Reprint from:

»ARZNEIMITTEL-FORSCHUNG«

»DRUG RESEARCH«

Arzneim.-Forsch. (Drug Res.) **25**, Nr. 2, 234—237 (1975)

Editio Cantor KG, Aulendorf i. W. (Western Germany)

From the Department of Ophthalmology, University Medical School, Szeged (Hungary)

The Parenteral Application of a New Metacycline Preparation, Tri-metacycline

By I. L. Kahán and H. Hammer

A tetracycline derivative which has proved very effective is metacycline (6-methylene-5-hydroxytetracycline), prepared first by Blackwood et al. [2] in 1961, by dehydrogenation of 5-hydroxytetracycline. Good results have been attained by the use of the commercially available capsules and syrup containing metacycline hydrochloride, primarily in thoracic surgery and in diseases of the respiratory system [1, 12, 14] but also in the most varied surgical, gastric, intestinal and other infections [4]. In spite of the effectiveness against similar microorganisms and the cross resistances due to their chemical similarities, certain microorganisms have proved more sensitive to some tetracyclines than to others. The Staphylococcus aureus strains, Pseudomonas aeruginosa and Klebsiella strains, Diplococcus pneumoniae and certain Escherichia coli strains exhibit a high sensitivity to metacycline [5, 7]. This justified the use of metacycline instead of other tetracycline varieties, despite the fact that these latter are commercially available in an injectable form, not causing gastrointestinal complaints and also applicable in unconscious and severe states, after transformation of the base compound to some water-soluble derivative. The high effectiveness of metacycline, both in in vitro tests and on administration in vivo, can almost certainly be explained by its good lipid solubility. It may be presumed that the preparation of a neutral aqueous solution with a concentration suitable for parenteral application has so far not succeeded just because of this high lipid solubility. Our investigations have shown that after conversion to tri-metacycline an aqueous metacycline solution can be obtained with high concentration (5—10%) and a pH of 7.4. (The tri-tetracyclines are complexes of the tetracyclines with three molecules of a recommended organic base.) The resulting solution exhibits a high antibiotic activity when examined in vitro or in vivo.

Materials and methods

The metacycline complex in the form of tri-metacycline was prepared directly before administration. The preparation was made up as follows:

Ampoule I: 50 mg metacycline hydrochloride (dry fill);
Ampoule II: the complexing agent dissolved in 1 ml water (the solution keeps indefinitely at room temperature).

After mixing the contents of the two ampules and mild shaking, the metacycline hydrochloride dissolves and a solution of pH 7.4 is obtained. This solution can be kept in a refrigerator for at least 24 h.

In vitro examinations

The in vitro antibiotic activity tests of tri-metacycline and metacycline were studied turbidimetrically using Bac. aureus varietas mycoides ATCC 9634, Staphylococcus aureus 8357 NCTO 42 and Escherichia coli K 12 as test microorganisms. The test was a 2-fold, serial dilution of the antibiotic in broth. The inoculum consisted of 0.2 ml of a 1×10^{-3} dilution of a culture incubated overnight at 37° C. Total final volume in each tube was 4 ml. The minimal inhibitory concentration (MIC) was recorded as the lowest concentration of the antibiotic inhibiting the growth of the microorganism, i.e. the last tube with clear broth.

In vivo examinations

For the study of i.v. and i.m. effectivities of the tri-metacycline injection preparation, the metacycline hydrochloride was in all cases administered in the tri-metacycline form as prepared above. Intravenous injections were used on a total of 28 male rabbits. 14 rabbits were given doses of 10 mg/kg, 8 rabbits doses of 15 mg/kg, and 6 rabbits doses of 20 mg/kg. Since no toxic effects at all were observed, the solution was injected in the shortest possible time into the marginal ear vein. The blood serum antibiotic level was determined by blood sampling after 1, 2, 4, 6 and 8 hours. The blood was centrifuged and the sera were examined by the agar-well diffusion method on the same day, using the Staphylococcus aureus 8357 NCTO 42 test microorganism. Levels were expressed in μg/ml based on comparison with the growth inhibition caused by known concentrations of the same drug (standards).

