 0.05$ for both male and female patients. The ISO advantage persisted in the drug-free period, and the female patients treated with ISO were the only patients in whom the total number of lesions continued to decrease markedly, to 43.1% after the treatment stopped, while in the other sub-groups the lesion number increased.

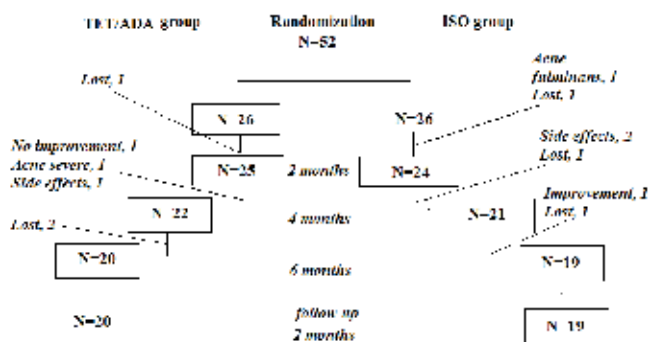


Fig. 1. Patients participating in the study and reasons for abandoning the study at different time-points. TET/ADA, tetracycline/adapalene; ISO, isotretinoin.

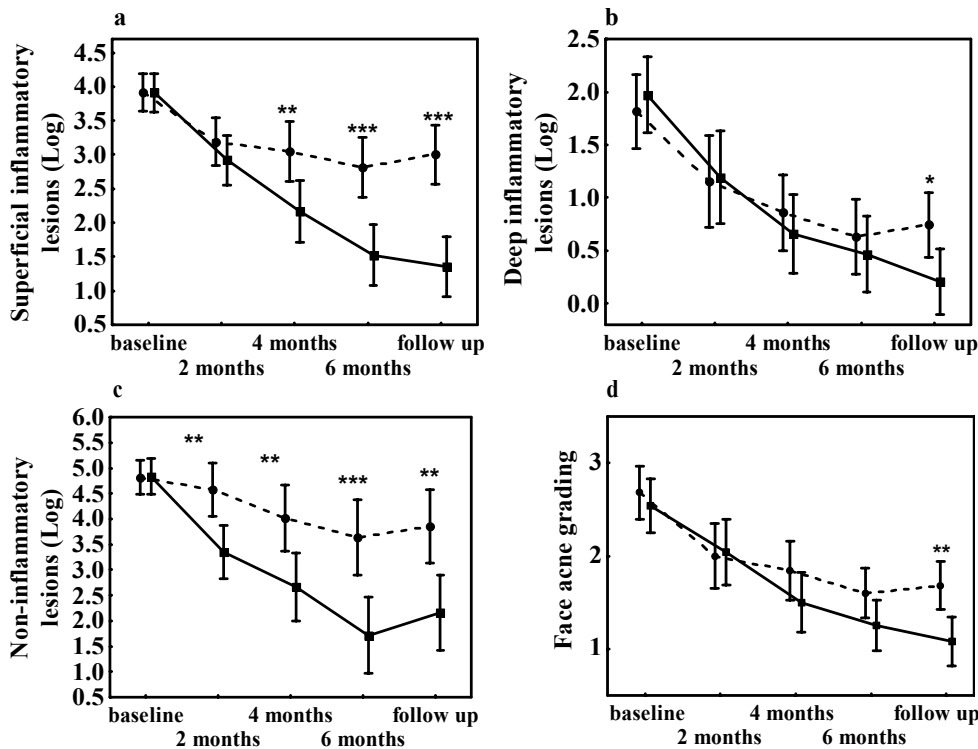


Fig. 2. Clinical results in patients treated with tetracycline/adapalene (●) or isotretinoin (■). Reduction in: (a) superficial inflammatory; (b) deep inflammatory; and (c) non-inflammatory lesions (mean natural log transformed number $\pm$ 95% confidence interval (CI) is shown). (d) Face acne grading (mean $\pm$ SD/SEM). Acne grading was given as a value from 1 to 3, where grade 1 = Leeds acne score 0 and 1; Grade 2 = Leeds acne score 2 or 3 and Grade 3 = Leeds acne score $\geq$ 4. * p < 0.05; ** p < 0.01; *** p < 0.001 for difference between treatment groups.

