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Effect of zinc gluconate on *propionibacterium acnes* resistance to erythromycin in patients with inflammatory acne: *in vitro* and *in vivo* study

Tetracyclines and macrolide antibiotics have been in use for acne treatment for more than 20 years. Since 1992 increasing resistance to these antibiotics, and especially to erythromycin, is reported with *Propionibacterium acnes.* Zinc salts have demonstrated their efficacy in inflammatory acne treatment as well as their bacteriostatic activity against *Propionibacterium acnes.* The objective of our work was firstly to determine whether the clinical anti-inflammatory efficacy of zinc salts was altered in the presence of erythromycin resistant strains *in vivo*, and secondly to study the *in vitro* and *in vivo* effect of zinc on the sensitivity of *Propionibacterium acnes* strains to erythromycin. Thirty patients with inflammatory acne were treated by zinc gluconate with a daily dose of 30 mg for two months and bacteriologic samples were taken at D0, D30 and D60. *In vivo,* this study displayed a reduction in the number of inflammatory lesions after a 2-month treatment whether or not *Propionibacterium acnes* carriage was present. Concurrently, *in vitro* addition of zinc salts in the culture media of *Propionibacterium acnes* reduced resistance of *Propionibacterium acnes* strains to erythromycin. Thus, association of zinc salts via a systemic route and topical erythromycin treatment seems an interesting option in the light of an increasing number of patients carrying erythromycin resistant *Propionibacterium acnes* strains.

Key words: acne, bacterial resistance, erythromycin, *P. acnes*, zinc gluconate

Antibiotics of the tetracycline and macrolide families have been in use for decades in the treatment of inflammatory acne. Their efficacy is the result of an antibacterial effect on *Propionibacterium acnes* (*P. acnes*) involved in acne lesions and an anti-inflammatory activity on pilosebaceous follicles with inhibition of polymorphonuclear chemotaxis, anti-lipase activity and inhibition of pro-inflammatory cytokines such as Il-6 and TNF-alpha. Since 1976, when Leyden *et al.* [1] published that the percentage of *P. acnes* resistant strains to antibiotics was at zero, the frequency of resistant strains in acne patients has inexorably increased, especially with erythromycin.

Thus, Eady *et al.* [2] reported a high rate of *P. acnes* resistant strains. Out of 486 acne patients, 178 (38%) were carriers of *P. acnes* strains resistant to at least one antibiotic. The most frequent was resistance to erythromycin (69.7%), 34.3% of *P. acnes* strains were resistant to tetracyclines, and 15.2% of strains harboured multi-resistance. These results have recently been confirmed in a European study by Ross [3].

Within this context, and considering the wide use of topical or oral erythromycin in acne treatment, recent guidelines have been proposed to try to limit the emergence of resistant strains [4]. Addition of zinc salts to erythromycin was one of these proposals as zinc has a proven efficacy in the treatment of inflammatory acne [5, 6] as well as antibacterial properties [7]. Zinc acts by inhibition of polymorphonuclear chemotaxis, it also inhibits 5 α-reductase and TNFα and in addition stimulates antiradical enzyme systems, mainly superoxyde dismutase. Furthermore, it modulates the expression of integrines [8].

There are few data regarding zinc and *P. acnes* and most are *in vitro* data. Holland *et al.* [9], comparing the evolution of resistance to erythromycin in *P. acnes* strains with or without *in vitro* addition of zinc, have demonstrated that the addition of zinc in the culture media can restore *P. acnes* sensitivity to erythromycin and they concluded in favour of zinc salts being added to erythromycin. Bojar *et al.* [10] demonstrated in other works that sensitivity to zinc salts was the same in erythromycin resistant and erythromycin sensitive *P. acnes* strains.