4 rabbits were killed after the 8-h blood sampling, and 4 rabbits 24, and 4 rabbits 48 h after the administration of tri-metacycline, by i.v. injection of air. 5 g each was taken from the lung, the liver, the kidney and the gluteal muscles, and 1 g from the spleen; these were homogenized and triple-extracted by the method of Schach v. Wittenau and Delahunt [16], and the antibiotic contents determined by the method employed with the serum.

The administration of tri-metacycline to humans was also achieved in 2 cases injecting i.v. a single dose corresponding to 220 mg and 350 mg metacycline (3 and 5 mg/kg), respectively, and the urine was examined after 48 and 96 h, and the blood serum levels after 1, 2, 4, 6, 8 and 24 h. Besides quantitative serial dilution test, the urine was also examined by chromatography followed by bioautography. 3 solvent systems were used:

I. benzene:nitromethane:pyridine (10:20:3) on a Whatman No. 4 paper saturated with McIlvain buffer, pH 3.5 ascending chromatography was carried out;

II. n-butanol:acetic acid:water (4:1:5) and

III. n-butanol:ammonium hydroxyde:water (4:1:5) solvent on a Whatman No. 1 paper saturated with 0.1 M disodium ethylene diaminetetraacetic acid [11] descending chromatography was carried out.

After chromatography the Staphylococcus aureus 8357 NCTO 82 test microorganism was used for the bioautography. For the sake of better visibility of the spots the chromatograms were treated with triphenyltetrazolium chloride yielding formazan after incubation.

The effects of i.m. injections were examined following administration of metacycline hydrochloride in the dose of 70 mg/kg to 10 rabbits. The blood sera were sampled 1, 2, 4, 6 and 8 h after injection, and the organs listed in connection with the investigation of the i.v. injection were examined 24 h after the administration in 4 rabbits, and 48 h after it in further 4 rabbits. The determinations were performed as previously.

Results

In vitro examinations

The in vitro effectivities of tri-metacycline and metacycline are given by the minimum inhibitory concentration (MIC) values in Table 1. Using Bac. cereus varietas mycoides ATCC 9634, Staphylococcus aureus 8357 NCTO 42 or Escherichia coli K 12, the MIC values of tri-metacycline were smaller than those of metacycline. The mathematical analyses of the differences of MIC values are highly significant.

In vivo examinations

On the administration of metacycline doses of 10, 15 and 20 mg/kg, the highest blood level was in all cases measured 1 h after the injection and similarly in all three cases, a blood level exceeding the therapeutic level in excess was measured even after 8 h. The slopes of the blood serum curves obtained on the application of the three different doses are roughly parallel to one another, however, running at different heights according to the doses (Fig. 1).

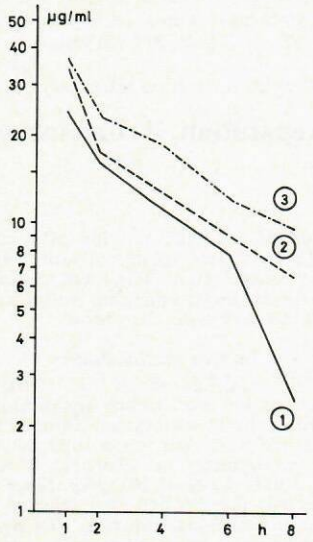

Fig. 1: Metacycline concentration (µg/ml) in sera of rabbits after a single dose of i.v. **1**: 10 mg/kg; **2**: 15 mg/kg and **3**: 20 mg/kg metacycline in the form of tri-metacycline.

