α-6-Deoxyoxytetracycline II. Activity in Chemotherapeutic Studies In the Mouse. (31798)

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In evaluations of antibacterial agents, one indication for continued testing is favorable activity against experimental infections in mice. It is important with related experimental compounds to emphasize distinctive properties that suggest advantages in clinical situations. In a study of tetracyclines in the dog, Schach von Wittenau and Yeary stressed superior absorption and tissue distribution after oral administration of the new antibiotic, α-6-deoxyoxytetracycline (DOOTC)*(1). A marked chemotherapeutic advantage for this agent over 6-methylene oxytetracycline (MOTC),† 6-demethylchlortetracycline (DMCT),‡ or tetracycline (TC)§ in experimental infections of mice also has been demonstrated after oral administration of these drugs(2). Both studies suggested possible clinical advantage for DOOTC over other tetracyclines.

In the present study, additional properties of DOOTC that appear to offer advantages for use in man, were demonstrated by two experimental protocols. These properties were retention of chemotherapeutically effective antibiotic concentrations, and capacity to quickly attain activity within the host. These properties were demonstrated by the capability of DOOTC and other tetracyclines to protect when administered at various times before (pre-infection) and at various times after the establishment of experimental infections in the mouse (post-infection).

Materials and methods. The antibiotics, DOOTC, MOTC, DMCT,‖ and TC, used in these studies were research quality hydrochloride salts.

Systemic infections in mice were produced by intraperitoneal inoculation of standardized cultures of *Staphylococcus aureus* 5 mp or *Pasteurella multocida.* Inocula of *P. multocida* were diluted in brain heart infusion broth. *S. aureus* 5 mp inocula were suspended in 5% hog gastric mucin to enhance virulence. Infection severity was 1-10 LD_{100}. The antibiotics were administered orally in a diluent composed of water and 1% carboxymethyl cellusol.

The two experimental protocols used are referred to as: (a) pre-infection therapy and (b) post-infection therapy. In the pre-infection protocol, the antibiotics were administered orally in a single dose to a large number of mice. At various time intervals later, a group (10 mice) was removed and inoculated with the test organism. Four days later, the percentage of animals alive was calculated for each time interval and antibiotic.

The post-infection protocol was the converse of the above. A large number of mice were infected, then at selected time intervals a group (10 mice) was removed and given a single oral dose of antibiotic. Percent protection was calculated after a 4-day holding period.

Each value in the various Figures represents the arithmetic average of 5 or 6 replicated experiments (50-60 mice). Mice in groups of 10 were housed in plastic disposable cages with food and water *ad lib.*

Results. Pre-infection. In the pre-infection protocol, efficacy was measured by the persistence of antibacterial effect after oral antibiotic administration demonstrated by prevention of death due to subsequent infection. A summary of data with *S. aureus* 5 mp as the infecting organism is presented in Fig. 1. The superiority of DOOTC over

* Chas. Pfizer and Co., Inc., has applied for the registered trade mark of Vibramycin.

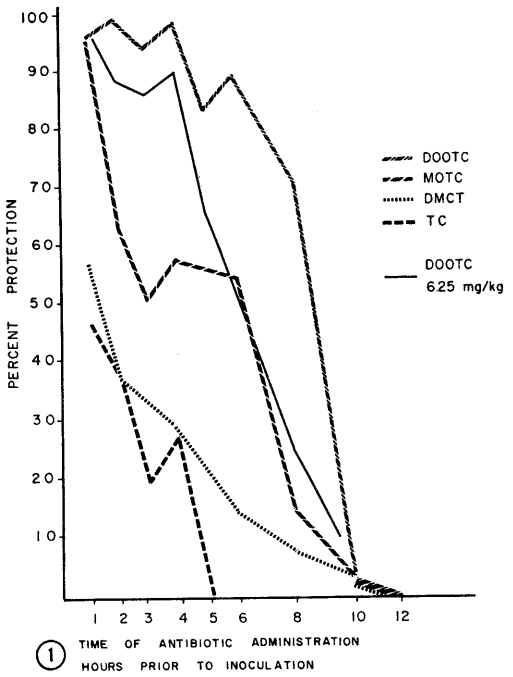
† Registered trade mark of Chas. Pfizer and Co., Inc., is Rondomycin.

