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Minocycline Induces an Increase in the Number of Excreting Pilosebaceous Follicles in Acne Vulgaris

A Randomised Study

I. BODOKH[1], Y. JACOMET[2], J. Ph. LACOUR[1] and J. P. ORTONNE[1]

[1]Department of Dermatology, Hôpital PASTEUR, University of Nice, and [2]Department of Pharmacology, Faculty of Medicine, University of Nice, France

The effects of treatment with minocycline 100 mg per day on sebaceous excretion in acne vulgaris using lipometry and Sebutape® were studied in 45 patients in an open study as well as in a randomised placebo-controlled study. In both studies a subclinical increase in sebaceous excretion was noted from the 28th day of treatment. This effect continued for 1 month after the end of treatment. The increase in sebaceous excretion was concomitant with an increase in the number of excreting pilosebaceous follicles. Minocycline may cause the follicles to become unblocked by acting on the factors responsible for ostial hyperkeratosis, in addition to an antibacterial effect on retentional acne lesions. ***Key word: seborrhoea.***

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I. Bodokh, Hôpital des Brousailles, Avenue des Brousailles, F-06400 Cannes, France.

Tetracyclines have been considered as the treatment of choice for acne vulgaris over the past 30 years. Many mechanisms have been proposed to explain their activity: a reduction in colonisation by Propionibacterium acnes (PA) due to their bacteriostatic action, an inhibition of lipase synthesis by PA; an anti-inflammatory effect, especially by a reduction in polymorphonuclear leukocytes chemotaxis, and a modulation in mononuclear cell activities (1–3). Those different mecha-nisms explain their efficacy on the acne inflammatory compo-nent. Apart from their lipase-inhibiting activity, which reduces the quantity of fatty acids in the sebum, the role of tetracyclines in sebaceous excretion has been poorly documented. This has led us to evaluate the action of minocycline (semi-synthetic cycline with a wide lipophilic spectrum) on sebaceous excretion in patients with acne vulgaris, in an open study as well as in a randomised double-blind, placebo-controlled study.

MATERIALS AND METHODS

Patients

The two studies were performed in patients with moderate acne vulgaris: grade 0.5 to grade 2 (4), and with a sebum excretion rate (SER) measured by alipometer on the forehead, of 0.2 μg/cm^2/min or more. The patients had not taken any treatment likely to interfere with acne or seborrhoea: especially no hormonal treatment, either oral or topical, or any oral isotretinoin, over the past 6 months preceding the study. During the selection visit, all patients received the same neutral dermatological cleansing bar to be used for washing their face, and were told not to apply any other product on their face throughout the study. Minocycline (Mynocine® from the Wyeth-Lederle Laboratories) was administered as 100 mg capsules with a meal. The placebo administered in the randomised study had the same presentation.

The first open study was carried out from January to May 1990: Twenty-one patients aged 15 to 30 years (11 males, 10 females) were treated with one capsule of minocycline every morning for 8 weeks. The clinical evaluation of acne and the measurements of seborrhoea were made on days 0, 28 and 56. The second study, the design of which was double-blind, randomised and placebo-controlled, lasted for 20 weeks. It was conducted from January until July 1991 in 24 patients aged 18 to 30 years (12 males and 12 females) who were examined every 4 weeks throughout the study. Twelve patients were randomised to receive one capsule of minocycline for the first 12 study weeks, the remaining 12 patients were given the placebo. After 12 weeks treatment was stopped, but measurements were made at weeks 16 and 20, i.e. 4 and 8 weeks after discontinuation of treatment. For both studies, ethics committee approval had been obtained, as well as written informed consent by the patients before inclusion in the study.

The measurement of seborrhoea was made using two non-invasive methods. The measurements were made at the same time of the day in order to avoid variations due to ambient temperature.