Erythromycin is widely used in France as a topical treatment for acne. In a primary study of the cutaneous bacterial flora of acne patients, Dreno *et al.* [11] showed that 95% of acne patients carry erythromycin resistant *S. epidermidis* strains and 63% carry *P. acnes* resistant strains. Presence of resistance in acne patients was associated with more frequent inflammatory lesions and, as in the study by Holland

et al. [9], there was no difference in zinc sensitivity amongst erythromycin resistant and erythromycin sensitive strains. A zinc concentration of 512 µg/mL inhibited every *P. acnes* strain. In this study, we assessed the evolution of *P. acnes* resistance to erythromycin by zinc gluconate with a daily dose of 30 mg of a Zn element for 2 months and its relation to the therapeutic response. Moreover, *in vitro P. acnes* sensitivity to zinc salts was checked.

Materials and methods

Patients

Thirty acne patients were selected according to: age above 12 years old, inflammatory acne of the face with more than 15 papules and/or pustules. These patients had been treated with topical erythromycin during the previous 12 months but should not have received either isotretinoin within the last two months, nor antibiotic by systemic route, nor combined cyproterone acetate and ethinyl-estradiol, nor zinc salts within one month, nor any topical anti-acne treatment including erythromycin within fifteen days before inclusion. This study was approved by the French national ethics committee (CCPPRB), written informed consent was mandatory.

These patients underwent bacteriologic sampling on the inclusion day (D0), then received 30 mg of zinc gluconate (Zn element) for two months in the form of two capsules of zinc gluconate before breakfast, or possibly at bed time. Patients were seen again at D30 and D60. When treatment was finished, another bacteriologic sampling was performed to assess change in resistance pattern of *P. acnes* to erythromycin with zinc gluconate treatment. Concurrently, inflammatory lesions on the face where numbered at D0, D30 and D60.

Bacteriologic study

Microbiological samples were obtained from each patient using the washings method of Williamson and Kligman [12] modified and standardized by Fleurette [13] and using an electric sampling device. 2.5 mL of a neutralizing washing fluid (Sörensen phosphate buffer pH 7.9 (100 mL), Triton X 100 (0.1 g), sodium thioglycollate (0.2 g), Tween 80 (3 g), sodium thiosulfate (0.3 g)) were poured into a glass cylinder with a 2 cm internal diameter which was applied to the skin. At the end of the electrical sampling device was a sterilizable rubber paddle which was inserted into the glass cylinder. This paddle rotated when the device was switched on, causing rubbing of the skin surface and dispersal of bacteria present in the washing fluid. The sampling time was short, about 20 seconds. Washings were collected using a syringe and transferred to a sterile bottle. A volume of 0.1 ml of washings, pure and diluted in sterile normal saline (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}), was inoculated by scraping on meat-yeast (MY) agars containing 2 µg/mL of furazolidone (Sigma) to inhibit the growth of staphylococci and on MY containing both 2 µg/mL of furazolidone and 4 µg/mL of erythromycin, in order to count total anaerobic bacteria and erythromycin-resistant anaerobic bacteria, after 7 days incubation at 37 °C in an anaerobic atmosphere. Colonies of *P. acnes* underwent biochemical identification based on the following tests: indol + or –, nitrate reduction, esculine hydrolysis, saccharose and maltose fermentation as well as testing for sensitivity to erythromycin (by diffusion on Wilkins-Chalgren agar). Thus, in the culture medium for *P. acnes* (Wilkins-Chalgren agar), a bacterial inoculum of 10^4 bacteria per 1 µL and a value of 512 µg/mL of erythromycin, which was adopted as the threshold of definition of resistance for *P. acnes* [14], were added. *P. acnes* was cultivated in anaerobic conditions for 5 days at 37 °C.

In vitro study of minimum inhibitory concentrations (MIC) of erythromycin with or without zinc

In a second step, 7 resistant strains of *P. acnes* at Day 0 before zinc gluconate treatment were selected. The MIC of erythromycin was determined with a dilution range for erythromycin between 0.03 and 512 µg/mL, with or without addition of zinc gluconate at dilution range of respectively 7.5, 15 and 30 mg/L zinc metal.

