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Entry of Four Tetracyclines into Saliva and Tears

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Although meningococci are susceptible to the tetracyclines by testing in vitro, oxytetracycline (OC) and doxycycline (DC) have failed to eliminate carriage, whereas minocycline (MC) has been effective. Because these congeners differ in lipophilicity, they and tetracycline (TC) were studied in volunteers by assay of serum, saliva, and tears obtained after 5 days of treatment. OC and TC were undetectable or attained concentrations subinhibitory for meningococci in saliva and tears. The concentrations of MC in saliva and tears were equal to or greater than the average minimal inhibitory concentration as long as 12 h post-dose. Near inhibitory concentrations resulted with DC at 100 mg/day; yet, doubling the dose to 100 mg/12 h did not yield concentrations that exceeded the average minimal inhibitory concentration for meningococci. The previous reports of failure or meager entry of DC and MC into saliva probably reflected extraction of these drugs in the paraffin chewed by subjects to stimulate salivary flow. The efficiency of entry of the tetracyclines into the secretions of the noninflamed upper respiratory tract correlates with lipophilicity at physiological pH, enabling prediction of meningococcal chemoprophylactic efficacy.

The ascendancy of sulfonamide-resistant *Neisseria meningitidis* in the United States (6, 8, 42, 51) and abroad (1, 36, 56) has not been of importance to therapy because meningococci remain susceptible to penicillin G (12, 19, 20, 30, 58). However, chemoprophylaxis is another matter, for the penicillins do not affect the carrier state (2, 17, 42, 51). Field trials with several other antimicrobics, also active against meningococci by testing in vitro, have shown either no useful effect (oxytetracycline, erythromycin, nalidixic acid, trimethoprim, ethoxzolamide, doxycycline, cephalexin, and coumermycin [2, 11, 13, 16, 17, 42, 45, 51]), or reduction in carriers (rifampin [3, 10, 14, 19, 26, 57] and minocycline [15, 26]).

The congeners of tetracycline available for therapy are active in vitro against meningococci (12, 15, 19, 20, 26). Yet, in the field, oxytetracycline (17) and doxycycline (16) failed to influence the carrier state, whereas minocycline reduced carriers by 95 to 84% (15, 26). To rationalize these findings and relate them to the physicochemical characteristics of the tetracyclines (9, 35, 54), the entry of oxytetracycline, tetracycline, minocycline, and doxycycline into saliva and tears was studied in normal volunteers.

The rationale for relating penetration of antimicrobic into saliva and tears to antimeningococcic chemoprophylactic efficacy has been presented elsewhere (28). Briefly, it is impractical to measure drug content in the mucous secretions that bathe the noninflamed nasopharyngeal site of carriage of *N. meningitidis*. Although saliva does not ordinarily wet the nasopharynx, tears pass into the nasopharynx as the normal route of drainage from the conjunctival sac. Moreover, agents known to be prophylactic—sulfapyrimidines (32, 49, 55) and rifampin (3, 10, 14, 19, 26, 57)—do attain concentrations inhibitory to meningococci in both saliva (16, 28) and tears (28). Paradoxically, when minocycline was applied in meningococcal chemoprophylaxis, either undetectable or subinhibitory concentrations were found in saliva (15, 26); tears were not studied in carriers. Meager entry of doxycycline into tears has been reported in children with trachoma (29).

MATERIALS AND METHODS

Tetracyclines. Capsules containing tetracycline hydrochloride (250 mg) and minocycline hydrochloride (100 mg) were supplied by Bruce H. Lloyd, M. D., Lederle Laboratories, Pearl River, N.Y. Oxytetracycline hydrochloride (250 mg) and doxycycline hyclate (100 mg) were provided by David J. Wolf, Pfizer Laboratories, New York, N.Y. Standard reference powder for each agent was supplied by the corresponding manufacturer.

Signatures attesting to informed consent were ob-

tained from the five participating volunteers, who were normal, healthy males not under treatment with antimicrobics and who ranged in age from 17 to 25 years. Each agent was taken perorally for 5 days according to the dosage schedule given in Table 1. Two days elapsed between drugs; the sequence of administration was: oxytetracycline, tetracycline, minocycline, doxycycline. Specimens of serum, saliva, and tears were collected before any agents were taken and at 2, 4, 8, 12 and 24 h after the final dose of each agent.