The lipometer (L'OREAL, France) (5) is an optical method allowing the measurement of the total mass of lipid deposited on a ground glass surface which has been pressed onto the skin with identical force on each occasion, by means of a dynamometric piston. The measurements were made on the forehead at the same site at each visit, and the following parameters were determined:

- total weight of lipids in the resting state (or casual level, CL)
- the value obtained after defatting the skin surface using ethanol (D1) (this had to be between 0 and 5 μg/cm^2)
- the mass of lipid found 1 h later (D2). Sebum excretion rate (SER) was therefore calculated according to the following formula:

$$\text{SER: } \frac{D2-D1}{60}$$

The skin temperature was measured at the end of each visit near the measured sites, immediately after D2 measurement was performed, using a skin thermometer (DIGIMED II, Industrie, Medizin-Messtechnik GmbH-RFA) in order to check that there were no major variations in cutaneous temperature.

The measurement of sebaceous excretion, follicle by follicle, was performed using the Sebutape® method (Hermal Pharmaceutical Industries, New York) (6). Sebutape® is a microporous, opaque, adhesive film which absorbs lipids. Surface lipids absorbed by Sebutape® are visible as a multitude of transparent blots the size of which depends on the level of follicular excretion. It was graded as 0, 1, 2, 3, 4 or 5 corresponding to the number of blots of lipid absorption and their surface areas, the evaluation being made in comparison with established reference patterns. Grade 0 corresponds to no sebaceous excretion and Grade 5 to maximum excretion. The Sebutape® was first assessed clinically using the reference patterns and then afterwards using an automated image analyser (Biocom-France). In order to determine the variations in pilosebaceous follicle excretion (PSFE), the following parameters were studied using the image analyser:

- the number of excreting follicles per unit of surface area
- the surface area of sebum on the Sebutape® defined as the quantity of lipids absorbed by the film over a given period of time: this is equivalent to the SER

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- the mean surface area of each lipid blot, which allows evaluation of the excretion intensity of a single pilosebaceous follicle

The image analyser consequently enabled us to make different measurements, and we arbitrarily selected three follicle populations having three different grey levels (GL): The first one of high grey level (HGL) corresponds to a small transparent blot, and the second of intermediate grey level value (IGL) and the third of low grey level value (LGL) correspond to a large transparent blot on the Sebutape® and thus to an area of high follicular excretion.

In order to select the same population of follicles at each visit, the Sebutape® was always applied to the same area of the forehead for 45 min. Thanks to a transparent guide made from tracing paper, the individual anatomical characteristics of each patient were drawn onto it at the first visit, and served as position markers for later visits.

Statistics

In the open study, the effects of treatment on sebaceous excretion were determined from CL, D2, and SER measurements, and the parameters defined by the image analyser (number of blots, surface area, and diameter). They were evaluated statistically by analysis of variance or by the paired *t*-test. The differences between the scores given by clinical evaluation of the Sebutape® values were compared using a multiple comparison test based on the non-parametric Kruskal-Wallis test.

In the randomised study, the results obtained by image analysis of the Sebutape® values were analysed at each visit both for minocycline-treated and for placebo-treated patients, using two statistical methods: mean values at each time-point were compared with mean values at day 0, and secondly by correlation and regression analysis. The statistical method for correlation and regression analysis was as follows: correlation study to determine whether or not the administration of minocycline or placebo has a time-related influence on the parameters; linearity study of the regression lines to justify a later comparison between the slopes of regression lines. This comparison enables the studied parameters to be separately identified. The final stage is a comparison between the slopes of regression lines, in order to discriminate between the variations in the parameters obtained under treatment with minocycline and treatment with placebo.

RESULTS

In order to compare the results more easily, an arbitrary value of 100 was given to the values obtained at day 0.

Open study

The mass of lipids in the resting state (CL) was increased on day 28: 147, and on day 56: 155 ($p<0.05$). The mass of lipid during one hour (D2) was increased on day 28: 141, and on day 56: 158 ($p<0.02$). The sebum excretion rates (SER) increased on day 28: 133, and on day 56: 158 ($p<0.02$).

These increases of CL, D2 and SER were also statistically significant between D0 and D28 and between D0 and D56 using Fischer's test at the 5% level.

The analysis of the scores obtained by clinical evaluation of the Sebutape® shows that the increase in seborrhoea between D0 and D58 was significant ($p<0.04$). The statistical analysis of the data corresponding to the number of blots, total surface area, and mean surface area per follicle showed that the difference in the means was significant between D0 and D56 ($p<0.001$) for all the parameters studied.