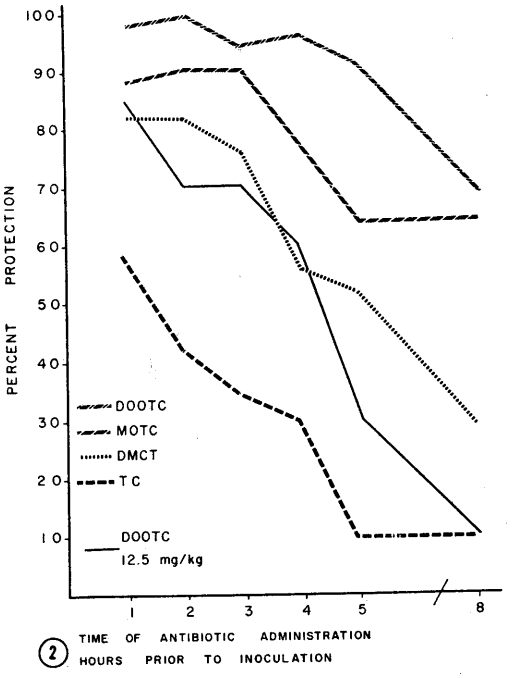
‡ Registered trade mark of Lederle Laboratories, American Cyanamid Co., Inc., is Declomycin.

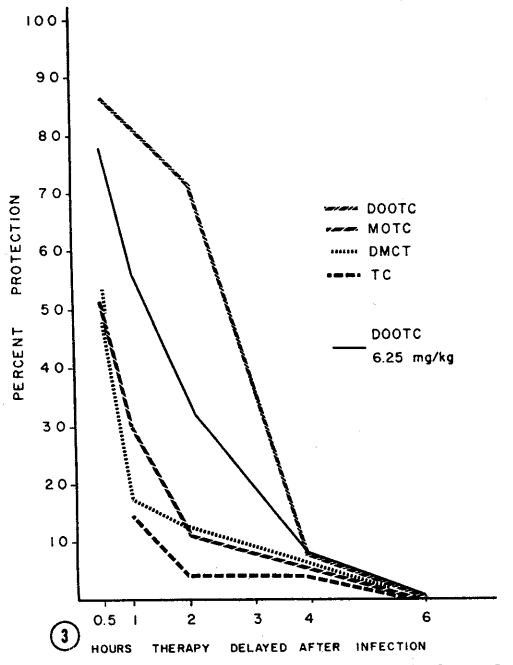
§ Registered trade mark of Chas. Pfizer and Co., Inc., is Tetracyn.

‖ We wish to thank Dr. B. W. Carey, Medical Director, Lederle Laboratories, Pearl River, N. Y., for the supply of DMCT.

other tetracyclines is clearly demonstrated. For example, with a single oral dosage of 12.5 mg/kg, protective activity of DOOTC persisted at a high level even when adminis-