The antibiotic contents of the organs of the animals killed 8 h after a single i.v. injection of 10 mg/kg metacycline are very high, with the highest values in the kidneys. In all the organs, the antibiotic values for the animals killed 24 h after the administration of tri-metacycline exceed even the 8-h values, by 60—280%. The considerable increases of the antibiotic contents of the kidneys and spleen are striking. On the other hand, the antibiotic contents of the organs 48 h after the administration were in all cases lower than the 24-h values. In the case of the lung, the liver and the muscle, the values measured were similar to the 8-h antibiotic level. The 48-h antibiotic contents of the kidneys comprised only about 60% of the 8-h kidney antibiotic level, but in contrast the antibiotic contents of the spleen were higher than the antibiotic level measured in the organ 8 h after the administration (Fig. 2).

The blood serum values in man following the i.v. application of 220 mg and 350 mg (3 and 5 mg/kg, respectively) in the form of tri-metacycline are shown in Fig. 3. Even 24 h later the antibiotic content of the blood exceeds the therapeutic

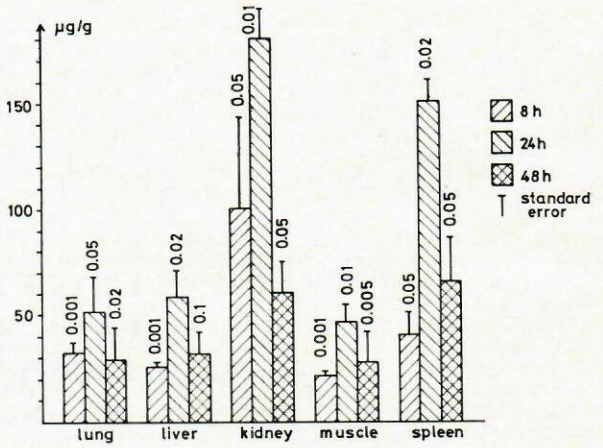

Fig. 2: Metacycline concentration (µg/g) in wet tissues of rabbits 8, 24 and 48 h after i.v. injection of a single dose of 10 mg/kg metacycline in the form of tri-metacycline (average of 4 experiments each). The figures over the columns give the probability p, which was in all cases smaller than the range indicated.

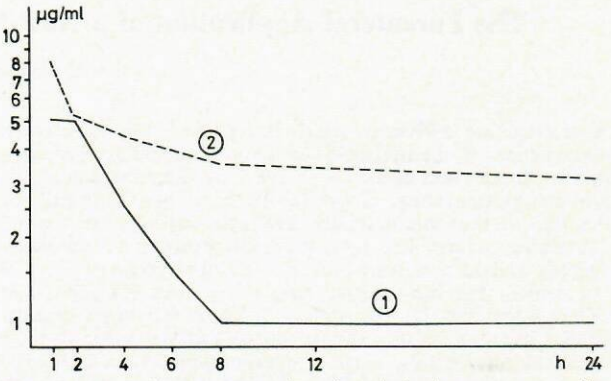

Fig. 3: Metacycline concentration (µg/ml) in human serum after a single dose of **1**: 220 mg (3 mg/kg); **2**: 350 mg (5 mg/kg) metacycline in the form of i.v. tri-metacycline.

level. Examination for the metacycline excreted with the urine indicated that the highest antibiotic excretion occurred on the 3rd day (Fig. 4). Chromatographic examination of the urine excreted on any day showed the presence of a yellow fluorescing spot migrating with the same R_f value as the metacycline and trimetacycline used as standards in the 3 solvent systems applied. R_fI: 0.49; R_fII: 0.81; R_fIII: 0.39. Bioautagraphic examination revealed antibiotic activity of the same (Fig. 5). A bluish fluorescent spot at the starting line caused by unknown materials derived from the urine exhibited no antibiotic activity.