Randomised study (Table I)

The analysis of the results shows that for the total surface area of sebum found on the Sebutape® as well as for the number of excreting follicles, the results obtained by clinical evaluation of the grade of Sebutape® using Reference Patterns increased in the minocycline treated group, whilst these parameters vary only slightly and irregularly in the placebo group. This increase began in the first month of treatment (Fig. 1). The increase continued throughout the study and lasted for one month after the end of treatment with minocycline. The statistical interpretation of the results by correlation and regression analysis shows a clear difference between the patients being treated with placebo and those being treated with minocycline. This difference began at day 0 and remained obvious until day 112 (Figs. 2 and 3). In the placebo group, no statistically significant variation was found for any of the different measurements. In patients treated with minocycline, a statistically significant increase in the surface area of sebum on the Sebutape®, and in the number of blots (or excreting follicles) was seen. This increase occurred irrespective of the three different grey levels selected: however, it was greater in the case of grey levels corresponding to follicles of intermediate excretion intensity and even more obvious in the case of grey levels corresponding to a low level of follicular excretion, than in grey levels corresponding to large transparent blots (to highly excreting follicles) which show only limited variation (Fig. 4).

DISCUSSION

Few detailed studies of seborrhoea after treatment with tetracyclines have been reported in the literature. Most of them have shown a reduction in sebum free fatty acid content with an associated increase in triglyceride content, the sum of the two remaining constant at about 60% of skin surface lipids (7–9).

More recently, a study comparing the sebum excretion

Table I. *Measurements of seborrhoea in the randomized study*

D: Day, M: Minocycline group, P: Placebo group

Randomized study		D0	D28	D56	D84	D112	D140
Clinical evaluation according to the reference patterns	M	100	135	140	120	125	110
	P	100	95	90	90	105	105
Number of excreting follicles	M	100	115	110	120	136	103
	P	100	98	95	92	90	95
Surface area of sebum absorption	M	100	129	157	164	171	114
	P	100	105	98	88	92	95

Arbitrary value of 100 was given to the value obtained at day 0.
The other results corresponding at the evaluation.

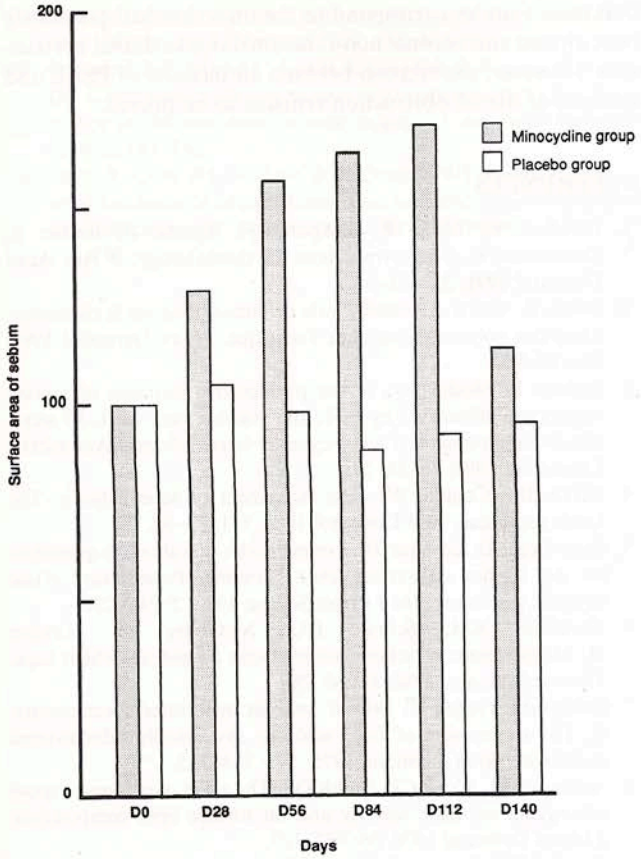

Fig. 1. Randomized study: clear increase in sebum excretion in the minocycline group (image analysis of Sebutape®).