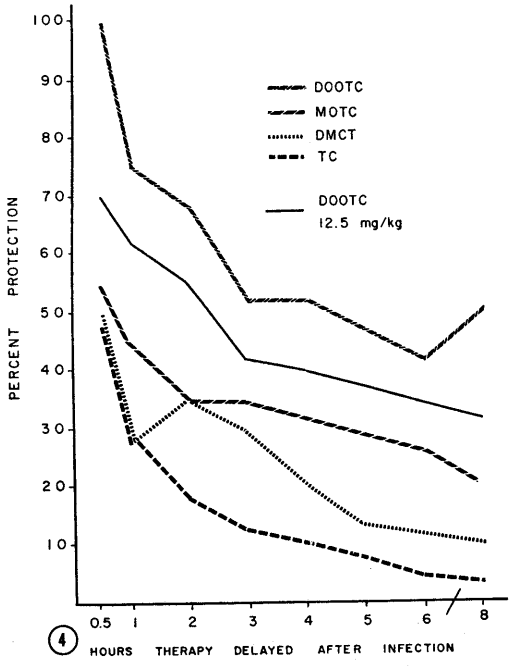


FIG. 1. Pre-infection protocol. Single oral dosage, 12.5 mg/kg. Infection: *S. aureus* 5 mp.
FIG. 2. Pre-infection protocol. Single oral dosage, 50 mg/kg. Infection: *P. multocida.*
FIG. 3. Post-infection protocol. Single oral dosage, 12.5 mg/kg. Infection: *S. aureus* 5 mp.
FIG. 4. Post-infection protocol. Single oral dosage, 50 mg/kg. Infection: *P. multocida.*

tered 8 hours prior to inoculation with *S. aureus, i.e.,* 72% of the mice were protected. MOTC, administered on an equal mg/kg basis with DOOTC 1 hour prior to experimental infection, conferred 95% protection on the mice. However, this decreased to about 55% when administered 6 hours prior to infection. The capacity of MOTC to protect was eliminated if administered 8 hours pre-infection, *i.e.,* only 15% protection was demonstrated. DMCT, when administered at 12.5 mg/kg 1 hour prior to infection, conferred about 60% protection; this diminished to only 15% at 6 hours, and to less than 10% at 8 hours prior to infection. TC, at 12.5 mg/kg given 1 hour prior to experimental infection, protected about 50% of the mice. The protective efficacy of TC decreased to about 30% when administered 4 hours prior, and was without activity at 5 hours prior to experimental infection with *S. aureus* 5 mp.

The comparison between the various tetracyclines is on an absolute basis, *i.e.,* each at the same dosage weight in mg/kg. Such data can be supplemented if a comparison is made on the basis of individual drug efficacy. To accomplish this, the dosage was related to a known capability of each antibiotic to protect against experimental infection. Thus, the oral 12.5 mg/kg dosage was related to oral PD_{50} values in conventional chemotherapeutic experiments(2). To clarify, against the *S. aureus* 5 mp infection, the relationship of $\frac{\text{12.5 mg/kg}}{\text{oral } PD_{50}, \text{ mg/kg}}$ for DOOTC is 2.6; 1.3 for MOTC, 1.1 for DMCT, and about 1.0 for TC. To obtain data for DOOTC, this antibiotic was also studied at a 6.25 mg/kg dosage. Even at this dosage, the protective effect of DOOTC clearly persists to a much higher degree and for a longer time than does that for DMCT or TC. Compared with MOTC at 12.5 mg/kg, DOOTC at 6.25 mg/kg conferred higher protection at up to 5 hours prior to initiation of infection. At the other time periods studied, the two are comparable.

Fig. 2 presents data obtained in the pre-infection protocol utilizing *P. multocida.* The single oral dosage of 50 mg/kg is approximately twice the PD_{50} (mg/kg) value for MOTC, DMCT, and TC in conventional chemotherapeutic experiments against this organism. DOOTC was studied at 50 mg/kg for comparative purposes and also at 12.5 mg/kg, *i.e.,* twice its approximate oral PD_{50} value(2). The antibacterial effect of DOOTC at 50 mg/kg persisted through 8 hours at a high degree by conferring 70% protection on the mice subsequently challenged with *P. multocida.* MOTC, at this same dosage, protected 63% of the mice at 8 hours prior to experimental infection. DMCT, at 50 mg/kg, was able to protect only 30% given 8 hours before inoculation with *P. multocida.* Tetracycline showed the least persistence. About 58% of the mice were protected by 50 mg/kg given 1 hour prior to inoculation with *P. multocida.* DOOTC is also included in Fig. 2 at an equal $\frac{\text{dose}}{\text{oral } PD_{50}}$ ratio to the other tetracyclines, (12.5 mg/kg). Thus, even at one-quarter the actual weight, persistence of DOOTC activity was superior to that of TC, and was comparable to DMCT in the 1- through 4-hour period although it fell below in the 4- to 8-hour period. MOTC at 50 mg/kg retained greater protective capacity throughout the time period than did DOOTC at one-quarter its dosage, *i.e.,* 12.5 mg/kg. Additional studies indicated that DOOTC at one-half the weight of MOTC was equivalent to the latter.

