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Some pharmacological aspects of a new water-soluble tetracycline

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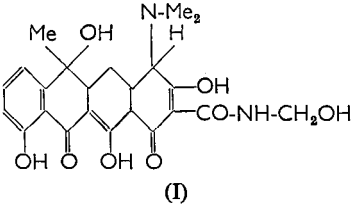
The pharmacological properties of a new derivative of tetracycline have been described. This water-soluble compound has a low toxicity, is stable, and lacks the local side-effects common to other derivatives of tetracycline.

PARENTERAL administration of tetracycline is limited by the poor water-solubility of the base and by the acid pH of solutions of its hydrochloride. Intravenously, tetracycline, oxytetracycline and chlortetracycline cause lesions in the tissues which may lead to phlebitis, whilst gastrointestinal disturbances and changes in the intestinal flora follow their oral administration.

A new tetracycline derivative, pyrolidinomethyltetracycline (PMT), was recently obtained through a carboxamido-substitution, using the Einhorn-reaction, and was shown to lack the local reaction side-effects.

Other experiments were made with 4-(2-hydroxyethyl)-diethylenediaminemethyltetracycline (Gradnik, Pedrazzoli & Ferrero, 1960). To increase water-solubility still further, tetracyclines were then coupled with amino-acids to yield compounds of lower toxicity (De Carneri, Coppi, Lauria & Logemann, 1961; Tubaro & Raffaldoni, 1961). Using an Einhorn-type reaction (Einhorn, 1905), tetracycline and formaldehyde have now been combined to form a water-soluble tetracycline (I, TMT or methylencycline), which may be administered orally or parenterally. This paper describes pharmacological studies with TMT.



(I)

Methods and materials

A comparison was made of the properties of tetracycline hydrochloride, pyrrolidinomethyltetracycline and the new water-soluble derivative.* All drugs are considered as tetracycline base for dosage purposes.

ACUTE TOXICITY

Swiss white mice (weight 18–20 g) were fasted for 12 hr and then given the antibiotic either by injection intraperitoneally (1 ml of a 1·8–8 mg/ml solution), intravenously (0·5 ml of a 5·6–7 mg/ml solution)

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* Monosodium salt.

and orally (1 ml of a 40–80 mg/ml solution or suspension). Deaths were noted over 7 days and the LD 50 values were calculated by the method of Litchfield & Wilcoxon (1949).

CHRONIC TOXICITY

This was determined in groups of 15 mice. Test animals were given daily 250 mg/kg of the drugs intraperitoneally in 0·5 ml of solution and control animals were given 0·5 ml of physiological saline solution. The groups were weighed every 3 days for the next 60 days. Total blood counts were made when the animals were killed.

EFFECT ON BLOOD PRESSURE AND RESPIRATION

Simultaneous recordings of blood pressure and respiration were made in 6 cats anaesthetised with pentobarbitone sodium (40 mg/kg). The drugs were given in doses of 0·01 and 25 mg/kg. Dogs similarly prepared received only the higher dose of the tetracycline.

EXPERIMENTAL INFECTION

Groups of 10 Swiss white mice (weight 18–21 g) were injected intraperitoneally with 0·5 ml of *Staphylococcus aureus* culture (Smith ATCC 13709, incubated for 5 hr in brain-heart broth Difco; the infecting doses contained $15 \times 10^6 \pm 3 \times 10^6$ cells as determined by plate counts), containing 1% (w/v) of sodium glycocholate and taurocholate (Amsterdam & Schneierson, 1954). The protective action of TMT and tetracycline was then determined using both intravenous and oral routes.

BLOOD LEVELS OF TETRACYCLINES IN DOGS

The drugs were administered orally (50 mg/kg) or intravenously (10 mg/kg), and blood samples were taken from the saphenous vein. A tube-dilution method in broth was used to determine the blood levels, with *Bacillus cereus var. mycoides* as test organism.

Results

ACUTE TOXICITY

The acute toxicity of the three tetracyclines was similar for each of the three routes studied (Table 1).