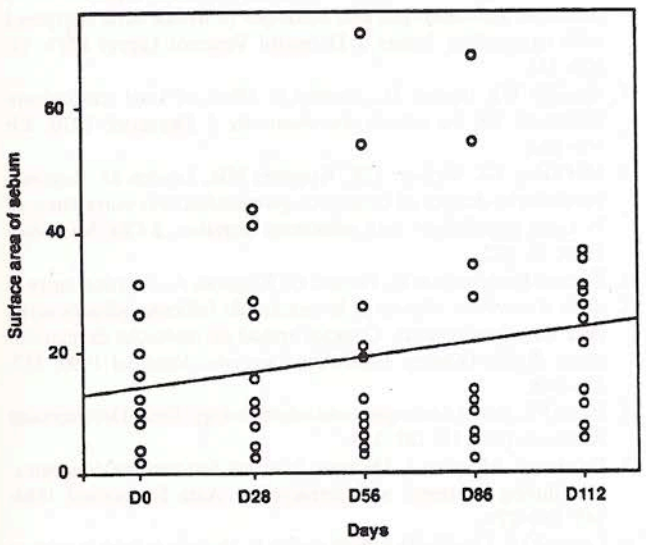

Fig. 2. Randomized study – Statistical analysis by correlation and regression: statistically significant increase in surface area of sebum.

suppressive effects of tetracyclines in association with anti-androgens and oestrogens (Diane®) showed a clear decrease in sebum excretion during treatment with Diane® and a non-significant increase during treatment with tetracyclines (10).

Our studies have shown that during treatment of acne vulgaris with minocycline there has been an increase in both seborrhoea and in the number of active pilosebaceous follicles,

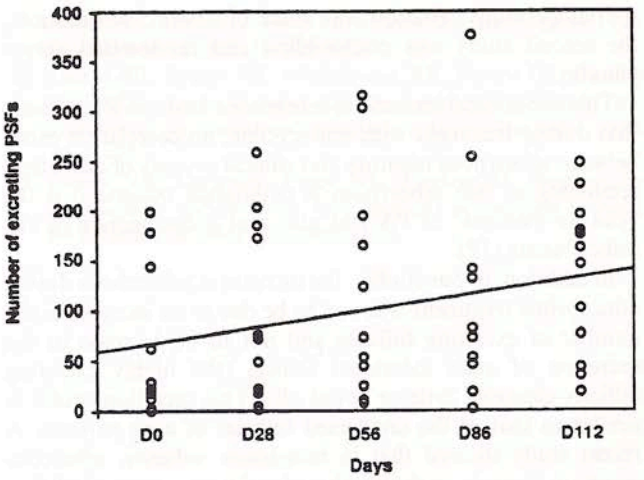

Fig. 3. Randomized study – Statistical analysis by correlation and regression: statistically significant increase in the number of excreting pilo-sebaceous follicles (PSF).