Post infection. Data obtained in the post-infection protocol, utilizing *S. aureus* as the infecting organism, are presented in Fig. 3. Compared on an equal weight basis (12.5 mg/kg) DOOTC is superior to the other tetracyclines in capacity to protect mice previously infected with *S. aureus* 5 mp. Administered 0.5 hour post infection, DOOTC protected 87% of the mice decreasing to about 72% at 2 hours post infection, to about 40% 3 hours post infection, and to about 8% 4 hours post infection.

Administration of MOTC and DMCT 0.5 hour post infection, resulted in about 54%

protection. A rapid decrease was observed when therapy was withheld 1 hour post infection. Both MOTC and DMCT protected about 13% of the mice when therapy was withheld 2 hours post infection and this decreased to <10% at 4 hours post infection. Tetracycline, at 12.5 mg/kg, when withheld 1 hour post infection, was capable of only 15% protection which decreased to about 5% when withheld 2 hours post infection.

Even at one-half the actual weight of the other tetracyclines, on a comparable $\frac{\text{dose}}{\text{oral PD}_{50}}$ basis, DOOTC at 6.25 mg/kg was able to produce a greater percent protection than the others (Fig. 3). Approximately 78% of the animals were protected by 6.25 mg/kg DOOTC administered 0.5 hour after infection. This percent protection decreased to 55% at 1 hour; to 33% at 2 hours and to about 8% 4 hours post infection. Protection was not demonstrated with any of the tetracyclines when drug dosage was withheld 6 hours post infection.

Fig. 4 presents data for the post-infection protocol utilizing *P. multocida* as infecting organism. The single oral dosage of 50 mg/kg represents about 2 for the $\frac{\text{dosage}}{\text{oral PD}_{50}}$ ratio. On a comparable weight basis (50 mg/kg) DOOTC again shows a superior chemotherapeutic effect over the other tetracyclines at up to 8 hours post infection with *P. multocida*. Even when withheld 8 hours post infection, DOOTC was able to protect 50% of the mice. MOTC, given 0.5 hour post infection was able to protect 54% of the mice. This level decreased to about 20% when DOOTC was given 8 hours post infection. At 50 mg/kg 0.5 hour post infection, DMCT protected 50% and then decreased to 10% when therapy was withheld 8 hours post infection. TC, at 50 mg/kg administered 0.5 hour post infection, also protected about 50% of the mice. However, the slope of decrease for TC was sharp, going to <10% when therapy was withheld 4 hours.

To place DOOTC on a comparable $\frac{\text{dose}}{\text{oral PD}_{50}}$ basis with the other tetracyclines, it was used at 12.5 mg/kg. Even at this dosage, which is only one-quarter the weight used of the other tetracyclines, DOOTC still provided greater and longer chemotherapeutic effect than did the other antibiotics.

Discussion. In chemotherapeutic experiments, protection against experimental disease is the result of a number of dynamic interrelated processes, among which are: (1) inherent antibacterial activity of the compound, (2) absorption, (3) transport and distribution, (4) serum binding, and (5) rate of excretion. When the combination of factors is favorable, effective concentrations are achieved within the host resulting in protection until the compound is eliminated or degraded. The pre-infection protocol is based on persistence of the compound within the host at concentrations conferring antibacterial activity thereby preventing death due to subsequent infecion. This persistence may be extrapolated to the clinical situation because fewer doses would be necessary, each remaining longer in the host so that an overall lower dosage might be indicated.