A better time evolution of the face acne grading was noticed for patients treated with ISO compared with those treated with TET/ADA (an interaction between time and treatment p = 0.008). Patients treated with either TET/ADA or ISO showed a decreased score, starting in the first 2 months and for the whole treatment period, but after the treatment stopped only those treated with ISO had a tendency to decreased face acne grading score (p = 0.052) (Fig. 2d). Comparisons between groups at different time-points demonstrated no difference between ISO and TET/ADA during the 6-month treatment period, but a significant difference after the follow-up period (p = 0.009). For both the back and chest there was an improvement in acne scores over time with both treatments (p < 0.001), but no difference was noticed between the treatments.

The maximum value for the DLQI questionnaire is 30, and the higher the score the more quality of life is impaired (14). Median ($\pm$ SD) DLQI scores were similar at baseline: 6.5 ± 3.9 for the ISO group and 6 ± 6 for the TET group. There was a significant improvement in the DLQI after 24 weeks of therapy in both treatment groups: 2.5 ± 2.7 in the ISO group, and 3 ± 5.3 in the TET/ADA group (p < 0.001). Analysing the gender difference, an improvement in quality of life was noticed for male patients in both groups (p < 0.01) and for female patients treated with ISO, but not for female patients treated with TET/ADA. The number of total lesions and mean DLQI score in female patients treated with antibiotics were found to have a positive correlation coefficient (r = 0.6, p = 0.05).

Microbiological results

Samples were collected from five different areas in order to collect a greater diversity of bacteria, and *P. acnes* from the face, back and chest were pooled. The microbiological results are reported in terms of the mean number of bacteria per square centimetre and the prevalence of patients in each group having a particular finding.

Both treatments produced a significant reduction in total *P. acnes* counts from baseline evaluation, but ISO demonstrated better antimicrobial efficacy than TET/ADA, starting from 2 months of treatment, and the dif-

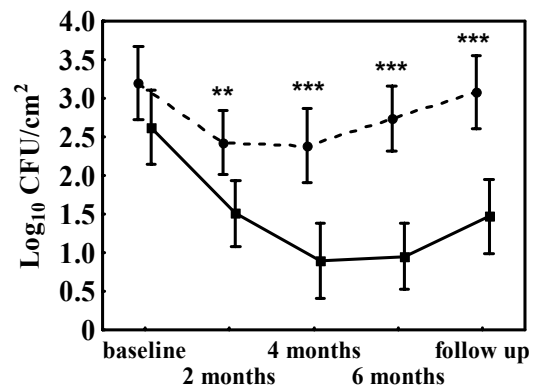


Fig. 3. Density of total *P. acnes* counts (mean $\pm$ 95% confidence interval (CI)), expressed as $\log_{10}$ CFU/cm² in patients treated with tetracycline/adapalene (TET/ADA) (●) or isotretinoin (ISO) (■). ** p < 0.01 *** p < 0.001. CFU, colony-forming units.

ference persisted for the duration of the study (Fig. 3).

There was no significant variation in the density of TET-resistant or CL-EM-resistant bacteria in either group, although the proportion of TET-resistant to total recovered bacteria increased in the TET/ADA group during the 6 months of treatment and did not decrease after the treatment stopped. In the ISO group the patients acquired TET-resistant strains during the 6-month treatment period. The patients treated with ISO who were carrying resistant TET bacteria were found to have had contact with acne patients or former acne patients ($r=0.4$, $p<0.05$). No strain was found to be LIN-resistant.

Twelve percent of patients from the TET/ADA group had acquired resistant strains after 6 months of treatment, while the number of patients carrying resistant bacteria was constant in the ISO group after a 6-month treatment period. Two months after the treatment stopped, one further patient in the TET/ADA group acquired resistant bacteria, while in the ISO group 2 patients had lost their resistant *P. acnes*. Thus, in general, the tendency was for patients treated with TET/ADA to gain resistant strains of *P. acnes* and for patients treated with ISO to preserve or lose them.