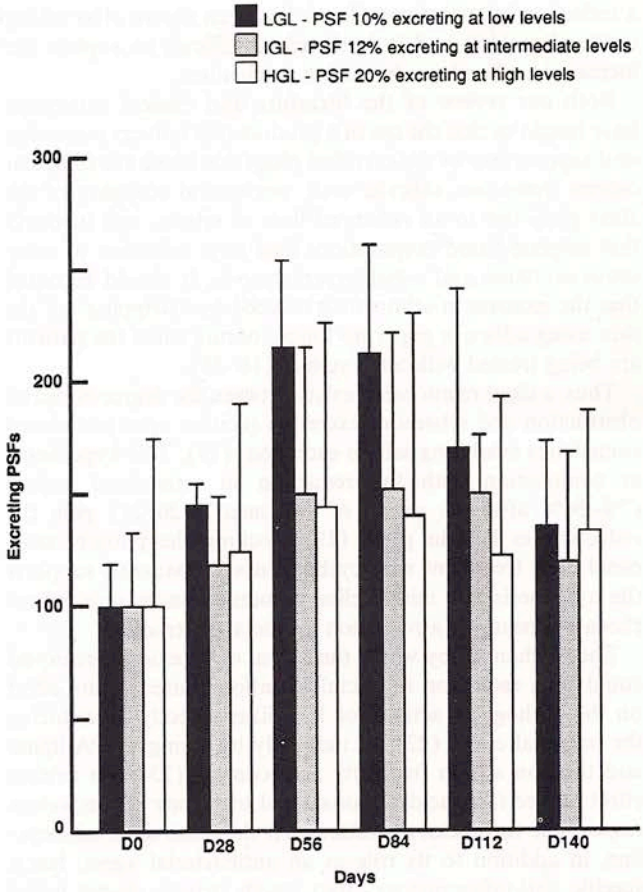

Fig. 4. Randomized study – Computerized image analysis of Sebutape®: clear increase in the number of follicles excreting at the intermediate (IGL) and low (LGL) levels in comparison to highly (HGL) excreting follicles: Recruitment of new excreting pilo-sebaceous follicles (PSF).

which was not due to a modification in the measurement conditions (11). The evaluations were made in the same room, at the same ambient temperature, at the same time of day; climatic conditions were the same for all of the patients and

the temperature variation was small or absent; furthermore, the second study was double-blind and randomised versus placebo.

This sub-clinical increase in seborrhoea leads us to conclude that during treatment with minocycline, no correlation exists between seborrhoea intensity and clinical severity of acne, thus reminding us that seborrhoea is pathogenic because it is the "culture medium" of PA and also that a dysfunction of the follicle exists (12).

In addition, in our studies, the increase in seborrhoea during minocycline treatment is found to be due to an increase in the number of excreting follicles and not to an increase in the excretion of each individual follicle (the highly excreting follicles changing little or not at all). This excretion profile is similar to that of the unaffected follicles of acne patients. A recent study showed that in non-acneic subjects, sebaceous excretion depends on the number of active or excreting follicles, whilst in acne patients, sebaceous excretion is due to the level of excretion from each individual follicle (13).

A hypothesis according to which minocycline interferes with androgen metabolism could be put forward (9, 14). However, a reduction in testosterone levels has been shown after taking tetracycline (15) and it is therefore difficult to explain the increase in seborrhoea by such a mechanism.

Both our review of the literature and clinical experience have taught us that the use of a product that induces regression or disappearance of the cornified plugs that block the follicular ostium (resorcine, salicylic acid, mechanical stripping of the skin) gives rise to an enhanced flow of sebum, and inversely that sulphur-based preparations lead to a reduction in sebaceous excretion and ostial hyperkeratosis. It should be noted that the increase in seborrhoea induced by "stripping" of the skin using adhesive paper no longer occurs when the patients are being treated with tetracyclines (16–18).

Thus, a close relationship exists between the degree of ductal obstruction and sebaceous excretion (neither open nor closed comedones exhibiting sebum excretion) (19). This hypothesis, in conjunction with the reduction in retentional lesions (7%–36% after 12 weeks of treatment) (20–21) plus the reduction in keratin plugs (19) blocking the pilosebaceous canal after treatment with cyclines in acne patients, supports the hypothesis that minocycline induces an increase in seborrhoea by means of a reduction in ductal obstruction.

The mechanism by which the ductal obstruction is removed could be a reduction in ductal irritation caused by its effect on the pathogenic activity of PA either directly by reducing the bacterial count (22), or indirectly by acting on PA lipase and thus on sebum free fatty acid content (23); the irritant effect of free fatty acids is considered to be one of the factors responsible for ductal alterations. At the same time, minocycline, in addition to its role as an antibacterial agent, has a specific anti-inflammatory effect which reduces ductal irritation. This anti-inflammatory activity has been demonstrated by different *in vitro* experiments to be due either to a reduction in the response of polymorphonuclear leukocytes to chemotactic factors, or to the inhibition of action of interleukin-1-beta on mononuclear cells (this interleukin stimulates these cells and induces cell division), or to the inhibition of lymphocyte transformation (from quiescent into active cells) by phytohaemagglutinin (1, 24, 25).

Minocycline induces a subclinical increase in seborrhoea by recruiting new excreting pilosebaceous follicles. It is probable that these follicles correspond to the ones that had previously been altered and become non-functional due to ductal obstruction. However, the relation between an increase of PSFE and removal of ductal obstruction remains to be proved.

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