DOOTC clearly persisted in the animal host at antibacterial concentrations considerably longer than did MOTC, DMCT, or TC. When *S. aureus* was used for infection, DOOTC was effective longer than DMCT and TC even at one-half their actual weight dosage. At one-half the dosage of MOTC, DOOTC was superior within the earlier hours and equivalent at the later time periods. The persistence of effective *in vivo* activity can also be emphasized by converting the data to a Chemoprophylactic Persistence Time 50% basis (CPT_{50}), *i.e.*, the time at which an antibiotic can be administered and still protect 50% of the animals against subsequent infection (Table I). DOOTC, after oral administration of 6.25 mg/kg, persists within the mouse for 4.90 hours at concentrations which will protect 50% from subsequent infection by *S. aureus*. The CPT_{50} for DOOTC is longer than the values for the other antibiotics used at twice this dosage.

TABLE I. Chemoprophylactic Persistence Time 50%. Pre-infection protocol.

Antibiotic	Oral dosage, mg/kg	CPT_{50} in hr, for infection*	
		S. aureus	*P. multocida*
DOOTC	50		>8
	25		6.65
	12.5	7.8	3.5
	6.25	4.9	
MOTC	50		6.1
	12.5	4.23	
	6.25	2.4	
DMCT	50		4.9
	12.5	2.2	
	6.25	<1	
TC	50		2.3
	12.5	<1	
	6.25	<1	

* Time in hr antibiotic administered before infection at which 50% protection from subsequent infection was conferred.

Data for the pre-infection protocol utilizing *P. multocida* have been treated similarly and are presented in Table I also. The CPT_{50} values for DOOTC are greater than those of the other 3 tetracyclines at one-half their dosage and greater than the CPT_{50} of TC at one-quarter of its dosage.

In the post-infection protocol, the demands on the antibacterial agent are more stringent than in the pre-infection protocol because the infection has had time to become established prior to treatment. In fact, mice infected with *S. aureus* are generally moribund about 4 hours after inoculation, and after 6 hours a high percentage are dead or dying. This type of response in mice is similar to that published recently by Kapral(3). In the *P. multocida* infection, deaths were not observed during the first 8 hours but occurred within an overnight period. Generally, 100% of the infected controls were dead within 36 hours. This response is comparable to that of the *P. multocida* infection studied by Neter *et al*(4).

As part of the pharmacodynamic processes mentioned above, the role of individual reaction rates, such as rate of absorption from the gastrointestinal tract, is of considerable importance in the post-infection protocol. Certainly in treatment of moribund mice infected with *S. aureus,* it is likely that the antibiotic which reaches effective chemotherapeutic concentrations in the shortest period will afford the greatest protection. Because the progress of disease with *P. multocida* is not as rapid as with *S. aureus,* the role of absorption rate is of less importance. The data in Fig. 3 indicate that DOOTC, at equivalent or one-half dosage of the other tetracyclines, gives greater protection after initiation of infection by *S. aureus* than do the other tetracyclines. As shown in Fig. 4, DOOTC at one-quarter the dosage of the other tetracyclines results in greater protection up to 8 hours after infection with *P. multocida.*

When the data are converted to Survival Time 50% (ST_{50}) values, *i.e.,* the delay in time at which drug administration will still protect 50% of the mice, the superiority of DOOTC at equal dosage with the other tetracyclines is evident (Table II). At one-half the actual weight dosage, the ST_{50} value of DOOTC in the *S. aureus* infection is greater than that of MOTC, DMCT, or TC. DOOTC, at only one-quarter the weight dosage in the *P. multocida* infection produced a ST_{50} greater than the other tetracyclines.

Summary. DOOTC, a new tetracycline antibiotic, was compared in protection studies in mice with MOTC, DMCT, and TC. Marked advantages of DOOTC over the other tetracyclines were demonstrated as follows: 1. Pre-infection protocol: DOOTC demonstrated greater Chemoprophylactic Persistence Time 50% values when administered at equal, at one-half, and, in one instance, at one-quarter the dosage used for the other tetracyclines against the Gram-positive organism *S. aureus* and the Gram-negative organism *P. multocida.* 2. Post-infection protocol: DOOTC demon-

TABLE II. Survival Time 50%. Post-infection protocol.