More patients in the TET/ADA group were found to carry resistant bacteria at baseline (28% TET-resistant, 44% CL-resistant, 24% EM-resistant) compared with the ISO group (16.6% CL-resistant, 12.5% EM-resistant and no patient carrying TET-resistant strains). After adjusting for baseline differences and by using logistic regression, there was no difference among the two groups with regard

to the carriage of TET- or EM-resistant strains after 6 months of treatment. During the same period, the odds for a patient in the TET/ADA group to carry CL-resistant strains as compared with the ISO group was 0.18 (95% confidence interval (CI)=0.05–0.71, $p=0.014$). The ROC curve showed a good sensitivity and specificity of the measure (Fig. 4a). Two months after the treatment had stopped, there was a significant difference between treatments, and the probability to carry resistant strains for CL and TET was higher in the TET/ADA group compared with the ISO group (odds ratio (OR)=0.06, 95% CI=0.013–0.37, $p<0.01$, OR=0.05, 95% CI=0.006–0.49, $p<0.001$, respectively) (Fig. 4b, c).

After 6 months of treatment, patients carrying TET-resistant *P. acnes* were also found more often to carry CL-EM-resistant *P. acnes* in both groups ($p<0.05$).

The carriage of TET-, CL- or EM-resistant *P. acnes* strains at least at one determination did not influence the clinical response, expressed as the total number of inflammatory lesions. The same response pattern was noticed for the patients colonized with susceptible bacteria (Fig. 5a) and for those patients carrying resistant *P. acnes* isolates at least at one examination during the study (Fig. 5b). No statistical correlation was found between the density or the presence of TET-resistant *P. acnes* and the clinical response in the TET/ADA group (number of lesions or acne grading score) at any time-point during the study and for any of the investigated regions (face, back or chest). The carriage of TET-, CL- or EM-resistant bacteria in any group did not affect the improvement in quality of life score.