Results

In vivo study

Among the 30 patients included in the study, 4 patients withdrew after the first month of treatment (2 with digestive disorders related to the treatment and 2 for personal reasons) and 6 patients were unable to have a second bacteriologic sampling.

Thus, finally, the statistical analysis concerned 20 patients with clinical and bacteriologic assessment at D0 and D60. Mean age was 19.4 ± 4.5 years, 63% of patients were female and 37% male. Every patient had received topical erythromycin during the preceding year. Mean duration of acne was 66.6 ± 38.1 months (min. 12 – max. 180). The clinical response on superficial inflammatory lesions (papules and pustules) is displayed in *table 1* and demonstrated a significant reduction as from D30 (–46.2% ± 28.5 $p < 0.001$) and further reduction at D60 (–57.3% ± 36.1; $p < 0.001$). Five patients presented digestive side effects (nausea, vomiting, stomach cramps), two of them requiring stopping of the treatment.

Table 1. Inflammatory lesions (papules and pustules) number evolution during 30 mg/day zinc gluconate treatment for 2 months.

		D0	D30	D60
Observed values	Mean	23.8	12.9	11.0
	s.d.	6.8	8.5	12.9
	median	21.5	11.5	8.0
	min.	15.0	2.0	0.0
	max.	43.0	38.0	52.0
	n	30	30	25
Evolution from D0 (%)	Mean		– 46.2	– 57.3
	s.d.		28.5	36.1
	median		– 46.5	– 63.6
	min.		– 92.6	– 100.0
	max.		18.2	52.9
	n		30	25
	p intra*		< 0.001	< 0.001

** : non parametric test*

Concurrently, according to the bacteriologic results before and after treatment, patients were divided in 4 groups as follows:

Before	After	
Resistant (R)	Resistant (R)	(7 patients) R-R
Sensitive (S)	Sensitive (S)	(8 patients) S-S
Resistant (R)	Sensitive (S)	(3 patients) R-S
Sensitive (S)	Resistant (R)	(2 patients) S-R

The relation between inflammatory lesions, number, evolution and erythromycin resistance in *P. acnes* is reported in *figure 1*.
In the R-R group, a significant reduction in inflammatory lesions number at D30 –48.3% ($p = 0.008$) and at D60 –57.1% ($p = 0.016$) was found. In the S-S group, a significant reduction at D30 –45.5% ($p = 0.012$) was confirmed at D60 with a –54.3% decrease ($p = 0.055$). There was no clinically relevant difference between the two groups according to the evolution of the inflammatory lesions.
In the R-S group, the decrease was –57.7% at D30 ($p = 0.125$) and –90.5% at D60 ($p = 0.25$). In the S-R group, the decrease was –30.1% at D30 ($p = 0.5$) and –43.9% at D60 ($p = 0.5$).

***In vitro* study**

A study of MICs with or without addition of zinc in the culture media was carried out on 7 *P. acnes* strains originated from 7 patients included in the study. These strains were selected due to their resistance to erythromycin at D0 (MIC ≥ 1024). Their MICs were compared on culture media with either erythromycin alone, or zinc gluconate alone or combination of erythromycin and zinc gluconate, in increasing concentrations. With increasing concentrations of zinc (7.5, 15 and 30 mg/L zinc metal) in the culture media (*table 2*), we observed a progressive reduction of resistance of *P. acnes* strains to erythromycin *in vitro*.

Discussion

At the clinical level, this study shows a decrease of acne lesions for a daily dose of 30 mg of gluconate zinc during 2 months with a reduction of 57.3% ± 36.1 ($p < 0.001$) of inflammatory lesions (papules and pustules). These results are similar to those observed in a randomised trial, knowing that the main objective of this study was at bacteriological

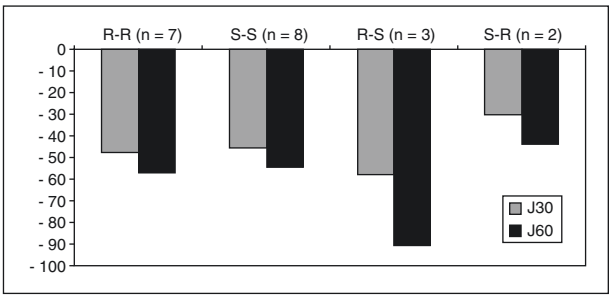

Figure 1. Variation in inflammatory lesions number according to bacteriologic results (median values).