The blood serum concentrations following the i.m. injection of tri-metacycline are shown in Fig. 6. In spite of the application of an antibiotic solution with a relatively high concentration, the antibiotic content of the blood serum scarcely exceeded 5 µg/ml, but in the first 8 h this blood antibiotic level remained constant. Examination of the antibiotic levels of the organs revealed marked antibiotic contents in the spleen, muscle and lung of the animals killed 24 h after administration of the i.m. injection. Compared to the high kidney antibiotic level observed after the i.v. injection, the metacycline content of the kidney after i.m. administration is low. When the organs of the animals killed were examined

Table 1: In vitro activity of metacycline and tri-metacycline.

Microorganism	Number of tests	Average MIC (µg/ml)		Δ MIC	$S_{\bar{x}}$	p
		Metacycline	Tri-metacycline			
Bac. cereus varietas mycoides ATCC 9634	8—8	0.356	0.147	0.21	0.04	<0.01
Staphylococcus aureus 8357 NCTO 42	8—8	0.440	0.230	0.21	0.06	<0.05
Escherichia coli K 12	8—8	0.525	0.425	0.10	0.03	<0.05

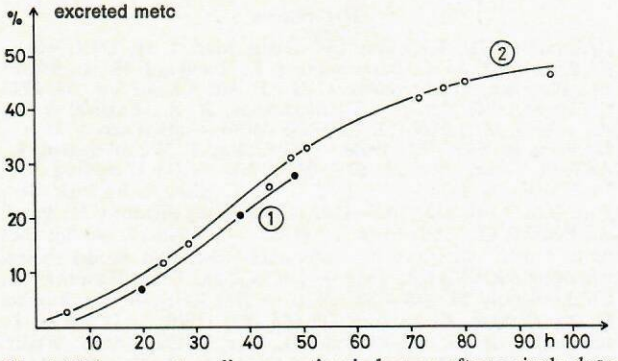

Fig. 4: Urinary metacycline excretion in human after a single dose of **1**: 220 mg (3 mg/kg); **2**: 350 mg (5 mg/kg) metacycline in the form of i.v. tri-metacycline expressed as percentage of the ingested dose.

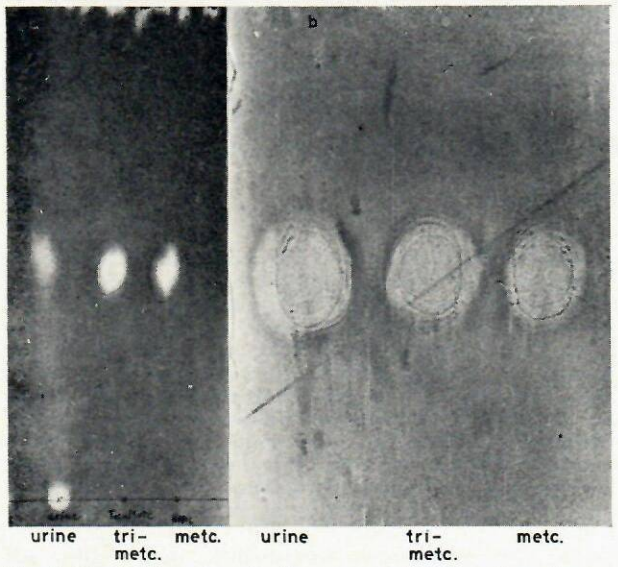

Fig. 5: Chromatography of urinary excreted metacycline after i.v. injection of tri-metacycline in humans. Left: chromatogram in u.v. light; right: bioautography. Solvent system I: benzene:nitromethane:pyridine (10:20:30).