TABLE 1. ACUTE TOXICITY (LD50 VALUES, mg/kg) OF THREE TETRACYCLINES IN MICE

Route of adm.	Tetracycline	PMT	TMT
Intravenous ..	160	150	170
Intraperitoneal ..	340	330	380
Oral	2,550	1,320	3,000

CHRONIC TOXCITY

The chronic intraperitoneal toxicity of the three tetracyclines is shown in Table 2.

Compound TMT was significantly less toxic than tetracycline or PMT and this was confirmed by the results of the body weight and blood counts.

TABLE 2. CHRONIC INTRAPERITONEAL TOXICITY (LD50 VALUES, mg/kg) OF THREE TETRACYCLINES IN MICE

Tetracycline	PMT	TMT
830 (750–920)	540 (460–640)	3,600 (3,270–3,960)

Mice treated daily with TMT at a dosage of 50 and 200 mg/kg intraperitoneally and 1 and 2 g/kg orally grew significantly more rapidly than controls. The blood counts showed no differences from control samples.

On the other hand, animals treated daily with tetracycline hydrochloride at a dosage of 20 mg/kg intraperitoneally grew significantly slower than controls. A dosage of 1 and 2 g/kg orally was lethal within 2–3 weeks. The blood counts did not differ from control values.

Weight curves of PTM intraperitoneally administered at the dosage of 50 and 20 mg/kg daily were similar to controls: 1 and 2 g/kg orally

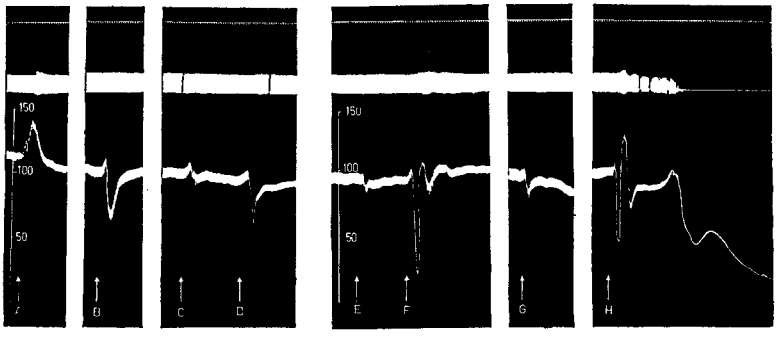

FIG. 1. Cat, female (3·2 kg) under barbiturate anaesthesia. From top to bottom: time (10 min interval), respiration, carotid blood pressure. A = adrenaline 5 μg/kg. B = histamine 5 μg/kg. C = TMT 10 and D, 25 mg/kg. E = tetracycline hydrochloride 10 and F, 25 mg/kg. G = PMT 10 and H, 25 mg/kg.

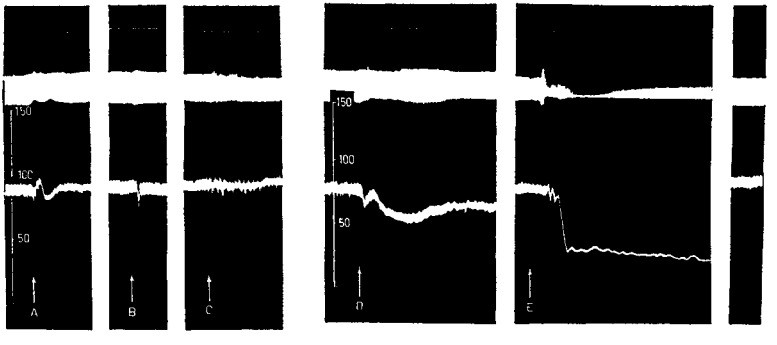

FIG. 2. Dog, female (8·4 kg) under barbiturate anaesthesia. From top to bottom: time (10 min interval), respiration, carotid blood pressure. A = adrenaline 2 μg/kg. B = histamine 2 μg/kg. C = TMT 25 mg/kg. D = tetracycline hydrochloride 25 mg/kg. E = PMT 25 mg/kg.