Specimens. Venous blood was collected in borosilicate glass, screw-capped tubes (15 by 125 mm); after clotting at room temperature for 1 h and standing in the refrigerator for 0.5 to 4 h, the sera were separated by centrifugation, transferred to snap-topped polycarbonate tubes (11 by 75 mm), and stored at −40 C.

Saliva was collected by two methods. First, volunteers sucked on clean, sterile pebbles and expectorated about 5 ml of saliva into sterile centrifuge tubes (stone-stimulated saliva or SS). Next, the volunteers chewed about 20 g of paraffin and a second specimen of saliva was collected (paraffin-stimulated saliva or PS) about 5 min after the first. PS specimens were always collected after SS specimens because bits of paraffin were expectorated for 30 to 60 min after chewing and collection. Storage of saliva overnight in the refrigerator was followed by centrifugation (20 min at 3,000 rpm); the clarified supernatants were transferred to sterile snap-topped polycarbonate tubes (11 by 75 mm) for storage at −40 C.

With the volunteers lying supine, sterile paper disks (concentration disks, sterile blanks, Difco Laboratories, Detroit, Mich.) were inserted into the inferior conjunctival sacs to stimulate the flow of tears. Tears were collected by aspiration by using sterile 0.5-ml pipettes, transferred to sterile snap-topped polycarbonate tubes (11 by 75 mm), and stored at −40 C.

Bioassay. A well-agar diffusion method for bioassay was employed (4). By using *Bacillus cereus* (ATCC 11778) inoculated as spores (*B. cereus* spore suspension, Difco Laboratories, Detroit, Mich.) added to molten nutrient agar (Difco Laboratories, Detroit, Mich.) to give a final concentration at 10^4 colony-forming units CFU/ml, the minimal measurable concentrations listed in Table 1 were obtained. To minimize the influence of non-drug variation, assays of specimens of serum and saliva were based on standards made up in pooled, pretreatment sera and salivas; assays of tears were referred to drug standards prepared in phosphate-buffered saline (18). All of the standards and specimens relating to a particular drug were set up at the same time. Each specimen was assayed in triplicate, and the results were averaged. The corresponding mean values of the five volunteers were then averaged, and the standard error of this mean was calculated; these data are plotted in Fig. 1-4.

RESULTS

Oxytetracycline was not detectable in salivas and attained concentrations in tears that would be subinhibitory for meningococci (Fig. 1).

Tetracycline was present in stone-stimulated, but not paraffin-stimulated, salivas. It was detectable in all specimens of tears but had fallen to levels at the limit of measurement by 24 h after the final dose (Fig. 2). The concentrations in salivas and in tears were uniformly

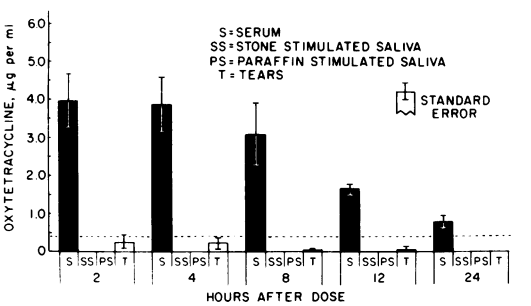

FIG. 1. *Concentration of oxytetracycline was measured in simultaneously collected serum, salivas, and tears obtained at intervals clustered about the final dose of 5 consecutive days of treatment (250 mg every 6 h, by mouth) in five volunteers. The dotted line marks 0.40 µg/ml, the average inhibitory concentration of oxytetracycline for Neisseria meningitidis (12).*

TABLE 1. *Dosage and minimal concentrations measurable by bioassay of four tetracycline congeners in serum, saliva, and tears*[a]

Congener	Dosage	Minimal measurable concentrations (µg/ml)			
		Serum	Saliva		Tears
			Stone	Paraffin	
Oxytetracycline	250 mg/6 h	0.25	0.13	0.13	0.13
Tetracycline	250 mg/6 h	0.20	0.20	0.13	0.13
Doxycycline	100 mg/24 h 100 mg/12 h	0.13	0.13	0.06	0.06
Minocycline	100 mg/12 h	0.06	0.06	0.06	0.03

[a] Salivary flow was stimulated by sucking a sterile pebble and by chewing paraffin.

lower than would be necessary to inhibit *N. meningitidis*.