Antibiotic	Oral dosage, mg/kg	ST_{50} in hr	
		S. aureus	*P. multocida*
DOOTC	50		3.8
	12.5	2.6	2.56
	6.25	1.27	
MOTC	50		1.65
	12.5	.80	
DMCT	50		.5
	12.5	.63	
TC	50		<.5
	12.5	<1	

strated greater Survival Time 50% values in every instance when administered at equal, at one-half, and at one-quarter the dosage used for the other tetracyclines. The persistence time of DOOTC at antibacterial concentrations within the host, and its chemotherapeutic efficacy in protecting mice from established infections, are advantageous properties and suggest possible advantages in clinical situations.

We are pleased to acknowledge the very capable technical assistance of Suzanne Little and Karin Carlsson in these studies.

1. Schach Von Wittenau, M., Yeary, R., J. Pharm. and Exp. Therap., 1963, v140, 285.
2. English, A. R., Proc. Soc. Exp. Biol. and Med., 1966, v122, 1107.
3. Kapral, F. A., Ann. N. Y. Acad. Sci., 1965, v128, 259.
4. Neter, E., Gorzynski, G. A., Cass, W. A., Proc. Soc. Exp. Biol. and Med., 1951, v76, 493.

Received August 30, 1966. P.S.E.B.M., 1967, v124.

Effect of Estrone and Estriol on Salivary Glands and Dental Caries In Female Rats.* (31799)

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Reports that administration of estradiol or diethylstilbestrol causes a decrease in number and diameter of the granular tubules of submandibular salivary glands(1) and an increase in incidence of dental caries(2,3) in the female rat have been confirmed by this laboratory. Estradiol, estrone and estriol are found in low concentrations in the blood and urine of women having normal menstrual cycles. The blood levels and daily urinary excretion of the 3 hormones rise markedly with increased duration of pregnancy(4,5). It stimulated the interest to determine whether estrone or estriol would affect the dental caries incidence (DCI) and the submandibular granular tubules (SGT) as those caused by exogenous estradiol or diethylstilbestrol in female rats. This investigation might shed light on the relationship between the hormones and DCI in pregnant women.

Materials and methods. Weanling female rats of the Sprague-Dawley strain weighing between 40 and 45 g were randomly divided into 4 groups. Each animal was housed in an individual raised wire screen cage. All of the animals were kept under the same environmental conditions, supplied with distilled water and fed, *ad libitum*, with cariogenic diet.† Beginning at 23 days of age, each animal of the 2 treated groups received daily subcutaneous injections of 0.2 mg estrone,‡ or 0.5 mg estriol§ in 0.05 ml of oil solution for 6 weeks. For comparison, a third treated group of rats was injected similarly with 0.02 mg estradiol benzoate.‖ A fourth group of rats was injected with oil alone and served as control.

At 65 days of age, the animals were sacrificed by chloroform inhalation. Submandibular, parotid, thyroid, and adrenal glands and ovaries were immediately removed, weighed, fixed in 10% formalin solution, and sectioned at 5 μ. Submandibular sections were stained by Lillie's Azure A-Eosin B method for the granules of the granular tubular cells. The number and diameter of the SGT were measured by the aid of square and linear micrometers. Parotid, thyroid, adrenal and ovarian sections were stained with hematoxylin and eosin. Heads were boiled in a pressure-

* This investigation was supported by USPHS research grant DE-01621 from Nat. Inst. of Dental Research, Nat. Inst. Health, Bethesda, Md.

† The composition of the diet was as follows: ground rice, 100 lb.; powdered whole milk, 45 lb.; powdered alfalfa, 4.5 lb.; sodium chloride, 1.5 lb.

‡ Theelin, Parke, Davis and Co., Detroit, Mich.

§ Estriol, Delta Chemical Works, New York.

‖ Estradiol benzoate, Schering Corp., New Jersey.