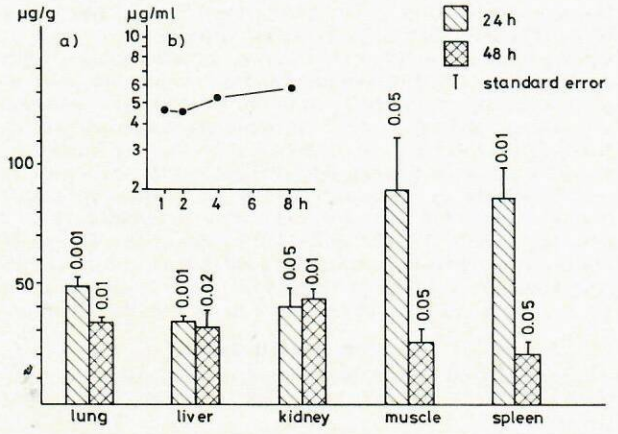

Fig. 6: a) Metacycline concentration (μg/g) of wet tissues of rabbits 24 and 48 h after i.m. injection; b) Metacycline concentration (μg/ml) in sera of rabbits after i.m. injection of a single dose of 70 mg/kg metacycline in the form of tri-metacycline (average of 4 experiments each). The figures over the columns give the probability p, which was in all cases smaller than the range indicated.

48 h after the i.m. administration of tri-metacycline, it was found that, compared to the 24-h values, the antibiotic level in the lung was somewhat lower, those in the muscle and spleen were substantially lower, and those in the liver and kidney were practically the same (Fig. 6).

Discussion

Of the various tetracycline derivatives which can be prepared from 5-hydroxytetracycline by various substituents in position 6, the one most simply and most economically to be obtained is metacycline. Although this has until now been commercially available in the form of capsules and syrup, the demand for its i.v. applicability is shown by the fact that 1 year after the preparation of metacycline Kunin [13] carried out blood serum examinations with an administration time of 15 min, using a 0.5% metacycline hydrochloride solution which might have been presumably acidic and also contained glucose. Our investigations indicate that the possibilities of the applicability of metacycline are increased substantially by its conversion to tri-metacycline.

Even after the administration of a comparatively low dose, the serum antibiotic values in our examinations are higher than those after infusions of dilute solutions or than those observed after oral administration [13].

Examination of the rabbit organs shows a strikingly high antibiotic level even after the administration of a metacycline dose of 10 mg/kg; these organ contents are practically not increased when the dose is raised [10]. Filidaro [8] observed the intensive concentration of metacycline in the rabbit's organ. In spite of this, the danger of accumulation is not to be feared, since the organ antibiotic contents of animals killed at various times after the administration show a decrease 48 h following the administration; this points to the gradual excretion of the antibiotic. The slower decrease of the antibiotic contents of the liver and spleen indicate a slight accumulation in the RES cells. The role of the spleen in the accumulation of drugs, and the favourable nature of their passage from there into the organism, was pointed out by Jancsó [9].

The lower but long-lasting antibiotic level in human blood serum can be attributed partly to the smaller dose, and partly to the different strength of the metacycline bond to human proteins. The slow excretion of tri-metacycline with the urine is due to the long-lasting blood-level. Chromatography and antibiotic activity demonstrated by bioautography point to the at least partial excretion of the tri-metacycline in an unchanged form, without occurrence of toxic degradation products. Attention has been drawn by Dimmling [6] to the importance of the excretion of tetracyclines with the urine.

Our investigations indicate that i.m. injection of tri-metacycline is generally less favourable than i.v. administration. The serum antibiotic values are relatively low, and accordingly the antibiotic levels of the kidneys, too, are low. The high spleen antibiotic content is almost certainly associated with the accumulation also observed on i.v. administration. In contrast to the antibiotic contents of other organs, the metacycline content of the lung equals the antibiotic value of animals killed 24 h after i.v. administration. Further examinations should be carried out to elucidate the most adequate routes of administration against pulmonary diseases, and to elucidate whether an aqueous solution of tri-metacycline might be suitably applied in the form of a spray (aerosol?).