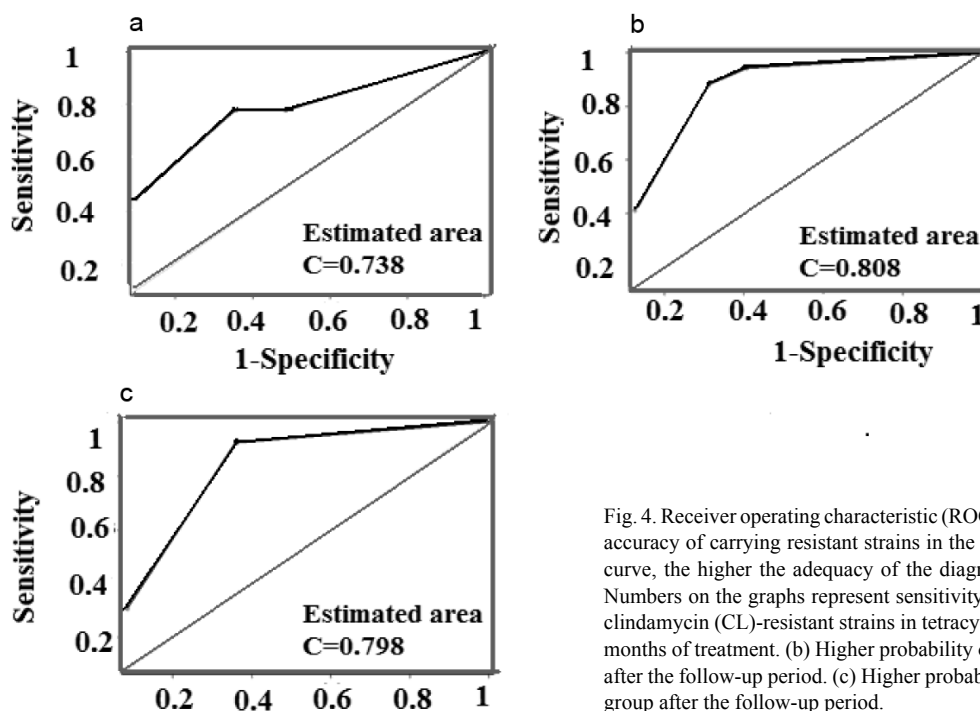


Fig. 4. Receiver operating characteristic (ROC) curves for the detection of the predictive accuracy of carrying resistant strains in the two groups. The larger the area under the curve, the higher the adequacy of the diagnostic test for the detection of resistance. Numbers on the graphs represent sensitivity and specificity. (a) Higher probability of clindamycin (CL)-resistant strains in tetracycline/adapalene (TET/ADA) group after 6 months of treatment. (b) Higher probability of CL-resistant strains in TET/ADA group after the follow-up period. (c) Higher probability of TET-resistant strains in TET/ADA group after the follow-up period.

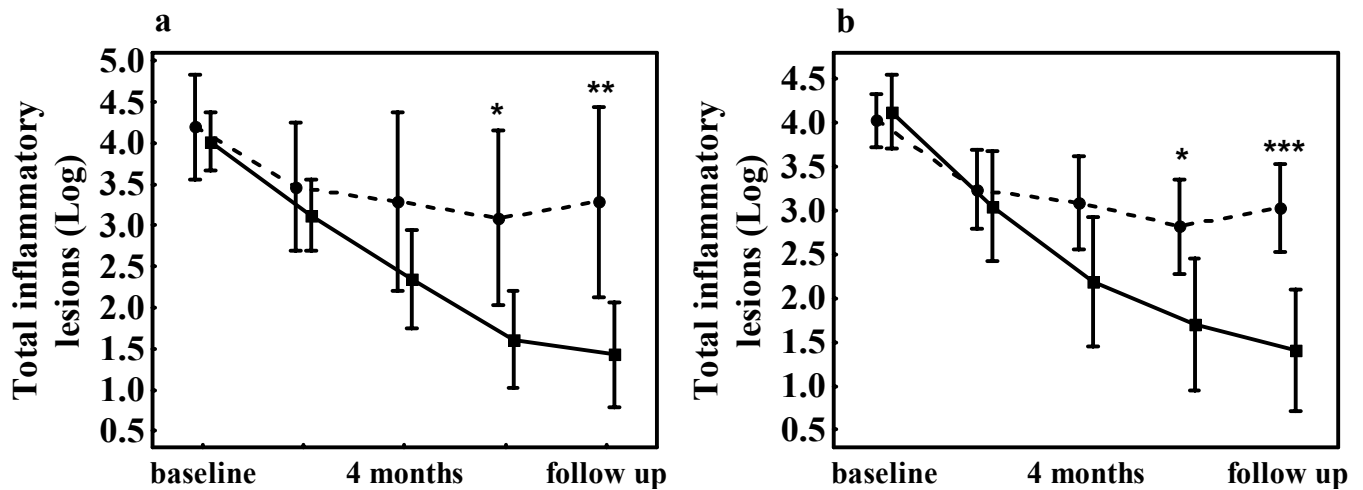


Fig. 5. (a) Changes over time in the total number of inflammatory lesions for patients colonized with susceptible *P. acnes* isolates during the study. $n = 4$ patients in tetracycline/adapalene (TET/ADA) (●) group; $n = 13$ patients in isotretinoin (ISO) group (■), (b) Changes over time in the total number of inflammatory lesions for patients colonized with resistant *P. acnes* isolates at least at one determination. $n = 21$ patients in TET/ADA group; $n = 11$ patients in ISO group. * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$.