SS contained concentrations of minocycline equal to or greater than the average minimal inhibitory concentration (MIC) for meningococci throughout the 24-h period of observation after the final dose (Fig. 3). In marked contrast, only the 2-h post-dose PS specimens contained minocycline in a mean concentration that nearly equalled the MIC. The concentrations in tears persisted at or above the MIC for 12 h post-dose.

Doxycycline was present in salivas and tears throughout the 24-h post-dose period of observation. However, the concentrations were uniformly lower than would be necessary to inhibit meningococci when the dose was 100 mg/day (Fig. 4a). Paraffin stimulation of salivary flow

FIG. 2. *Concentration of tetracycline was measured in simultaneously collected serum, salivas, and tears obtained at intervals clustered about the final dose of 5 consecutive days of treatment (250 mg every 6 h, by mouth) in five volunteers. The dotted line marks 0.60 µg/ml, the average minimal inhibitory concentration of tetracycline for Neisseria meningitidis (12, 19, 20).*

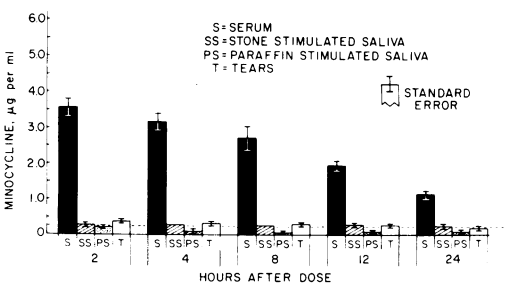

FIG. 3. *Concentration of minocycline was measured in simultaneously collected serum, salivas, and tears obtained at intervals clustered about the final dose of 5 consecutive days of treatment (100 gm every 12 h, by mouth) in five volunteers. The dotted line marks 0.25 µg/ml, the average minimal inhibitory concentration of minocycline for Neisseria meningitidis (12, 15, 19, 26).*

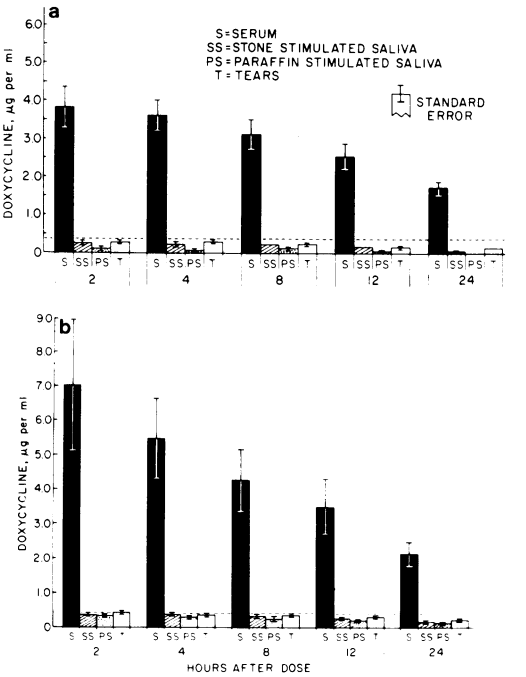

FIG. 4. *Concentration of doxycycline was measured in simultaneously collected serum, salivas, and tears obtained at intervals clustered about the final dose of 5 consecutive days of treatment. (a) 100 mg every 24 h; (b) 100 mg every 12 h, by mouth, in five volunteers. The dotted line marks 0.38 µg/ml, the average minimal inhibitory concentration of doxycycline for Neisseria meningitidis (12, 16).*

was once again associated with a lower concentration than was found with stone stimulation. Doubling the dose of doxycycline (100 mg/12 h) yielded concentrations that matched the mean MIC for meningococci in SS 4 h post-dose (Fig. 4b). At 2 and 4 h, the mean concentrations in tears were equal to or greater than the average MIC.

DISCUSSION

The inviolate regions of the tetracycline molecule—those structures which are essential to antimicrobial activity and hence are present in every congener—consist of three acidic functional groupings (Fig. 5): tricarbonylmethane, dimethylammonium, and phenolic diketone (54). The pK_a's of these groupings are affected measurably (35), but not to an extent that is physiologically significant, by the structural modifications that characterize specific congeners (R_1 through R_4 in Fig. 5). Thus, the tricarbonylmethane moiety has a pK_a of 3.27 for oxytetracycline and 3.30 for tetracycline; the