In the evaluation of the results of the above examinations, consideration must be paid to the distribution of tri-metacycline between lipids and water. Our investigations [10] have shown the considerable increase of water solubility of metacycline after conversion to tri-metacycline, while at the same time its lipid solubility decreases by only about 20%, similarly as for those of the other tri-tetracyclines. Following the transformation of the parent compound to tri-metacycline, the resulting "optimum lipid solubility" [3] increases its biological value. Just like the other tri-tetracyclines but in contrast to the parent compounds, tri-metacycline is not injurious to the tissues, and therefore its local application is very advantageous, even its ophthalmological administration is recommended [15]. The aqueous solution does not contain any form of organic solvent, macromolecule or stabilizing material at all, and this fact contributes to the non-toxic nature of the injectable tri-metacycline solution.

Mathematical analysis performed by K. Boda, Central Laboratory, and the technical assistance in chromatography and bioassay by S. Mindszenty is greatly acknowledged.

Summary

Tri-metacycline, one of the new tetracycline complexes (tri-tetracyclines), is prepared by mere dissolution of metacycline hydrochloride in an aqueous solution of the complexing agent. In vitro and in vivo studies show a high antibiotic activity. Significantly lower MIC values were found for tri-metacycline than for the parent compound. Parenteral administration resulted in high sera and tissue values, without signs of accumulation; excretion via the kidneys was proved.

Zusammenfassung

Parenterale Anwendung eines neuen Metacyclinpräparates, Tri-metacyclin

Tri-metacyclin, ein neuer Tetracyclinkomplex (Tri-tetracycline), wird allein durch Lösung von Metacyclin-hydrochlorid in einer wäßrigen Lösung des Komplexbildners gewonnen. Die Untersuchungen in vitro und in vivo zeigen hohe antibiotische Aktivität. Die mittleren Hemmkonzentrationen liegen bei Tri-metacyclin signifikant niedriger als bei der Ausgangssubstanz. Die parenterale Verabreichung ergab hohe Serum- und Gewebespiegel ohne Anzeichen einer Kumulation. Es wurde nachgewiesen, daß die Ausscheidung über die Nieren erfolgt.

References

[1] Bamberg, H., Schliewen, Ch., Dtsch. Med. J. **18,** 234 (1966) — [2] Blackwood, R. K., Beereboom, J. J., Rennhard, H. H., Schach von Wittenau, M., Stephens, C. R., J. Am. Chem. Soc. **83,** 2773 (1961); **85,** 3949 (1963) — [3] Blackwood, R. K., English, A. R., Adv. Appl. Microbiol. **13,** 237—265 (1970) — [4] Bolanos, M. G., Medicina **35,** 695—699 (1967) — [5] Chang, T. W., Weinstein, L., Antibiot. Chemother. **12,** 676—688 (1962) — [6] Dimmling, Th., Med. Klin. **67,** 1632—1637 (1972) — [7] English, A. R., Proc. Soc. Exp. Biol. Med. **122,** 1107—1112 (1966) — [8] Filadoro, F., Pezzi, R., Renzini, G., Antibiotica **1,** 1 (1963) — [9] Jancsó, N., Speicherung, Stoffanreicherung im Retikuloendothel und in der Niere, Akadémiai Kiadó, Bp., 1955 — [10] Kahán, I. L., Hammer, H., Chemotherapy **20,** 148—155 (1974) — [11] Kelly, R. G., Buyske, D. A., Antibiot. Chemother. **10,** 604—607 (1960) — [12] Kiss, J., Faragó, E., Bacsa, S., Schnitzler, J., Orv. Hetilap (Ärztl. Wschr. ung.) **110,** 2759—2763 (1962) — [13] Kunin, C. M., Exp. Biol. Med. **110,** 311—315 (1962) — [14] Milek, E., Grantová, H., Dtsch. Med. J. **18,** 393—395 (1967) — [15] Pápai, I., Kahán, I. L., Hammer, H., to be published — [16] Schach von Wittenau, M., Delahunt, C. S., Pharm. Exp. Ther. **152,** 164—169 (1966) — [17] Selzer, G. B., Wright, W. W., Antibiot. Chemother. **7,** 292 (1957)

For the authors: Dr. Ilona Kahán, Department of Ophthalmology, University Medical School, Szeged (Hungary)