Side-effects

In the ISO group the most common side-effects were dry skin (91.4%), cheilitis (95.8%), dry eyes (75%) and epistaxis (54%) after 2 months of treatment. The rate of side-effects had changed after 6 months of therapy: dry skin and cheilitis (92%), dry eyes (58%) and epistaxis (42%), and the symptoms disappeared completely after discontinuation of the treatment in 83% of cases. Two patients stopped the treatment because of severe xerosis. One patient developed acne flare early in the treatment and had to stop the treatment (18). Three patients in this group complained of "tiredness" or "fatigue". They were carefully monitored, but the symptoms disappeared after the treatment stopped. One male patient presented a transitory moderate increase in liver tests (aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT)), which became normal when the tests were repeated. However, most ISO-treated patients experienced transitory increases in triglycerides and cholesterol, but within the normal limits.

Ten percent of patients in the TET/ADA group complained of transitory nausea or abdominal pain during the treatment and one patient abandoned the study because of gastro-intestinal disturbances. Fifteen percent of patients experienced dry skin, itching and redness at least at one time-point during the study.

DISCUSSION

ISO is considered a very efficient acne treatment, but has a wide variety of possible side-effects. It is of interest to compare the clinical and bacteriological response after ISO therapy with another efficient alternative treatment that does not have such risks. This alternative treatment in the case of moderate/severe acne is the

combination of an oral antibiotic and a topical retinoid followed by topical retinoid as maintenance therapy. There is one study comparing only the clinical efficacy of ISO and TET (19), excluding an important group of acne patients, women of childbearing potential, and not taking into consideration the bacteriological aspect. Also, Lester et al. (19) have administered antibiotic without any associated topical treatment, which is no longer recommended (5).

In our open study, both treatments improved the clinical condition, although ISO was more effective in the treatment of the majority of lesions, had a faster onset of action and, after the discontinuation of therapy, was found to have better long-term efficacy compared with TET/ADA. Acne grading implies a certain grade of subjectivity and, using this evaluation method, the difference between treatments became apparent after therapy was discontinued for the face lesions, while for the back and chest there was no difference in response between the ISO group and the TET/ADA group, probably because the acne in patients entering the study was not so severe on the back and chest compared with the face.

Female patients in the ISO group received contraceptive treatment with anti-seborrhoeic activity for the study duration and one month after the ISO stopped; therefore the clinical results were also presented for gender groups. This treatment did not influence the response during the study, but in the drug-free period these patients were the only sub-group in which the number of lesions continued to decrease.

TET has multiple mechanisms of action in acne patients: antibacterial, inhibitory of the production of pro-inflammatory mediators produced by propionibacteria, inhibitory of tissue destructive enzymes, antioxidant activity, modulation of non-specific or innate

immunity and modulation of cytokine production, but it is not known which mechanism is the most relevant in acne patients (20). ISO had a better indirect effect in reducing the propionibacteria number, and this effect persisted for the follow-up period. This outcome may be the result of altering the follicular microclimate (15). No correlation was found between this reduction and clinical efficacy (acne grading or number of lesions), which is in agreement with Cove et al. (21), who have shown that effectiveness of oral antibiotics in treating the disease cannot be explained by a reduction in the number of viable bacteria.

On the other hand, the population of resistant bacteria from the skin was not significantly affected by the treatment with either TET/ADA or ISO. This result may also prove that once a patient becomes colonized with resistant bacteria, this is stabilized in the population and may persist a fairly long time regardless of the treatment (22).

The absence of TET-resistant strains in the ISO group and their presence after 6 months can be explained by a transfer of resistant bacteria from close contacts and it was found that more patients in this group had been in contact with acne patients, possible carriers of resistant propionibacteria (7). It was also shown that *P. acnes* can survive for long periods at room temperature in air and may be transferred between individuals via inanimate objects (23).

