Effect of Doxycycline on the Generation of Reactive Oxygen Species:

A Possible Mechanism of Action of Acne Therapy with Doxycycline

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On the basis of a recent report that minocycline is effective in the treatment of acne inflammation by acting directly as an antioxidant on infiltrating neutrophils, we investigated whether doxycycline might also be capable of reducing the generation of reactive oxygen species, using human neutrophils and a cell-free, xanthine-xanthine oxidase system. The species investigated are superoxide radical anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH·). Doxycycline significantly reduced the levels of O_2^-, H_2O_2 and OH· generated by both systems. Our results seem to suggest that the clinical effectiveness of doxycycline in the treatment of acne inflammation is due partly to its antioxidant effect on neutrophils. *Key words: Antioxidant action; Neutrophil; Tetracyclines.*

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Tetracyclines, such as minocycline and doxycycline, are an effective treatment for acne vulgaris. We have recently reported that minocycline inhibits neutrophil reactive oxygen species (ROS) generation, which supports the hypothesis that the agent is effective in acne treatment not only by reducing the numbers of *Propionibacterium acnes* (*P. acnes*), but also by inhibiting neutrophil-generated ROS which induce a chemical insult to the integrity of follicular epithelium in acne (1). We have more lately found that reduced levels of linoleic acid in acne comedones, comparable with normal hair follicles, which effectively inhibits neutrophil ROS generation, contributes to an exacerbation of acne inflammation (2).

Although doxycycline has been reported to have a ROS-reducing effect (3), the effect on the levels of all kinds of ROS generated both by neutrophils and in the xanthine-xanthine oxidase system has not yet been examined.

In the present study, we investigated whether doxycycline might be capable of reducing the generation of ROS, including superoxide radical anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH·), using human neutrophils and a cell-free, xanthine-xanthine oxidase system. The results revealed that doxycycline does in fact effectively inhibit the levels of all kinds of ROS generated in both systems.

MATERIALS AND METHODS

Chemicals: Doxycycline (Taito Pfizer Pharmaceuticals Ltd, Japan) was added to the following neutrophil function assay systems in concentrations of 0.005, 0.05, 0.5, 5 and 50 µg/ml.

Neutrophil preparation: Neutrophils isolated from heparinized venous blood from healthy volunteers by a modification of a previously described method (4) and suspended in Krebs Ringer phosphate buffer (KRP) were preincubated at 37°C for 10 min with opsonized zymosan (Sigma).

Neutrophil ROS generation assay: The assay method for ROS has been detailed in our previous report (2). Briefly, the formation of O_2^- was determined by measuring ferricytochrome *c* (Type III, Sigma) reduction induced by O_2^- produced from neutrophils stimulated with opsonized zymosan. H_2O_2 generation was measured by quantifying the weakening of fluorescence intensity of scopoletin (Sigma) due to its peroxidase-mediated oxidation by H_2O_2 (5). OH· was quantitated by taking the amount of ethylene gas formed from α-keto-methiolbutyric acid (KMB) (Sigma) plus the neutrophil-generated OH· (6).

ROS generation assay in the xanthine-xanthine oxidase system: All ROS were also measured in the xanthine-xanthine oxidase system. Instead of adding neutrophils and opsonized zymosan, 0.1 mM hypoxanthine (Sigma), 1.25 mM EDTA and 16.5 µM ferricytochrome *c* were mixed in a total volume of 2 ml (125 mM phosphate buffer). Thereafter, approximately 0.006 units/ml dialysed xanthine oxidase (Sigma) was added to generate ROS (7).