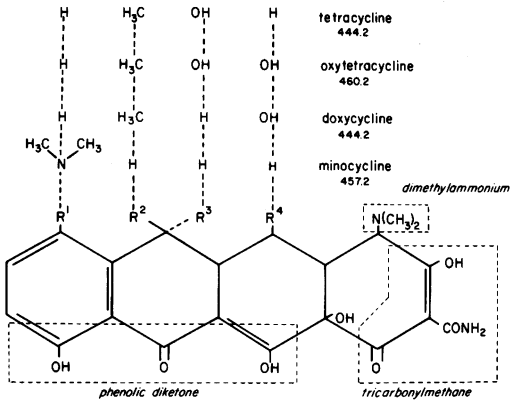

FIG. 5. *Inviolate regions of the tetracycline molecule contain three acidic groupings which are not influenced in pK_a to a biologically significant degree by the substituents, R_1-R_4, that distinguish the various congeners of the tetracyclines. The characteristic structures and the molecular weights of the congeners studied in the present work are also indicated.*

phenolic diketone moiety has a pK_a of 7.32 for oxytetracycline and 7.68 for tetracycline; and the dimethylammonium grouping has a pK_a of 9.11 for oxytetracycline and 9.69 for tetracycline (54). From these values, it is apparent that the tetracyclines will be ionized throughout the physiological range of pH—cationic at acidic pH, dipolar ionic at neutral pH (tricarbonylmethane, negatively charged; dimethylammonium, positively charged; and phenolic diketone, unionized), and anionic at alkaline pH.

Passive enteric absorption should be favored when a tetracycline congener is dipolar ionic because that is the ionization state dictated by the pH of the blood. Absorption of tetracycline is, in fact, maximal in the duodenum (pH 5.8 to 7.6), less in the ileum (pH 6.8 to 7.3), still less in the stomach (pH 1.5 to 2.5), and almost nondetectable in the colon (43).

Movement of the tetracyclines from the blood into various extravascular liquids should be favored by a nearly neutral pH. The pH of saliva (5.6 to 7.6) and tears (7.3 to 7.7) would appear to be conducive to passive diffusion. However, both saliva and tears are true secretions, and other factors appear to be important.

Binding to serum proteins probably does not affect the entry of the tetracyclines into saliva and tears. The extent of such binding varies not only with the congener but also with the investigator (Table 2). Moreover, it does not correlate with the half-lives in the blood—doxycycline has the longest half-life and yet is bound about as extensively as tetracycline and less extensively than minocycline.

In contrast, lipophilicity appears to be a primary determinant. From the chemical structures of the four congeners that were studied (Fig. 5), it is apparent that the substituents characteristic of minocycline are least polar —minocycline should be the most lipophilic compound of the group. That this is indeed the case is shown in the plot of *n*-octanol-aqueous buffer distribution as a function of pH (Fig. 6). The minimal lipophilicity of the more polar oxytetracycline and tetracycline is also evident.

The consequences of these variations in lipophilicity are several. Enteric absorption is augmented as lipophilicity increases. Thus, both doxycycline and minocycline are clinically effective at lower dosage than is required with

TABLE 2. *Renal clearance, half-life, and binding to serum proteins of four tetracycline congeners*[a]

Congener	Renal clearance (ml/min/1.73 m²)	$T_{1/2}$[b] in blood (h)	Bound to serum proteins (%)
Oxytetracycline	~98	9.2	10–40
Tetracycline	50–73	7.5–8.5	20–67
Doxycycline	16–33	15.1–24	25–93
Minocycline	6.3–9.2	13.4–26.3	76–83

[a] As derived from several reports (5, 7, 21, 23, 33, 34, 38, 39, 41, 44, 46, 48, 52, 59).

[b] $T_{1/2}$, Half-life.

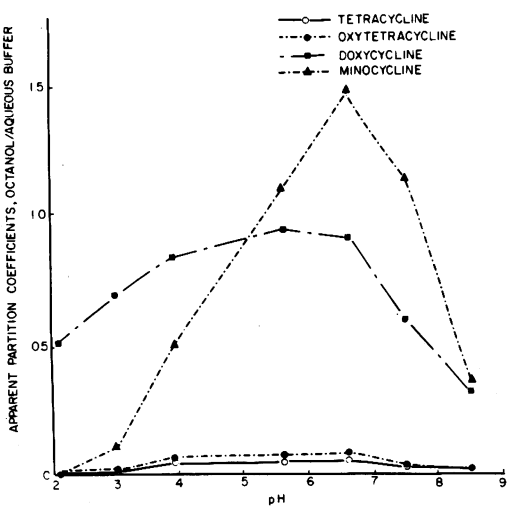

FIG. 6. *Lipophilicity of four tetracyclines, as a function of pH, can be adduced from n-octanol/aqueous buffer partition (plot of data published in reference 9).*

either oxytetracycline or tetracycline. As one desirable consequence, there is less residual drug in the gut and hence less perturbation of the colonic microbiota (22, 27).