In contrast with previous information, which stated that resistant strains are overgrown by sensitive ones when the treatment has stopped, the cost of resistance may be ameliorated by compensatory mutations. Consequently, in competition with susceptible strains, low-fitness resistant clones may survive and persist in the bacterial population (24, 25). This finding has been proved in a clinical setting for another skin bacterium, *Staphylococcus aureus* (26). Mills et al. (22) have also found that after 3 months of topical EM treatment, the prevalence of the resistance did not significantly increase, but during the drug-free period more patients continued to carry EM-resistant *P. acnes* on the skin compared with the placebo group. They have also found that the density of EM-resistant *P. acnes* strains was unaffected by EM treatment. In the present study, similar data were found regarding TET-resistant strains after oral antibiotic treatment. An interesting point of view would be that longer periods of treatment, which are usually required in acne patients, might increase the possibility of making a resistant phenotype permanent (22). There was no difference in the prevalence of patients carrying TET-resistant bacteria after 6 months of treatment, probably because the TET concentration was high enough in the pilosebaceous follicle to inhibit all the strains. After the follow-up period more patients in the TET/ADA group carried TET-resistant strains, possibly because, while the inhibitory effect of TET

on *P. acnes* had disappeared, the indirect antibacterial effect of ISO was maintained. The higher probability of carriage of CL-resistant bacteria in the TET/ADA group can be explained by a selection of CL-resistant strains, commonly present on the skin of acne patients, due to the fact that this is a frequently prescribed topical treatment in Sweden.

From another point of view, it was speculated that TET could be a driving force and an inductor of macrolide-lincosamide-streptogramin B resistance (27). The combination between an *erm* gene and a *tet* gene (as a CL/EM, respective TET determinant of resistance) would be a possible event in the *P. acnes* genome, and this association has been described previously on a transposon in *Streptococcus pyogenes* (27). This could explain why patients carrying TET-resistant strains were more often found to be colonized with CL- and EM-resistant bacteria.

The lack of correlation between the clinical response and the presence of resistant bacteria could be explained by the high level of initial resistance found in the population (22). All acne patients are colonized with both susceptible and resistant isolates, and not always those colonized with resistant strains on the skin surface contain resistant *P. acnes* deep in the infundibulum, where acne pathogenicity takes place (20). Antibiotic resistance could be a possible contributor to therapeutic failure in some acne patients, but the interaction between the antibiotic and the pilosebaceous follicle is very complex (1, 28). If the antibiotic concentration within the follicle exceeds the MIC of the antibiotic for the *P. acnes* residing inside, the antibacterial effect will be enough to destroy even bacteria considered to be resistant by *in vitro* MIC determination, and consequently the bacteria may be clinically susceptible and the density of resistant strains will not increase (20). The patients' outcome following TET treatment is much more dependent on follicular levels by comparison with EM treatment, due to the fact that the MIC of EM for resistant strains is much higher than the follicular concentration of the drug that could be reached by oral administration of the drug (20). The sebum excretion rate is also pronounced in the majority of acne patients and is an important parameter affecting the final antibiotic concentration within the follicle (29). In addition to these observations, the ADA used in addition to TET enhanced the clinical response by targeting both non-inflammatory lesions and inflammation (4). Ozolins et al. (1) have found that colonization with tetracycline-resistant bacteria significantly decreased the proportion of treatment responses with oral tetracycline, but in that case no topical retinoid was added to the treatment. Interesting data came from Webster et al. (30) who have proved the anti-inflammatory capacity of antibiotics and have shown that sub-minimal inhibitory concentrations of antibiotic, which do not suppress the growth of *P.*

acnes, can inhibit the inflammatory capacity of bacteria. Taking into consideration all these factors, it is difficult to establish the causal link between resistance and clinical response.

The quality of life improved after therapy with both treatments and for both genders, for the patients carrying susceptible or resistant bacteria, with the exception of female patients treated with oral antibiotic plus topical retinoid. The female patients receiving ISO and whose quality of life improved significantly also received oral contraceptives that are also effective as acne treatment. Sometimes different methods of grading used by dermatologists do not match with patients' assessment regarding clinical benefit, but in the case of female patients from the TET/ADA group, the quality of life score was found to correlate with the total number of lesions.

Adverse effects followed the expected patterns for both drugs and the combination oral antibiotic plus local retinoid was better tolerated than oral ISO. Skin and mucosal side-effects made ISO patients easy to recognize and the study was therefore not blinded.