Triplicate assays were performed in each experiment; the results are expressed as means ± SD of replicate assay. Statistical significance was ascertained by Student's *t*-test.
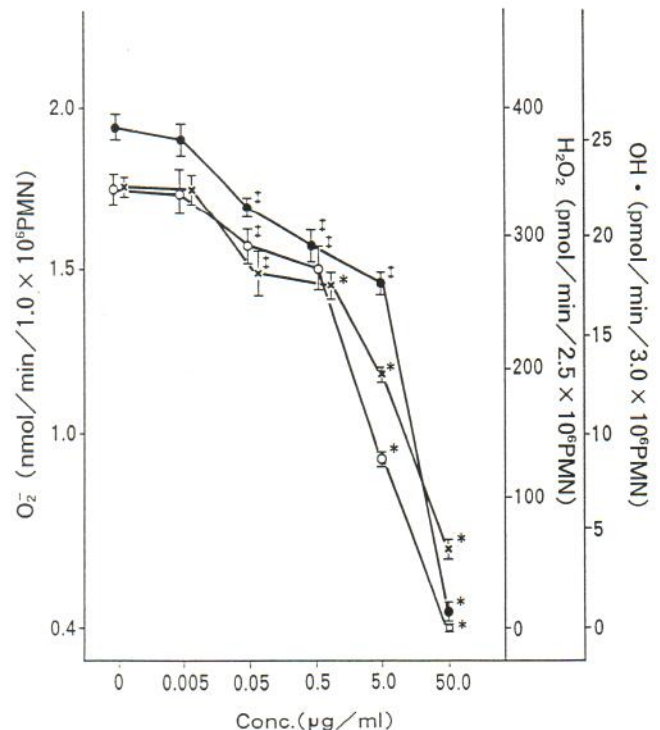

Fig. 1. Effect of doxycycline on ROS generation by neutrophils. ●, O_2^-; ○, H_2O_2; X, OH· levels. PMN denotes polymorphonuclear leukocytes. ‡$P < 0.01$ vs control, *$P < 0.001$.
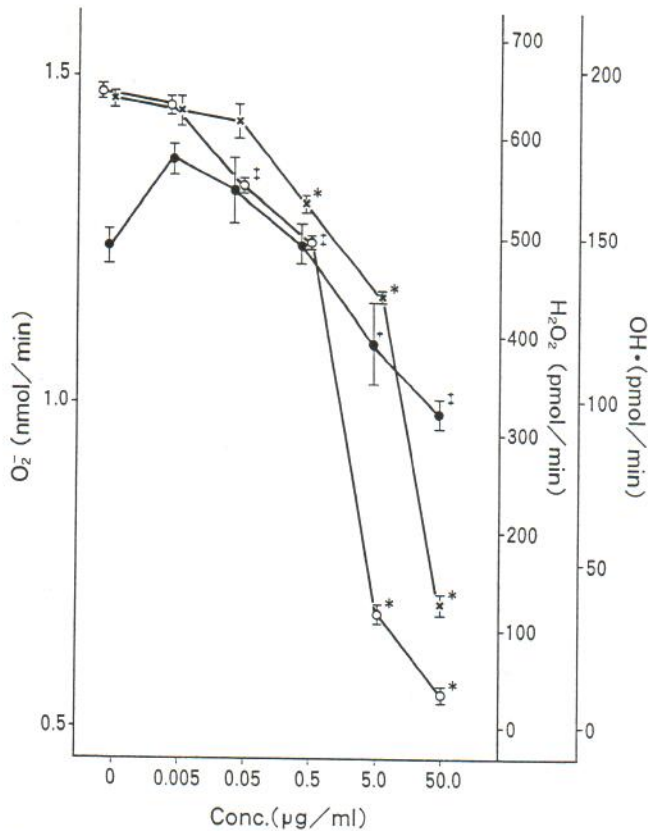
Fig. 2. Effect of doxycycline on ROS generated in the xanthine-xanthine oxidase system. $+P<0.05$ vs control. For other symbols, see caption to Fig. 1.

RESULTS AND DISCUSSION

The present study demonstrated that doxycycline significantly reduced the levels of ROS generated both by neutrophils and in a cell-free system in a dose-dependent manner (Figs 1, 2). Although doxycycline has been reported to reduce O_2^- and H_2O_2 (3), we found that the agent additionally inhibited OH·, which is one of the most toxic ROS. The results obtained in this study probably indicate that doxycycline does not *inhibit* the neutrophil metabolism *to produce* ROS, but rather scavenges the ROS generated. These findings seem to suggest that doxycycline is effective in the treatment of acne not only by inhibiting the growth of *P. acnes,* but also by acting as antioxidants on infiltrating neutrophils as observed with minocycline (1).

We have recently reported (1) that tetracycline, oxytetracycline and minocycline inhibit the level of certain kinds of ROS (O_2^-, H_2O_2, OH·), generated both by neutrophils and in a xanthine-xanthine oxidase system. In contrast, our results showed that doxycycline reduces the level of every kind of ROS generated by both systems. This seems to suggest that doxycycline possesses the most potent antioxidant action within the tetracycline group. Therefore, it is likely that doxycycline is the drug of choice in the treatment of inflammatory acne.

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