However, more than enhanced absorption is implicated in the clinical validity of smaller dosage with minocycline and doxycycline. Both exhibit a reduced renal clearance and a prolonged half-life as compared with oxytetracycline and tetracycline (Table 2). Non-renal, in part catabolic, disposal appears to be a major means of elimination of minocycline and doxycycline (31, 47, 59). With doxycycline, the half-life is essentially unchanged regardless of the state of renal function, a point of clinical significance because this congener may be safely given to patients with renal failure (24, 25, 37, 39-41, 53).

With minocycline, there is relative ease of passage into the central nervous system in the absence of inflammation (31, 38, 50)—another consequence of lipophilicity in the near physiological range of pH. Possibly this phenomenon is related to the occurrence of lightheadedness, dizziness, and headache experienced by some persons taking minocycline (15, 26; one of the five volunteers of the present study).

Lipophilicity may have been responsible for the reported failure of detection, or the finding of subinhibitory concentrations, of minocycline in the salivas of volunteers (15, 26). In such studies, salivary flow was stimulated by chewing paraffin, an effective method for extracting tetracyclines from saliva, according to the data presented in Figures 1-4.

Differences in the efficiency of entry of the four tetracyclines into saliva and tears may also be related primarily to lipophilicity. Minocycline, the congener of maximal lipid solubility over the pH range of 5.5 to 7.5 (Fig. 6), attained concentrations in saliva and tears that are known to be inhibitory to meningococci (Fig. 3). Minocycline has been proven in the field to reduce the frequency of meningococcal carriage (15, 26).

Doxycycline is a tetracycline congener of less lipophilicity than minocycline in the pH range of 5.5 to 7.5 (Fig. 6). It failed to attain concentrations in saliva and tears that would be inhibitory to meningococci when given to volunteers in the usual, recommended dose of 100 mg/day (Fig. 4a), and barely reached the average meningococcal MIC when the dose was doubled to match the usual, recommended dose of minocycline (Fig. 4b).

Doxycycline has been reported to be ineffective in the treatment of carriers (16). Although the dose that was used was not specified (16), it was probably not greater than 100 mg/day because the maximal serum concentration was reported as 1.84 μg/ml; the average maximal serum concentration in our volunteers was 3.82 $\pm$ 0.53 μg/ml 2 h after the final dose. Salivary concentrations were quite low (0.014 to 0.115 μg/ml), possibly as a consequence of extraction in paraffin during collection of the specimens (16).

In a study of 18 American Indian children given doxycycline in the treatment of trachoma (29), this antimicrobial was not detected in tears obtained from eight patients. There were 10 specimens with measurable doxycycline; the average concentration was 0.03 μg/ml, with concomitant serum concentrations equal to or greater than 2.0 μg/ml (tears/serum ratio, about 0.015). In our volunteers, comparable average serum concentrations (2.54 $\pm$ 0.33 μg/ml) were present 12 h after the final dose (average dose: 1.4 mg per kg of body weight per day); the corresponding average concentration in the tears was 0.179 $\pm$ 0.017 μg/ml (tears/serum ratio, 0.071). Doubling the dose of doxycyline in our volunteers also yielded comparable average serum concentrations (2.13 $\pm$ 0.34), but at 24 h after the final dose; the corresponding average concentration in the tears was 0.21 $\pm$ 0.03 μg/ml (tears/serum ratio, 0.099). It is probably fruitless to try to reconcile these differences. Different methods for collecting and assaying doxycycline in tears were used. Probably more important are the differences in the subjects: American Indian children with trachoma and young adult whites without eye infections.

Tetracycline and oxytetracycline are relatively lipophobic (Fig. 6). Penetration into saliva and tears was either immeasurably slight or meager, despite the administration of 250 mg every 6 h. Oxytetracycline was not effective in the treatment of carriers of *N. meningitidis* (17). It is probable that tetracycline would be equally ineffective.

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