In a European Directive a stricter regimen for prescribing systemic ISO for acne has been introduced recently (31). Generally, ISO proved to have a faster effect and a prolonged remission after treatment. The study has indirectly shown that the anti-inflammatory properties of the tetracyclines could be important in the treatment of inflammatory acne, and the combination of oral TET and a topical retinoid may be a good option for acne patients, despite the presence of resistant *P. acnes* on the skin. The carriage of resistant strains also seems to be a persistent event. *P. acnes* antibiotic resistance does not necessarily mean that acne will be resistant, but it is important to prevent development of resistance and accumulation of resistant *P. acnes* since, after acne patients are colonized with resistant strains, these strains may persist indefinitely and become reservoirs of resistant bacteria.

In conclusion, the combination TET/ADA followed by topical ADA as a maintenance treatment proved to be a good alternative to ISO in patients for whom there is a contraindication to its use, or who do not want to use ISO due to its possible side-effects.

ACKNOWLEDGEMENTS

This study was supported in part by Edward Welander and Finsen Foundations, and by F. Hoffman-La Roche Ltd Co. The source of funding for the study had no role in collecting and interpreting the data or in writing the paper.

Conflict of interest: none to declare.

REFERENCES

1. Ozolins M, Eady EA, Avery AJ, Cunliffe WJ, Li Wan Po A, O'Neill C, et al. Comparison of five antimicrobial regimens

- for treatment of mild to moderate inflammatory facial acne vulgaris in the community: randomised controlled trial. *Lancet* 2004; 364: 2188–2195.
2. Gollnick HP, Graupe K, Zaumseil RP. Comparison of combined azelaic acid cream plus oral minocycline with oral isotretinoin in severe acne. *Eur J Dermatol* 2001; 11: 538–544.
3. Cunliffe WJ, Holland KT, Bojar R, Levy SF. A randomized, double-blind comparison of a clindamycin phosphate/benzoyl peroxide gel formulation and a matching clindamycin gel with respect to microbiologic activity and clinical efficacy in the topical treatment of acne vulgaris. *Clin Ther* 2002; 24: 1117–1133.
4. Dreno B, Bettoli V, Ochsendorf F, Layton A, Mobacken H, Degre H. European recommendations on the use of oral antibiotics for acne. *Eur J Dermatol* 2004; 14: 391–399.
5. Gollnick H, Cunliffe W, Berson D, Dreno B, Finlay A, Leyden JJ, et al. Management of acne: a report from a Global Alliance to Improve Outcomes in Acne. *J Am Acad Dermatol* 2003; 49: S1–S7.
6. Goldsmith LA, Bologna JL, Callen JP, Chen SC, Feldman SR, Lim HW, et al. American Academy of Dermatology Consensus Conference on the safe and optimal use of isotretinoin: summary and recommendations. *J Am Acad Dermatol* 2004; 50: 900–906.
7. Ross JI, Snelling AM, Carnegie E, Coates P, Cunliffe WJ, Bettoli V, et al. Antibiotic-resistant acne: lessons from Europe. *Br J Dermatol* 2003; 148: 467–478.
8. Oprica C, Nord CE. European surveillance study on the antibiotic susceptibility of *Propionibacterium acnes*. *Clin Microbiol Infect* 2005; 11: 204–213.
9. Oprica C, Emtestam L, Lapins J, Borglund E, Nyberg F, Stenlund K, et al. Antibiotic-resistant *Propionibacterium acnes* on the skin of patients with moderate to severe acne in Stockholm. *Anaerobe* 2004; 10: 155–164.
10. Dreno B. Pathophysiology of acne. *Presse Med* 2005; 34: 537–539.
11. Katsambas A, Papakonstantinou A. Acne: systemic treatment. *Clin Dermatol* 2004; 22: 412–418.
12. Niebyl JR. Antibiotics and other anti-infective agents in pregnancy and lactation. *Am J Perinatol* 2003; 20: 405–414.
13. Burke BM, Cunliffe WJ. The assessment of acne vulgaris – the Leeds technique. *Br J Dermatol* 1984; 111: 83–92.
14. Finlay AY, Khan GK. Dermatology Life Quality Index (DLQI) – a simple practical measure for routine clinical use. *Clin Exp Dermatol* 1994; 19: 210–216.
15. Orfanos CE, Zouboulis CC. Oral retinoids in the treatment of seborrhoea and acne. *Dermatology* 1998; 196: 140–147.
16. National Committee for Clinical Laboratory Standards. Methods for antimicrobial susceptibility testing of anaerobic bacteria. Approved standard – Sixth edition. NCCLS document M11-A6. Wayne, PA: NCCLS, 2004.
17. SAS/STAT Software. Change and enhancements through release 6.12. Cary, NC: SAS Institute Inc., 1997.
18. Chivot M. Acne flare-up and deterioration with oral isotretinoin. *Ann Dermatol Venereol* 2001; 128: 224–228.
19. Lester RS, Schachter GD, Light MJ. Isotretinoin and tetracycline in the management of severe nodulocystic acne. *Int J Dermatol* 1985; 24: 252–257.
20. Eady AE, Cove JH, Layton AM. Is antibiotic resistance in cutaneous propionibacteria clinically relevant? implications of resistance for acne patients and prescribers. *Am J Clin Dermatol* 2003; 4: 813–831.
21. Cove JH, Cunliffe WJ, Holland KT. Acne vulgaris: is the bacterial population size significant? *Br J Dermatol* 1980; 102: 277–280.
22. Mills O Jr, Thornsberry C, Cardin CW, Smiles KA, Leyden JJ.