Table 2. Evolution of erythromycin MICs when varying concentrations of zinc gluconate are present in the culture media

	Number of strains				
Median starting Dilution value	E-MIC (mg/L)	Zinc-MIC (mg/L)	E+Zinc 50-MIC	E+Zinc 100-MIC	E+Zinc 200-MIC
<= 0.03					7
256		1		3	
512		6	1	3	
>= 1024	7	0	6	1	

level. Our study demonstrates that the efficacy of zinc gluconate in patients is not related to the presence or not of resistant strains of *P. acnes* on the skin of acne patients. Indeed, a similar reduction in the number of inflammatory lesions during the first and second months of treatment was found, in the two groups of patients with either resistant *P. acnes* strains at inclusion and at the end of treatment (R-R) or sensitive *P. acnes* strains (S-S). Efficacy on inflammatory lesions was not altered during zinc gluconate treatment in patients with resistant bacterial strains as compared to patient without resistant strains to *P. acnes.* In the two patient groups with a modified resistance pattern of *P. acnes* during zinc gluconate treatment (S-R and R-S), no definitive conclusion can be drawn as patient number is small *(figure 1)*, but the evolution of patient groups looks similar during zinc salt treatment. This study is the first *in vivo* work studying the relation between resistant or non-resistant *P. acnes* strains on the skin of acne patients and the clinical response to zinc salts, demonstrating independence between zinc salt efficacy and resistance strain carriage. These results differ from those with erythromycin where a relation between clinical failure and bacterial strains resistant to the antibiotic was established in three studies [15-17]. Moreover, our bacteriologic *in vitro* study of erythromycin MICs demonstrates that the addition of increasing doses of zinc gluconate from 15 to 30 mg/L (expressed as zinc metal) decreases resistance in *P. acnes* strains, thus confirming the results of Holland *et al*. [9] and Bojard [10] which showed that erythromycin resistant *P. acnes* strains can be sensitive to zinc salts *in vitro*.
The increased sensitivity of *P. acnes* to erythromycin when zinc salts are present has led some authors to conduct clinical studies targeting the clinical efficacy of combined topical zinc and erythromycin. Results are still scarce and under debate. In 1980, Feucht *et al.* [18] tried 4% erythromycin combined either with zinc acetate 1.2% (lotion) or zinc octoate 1.2% (gel). After a ten-week treatment, efficacy was superior to placebo and equivalent to 500 mg of tetracycline.
In 1989, Habbema *et al.* [19] showed the superiority of combined topical zinc + erythromycin 4% over erythromycin 2% alone on acne lesions after a 12-week treatment. Strauss *et al.* [20], comparing zinc acetate (1.2%) and vehicle combined with erythromycin (4%) showed a significant reduction of the number of bacterial strains in the zinc erythromycin group (98% *versus* 43%). This was associated with a more pronounced reduction (69%) of skin surface free fatty acids. From a clinical point of view, inflammatory lesions were significantly reduced in the

zinc-erythromycin group compared to control after 8 weeks of treatment (69% vs. 9%). One of the zinc modes of action would be to increase the residence time of erythromycin on the skin [21].

In conclusion, our study demonstrates that the efficacy of zinc gluconate on inflammatory lesions in acne is not related to existing *P. acnes* resistant strains as it is for erythromycin. It confirms that, *in vitro*, erythromycin resistant *P. acnes* strains can be inhibited by zinc gluconate in a dose dependant relationship. Thus, combining zinc salts to a topical antibiotic can be an interesting option both from a bacteriological and a clinical point of view in the treatment of inflammatory lesions of acne, as proposed in some recent papers for the prevention of bacterial resistance in acne patients. ■

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