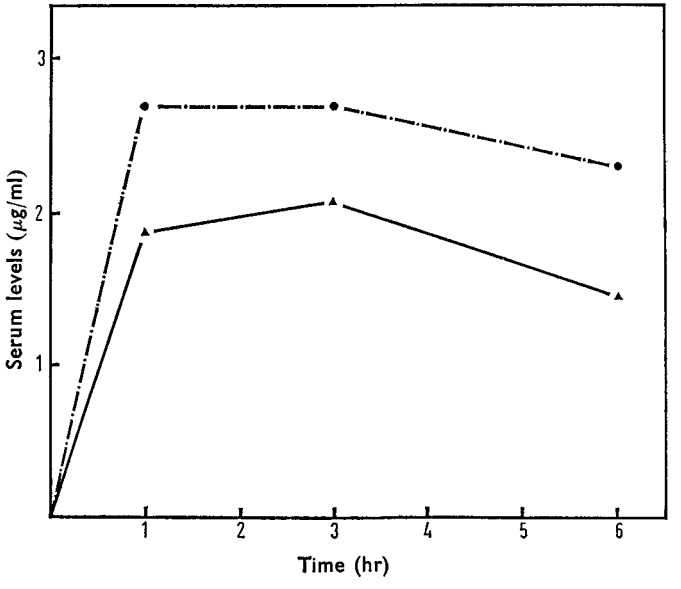

FIG. 3. Blood serum levels in dogs following one single oral dose of 50 mg/kg of tetracycline hydrochloride and TMT.

●—·—·—·—● TMT

▲——————▲ Tetracycline hydrochloride

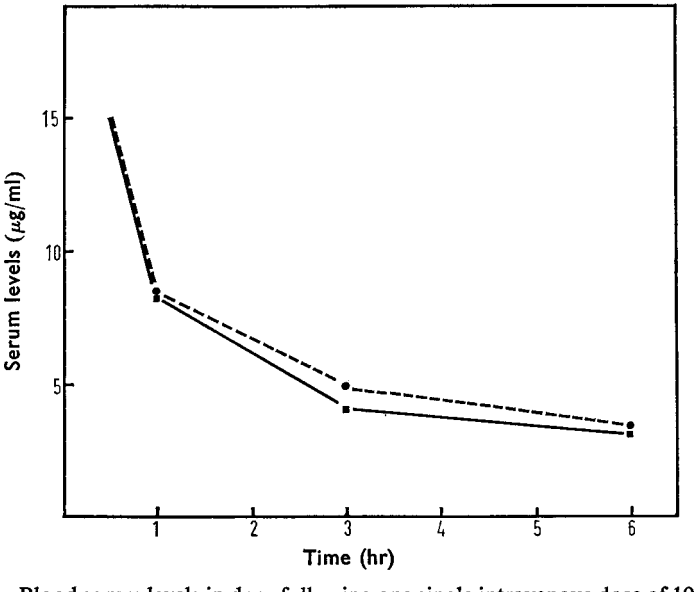

FIG. 4. Blood serum levels in dogs following one single intravenous dose of 10 mg/kg of TMT and PMT.

●— — — —● TMT

■——————■ PMT

administered was lethal in 7–14 days. Again the blood counts showed no differences from control samples.

ACTION ON BLOOD PRESSURE AND RESPIRATION

Both in the dog and in the cat the action of TMT on the arterial blood pressure and respiration was less than that of tetracycline. Doses of 25 mg/kg produced only a transient fall in blood pressure in the cat (Fig 1) and no change in the dog (Fig 2) whereas corresponding doses of tetracycline were nearly lethal.

EXPERIMENTAL INFECTION

Tetracycline and TMT were equally effective in protecting mice against the *Staph. aureus* infection. The ED50 values of tetracycline, 8·8 (5·9–13·0)* mg/kg orally and 8·6 (5·9–12·5)* mg/kg intravenously did not differ significantly from those of TMT, 9·0 mg/kg (6·2–13·0)* orally and 5·6 (4·1–7·6)* mg/kg intravenously.

BLOOD LEVELS OF TETRACYCLINES IN DOGS

The blood levels of tetracycline and TMT after oral administration are shown to be similar in Fig 3.

In Fig 4, blood serum levels after a single intravenous dose of 10 mg/kg of TMT and PMT are shown also to be similar.

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* Confidence limits for a 19/20 probability.