- Bacterial resistance and therapeutic outcome following three months of topical acne therapy with 2% erythromycin gel versus its vehicle. *Acta Derm Venereol* 2002; 82: 260–265.
23. Qureshi A, Ross JI, Snelling AM, Eady EA, Cove JH. Survival of antibiotic-resistant *Propionibacterium* acnes in the environment. 14th European Congress of Clinical Microbiology and Infectious Diseases 2004, Prague, poster presentation.
 24. Andersson DI. Persistence of antibiotic resistant bacteria. *Curr Opin Microbiol* 2003; 6: 452–456.
 25. Morell V. Antibiotic resistance: road of no return. *Science* 1997; 278: 575–576.
 26. Nagaev I, Bjorkman J, Andersson DI, Hughes D. Biological cost and compensatory evolution in fusidic acid-resistant *Staphylococcus aureus*. *Mol Microbiol* 2001; 40: 433–439.
 27. Nielsen HU, Hammerum AM, Ekelund K, Bang D, Pallesen LV, Frimodt-Moller N. Tetracycline and macrolide co-resistance in *Streptococcus pyogenes*: co-selection as a reason for increase in macrolide-resistant *S. pyogenes*? *Microb Drug Resist* 2004; 10: 231–238.
 28. Eady EA, Cove JH, Holland KT, Cunliffe WJ. Erythromycin resistant propionibacteria in antibiotic treated acne patients: association with therapeutic failure. *Br J Dermatol* 1989; 121: 51–57.
 29. Eady EA, Cove JH, Blake J, Holland KT, Cunliffe WJ. Recalcitrant acne vulgaris. Clinical, biochemical and microbiological investigation of patients not responding to antibiotic treatment. *Br J Dermatol* 1988; 118: 415–423.
 30. Webster GF, Leyden JJ, McGinley KJ, McArthur WP. Suppression of polymorphonuclear leukocyte chemotactic factor production in *Propionibacterium* acnes by subminimal inhibitory concentrations of tetracycline, ampicillin, minocycline, and erythromycin. *Antimicrob Agents Chemother* 1982; 21: 770–772.
 31. Layton AM, Dreno B, Gollnick HPM, Zouboullis CC. A review of the European Directive for prescribing systemic isotretinoin for acne vulgaris. *J Eur Acad Derm Venereol* 2006; 20: 773–776.