Metal-Acid Complexes with Members of the Tetracycline Family
II. Development of Stable Preconstituted Parenteral Formulations

By EDWARD G. REMMERS, WILLIAM C. BARRINGER, GEORGE M. SIEGER, and ALBERT P. DOERSCHUK

Stable preconstituted liquid formulations suitable for intramuscular and intravenous administration have been prepared using aluminum-calcium-gluconate complexes of tetracycline and 6-demethylchlortetracycline. These formulations are well tolerated at therapeutic levels and give adequate blood levels. The most desirable preparations contain the antibiotic-aluminum-calcium-gluconate complex of molar ratio 1:3:1:6 in 50 to 75 per cent propylene glycol at pH 8.5. Such formulations retain their initial potency at room temperature and elevated temperatures for prolonged periods.

IN THE FIRST publication in this series (1), the pharmaceutical properties of metal-acid complexes with members of the tetracycline family were described. These properties were considered of sufficient interest to develop suitable parenteral formulations of these complexes. The purpose of this publication is to describe the development of stable preconstituted parenteral formulations with this class of preparations.

EXPERIMENTAL

Preparation of Metal-Acid Complexes.—The method of preparing metal-acid complexes has been described (1).

Preparation of Aqueous Propylene Glycol Formulation.—The metal-acid complex was dissolved completely with stirring in a fraction of the total amount of distilled water to be used. The desired amount of propylene glycol was then added. Sodium formaldehyde sulfoxylate was dissolved in a small amount of distilled water and was added to the formulation. The pH was elevated to 8.0–8.5 with monoethanolamine, and the formulation brought to the proper volume with distilled water. The solution was then filtered through a fine sintered-glass filter and sealed in ampuls under a nitrogen atmosphere.

Blood Level Studies.—The procedure for intramuscular administration has been described (1); the cephalic vein was used for intravenous administration.

RESULTS AND DISCUSSION

Screening Studies.—A large number of metal-acid complexes with varying constituents and molar *ratios* were prepared for evaluation by criteria of complex formation previously described (1), solubility studies at neutral and slightly alkaline pH's, alkaline stability studies (2), and blood level and blood pressure depression studies. These screening experiments indicated the existence of the following important relationships for these preparations: (*a*) on intramuscular and intravenous administration, the aluminum-calcium-gluconate complexes of demethylchlortetracycline (DMCTC) and tetracycline (TC) exhibited high blood levels; (*b*) for good complex formation, it is necessary to use a minimum of 3 molar parts of aluminum; (*c*) the solubility of the complex under neutral or slightly alkaline conditions is reduced as the molar ratio of calcium is increased; and (*d*) a minimal amount of calcium (approximately 1 molar part) is needed to impart enhanced alkaline stability to the complex and to prevent blood-pressure depressions after intravenous administration.

Because of these relationships, most of the formulation studies were performed on the DMCTC and TC-aluminum-calcium-gluconate complexes having a molar ratio of 1:3:1:6.

Blood Levels After Intramuscular Administration.—Table I presents the results of a blood level experiment designed to investigate the effect of propylene glycol content in a formulation containing DMCTC-aluminum-calcium-gluconate (molar ratio 1:3:1:6) following intramuscular administration in dogs. The data indicate that no statistically significant differences in blood levels [measured by area under the 24-hour blood-level curve, AUC (3)] could be detected among formulations ranging from 0 to 75% propylene glycol.

No gross irritation was noted at 4 and 7 hours following intramuscular administration. At 24 hours after administration, one dog from each group receiving 25, 50, and 75% propylene glycol limped slightly.

A second blood level experiment was designed to determine the effect of pH of the aqueous propylene glycol formulation on the blood levels obtained with the DMCTC-aluminum-calcium-gluconate complex (molar ratio 1:3:1:6) when administered intramuscularly to dogs. The results of this experiment are summarized in Table II. The data indicate that the pH of the formulation exerts a statistically significant effect on blood levels. As the pH of the formulation is lowered from 8.5 to 4.0, blood levels tend to increase. The major portion of the increase occurs between pH 5.5 and 4.0.

In this experiment, gross irritation was noted in only one dog which received the formulation at pH 4.0. At 4 and 7 hours following intramuscular administration, the dog limped but had completely recovered 24 hours afterward.

Blood Levels After Intravenous Administration.—Table III compares the results obtained with DMCTC-aluminum-calcium-gluconate complexes in 56% propylene glycol with a DMCTC·HCl-ascorbic acid formulation in dogs. The data indicate that the metal-acid complexes in the aqueous propylene gly-

Received May 14, 1964, from the Pharmaceutical Product Development Section, Lederle Laboratories, American Cyanamid Co., Pearl River, N. Y.

Accepted for publication July 20, 1964.

Biological studies were performed under the direction of Dr. J. J. Corbett and Mr. N. Anagnostakos, Biological Assay Development Laboratory.

Tetracycline and 6-demethylchlortetracycline are marketed as Achromycin and Declomycin, respectively, by the American Cyanamid Co., Pearl River, N. Y.

TABLE I.—EFFECT OF PROPYLENE GLYCOL CONTENT ON BLOOD LEVELS[a] IN DOGS FOLLOWING INTRAMUSCULAR ADMINISTRATION OF A DMCTC-METAL-ACID COMPLEX

DMCTC-Aluminum-Calcium-Gluconate Complex (1:3:1:6), pH[b] 8.3	Hr. After Injection[c] 1	4	7	24	AUC, mcg./Hr./ml.	95% Confidence Limits
Propylene glycol, 75%	6.24	3.87	2.10	0.74	50.0	38.5–65.0
Propylene glycol, 50%	5.64	4.77	3.18	0.95	63.2	48.6–82.1
Propylene glycol, 25%	5.40	3.28	2.03	0.73	48.2	37.1–62.7
No propylene glycol	6.60	3.60	2.24	0.68	52.5	40.4–68.3

[a] Based on microbiological assays expressed as micrograms per milliliter tetracycline·HCl. Average of three dogs per group. [b] pH adjusted with monoethanolamine after addition of 0.5% sodium formaldehyde sulfoxylate. [c] Dose = 1.5 mg. DMCTC·HCl/lb. body weight.

TABLE II.—EFFECT OF pH ON BLOOD LEVELS[a] IN DOGS FOLLOWING INTRAMUSCULAR ADMINISTRATION OF DMCTC-METAL-ACID COMPLEX

DMCTC-Aluminum-Calcium-Gluconate Complex (1:3:1:6) in 70% Propylene Glycol	Hr. After Injection[c] 1	4	7	24	AUC, mcg./Hr./ml.	95% Confidence Limits
pH[b] 4.0	11.2	7.1	7.2	1.17	112.5	83.1–152.2
pH 5.5	8.0	4.35	3.8	0.71	71.0	52.5–96.1
pH 7.0	6.56	4.2	3.85	0.64	69.0	51.0–93.4
pH 8.5	4.0	5.25	3.6	0.78	60.8	44.9–82.3

[a] Based on microbiological assays expressed as micrograms per milliliter tetracycline·HCl. Average of three dogs per group. [b] pH adjusted with monoethanolamine after addition of 0.5% sodium formaldehyde sulfoxylate. [c] Dose = 1.5 mg. DMCTC·HCl/lb. body weight.

TABLE III.—BLOOD LEVELS[a] IN DOGS FOLLOWING INTRAVENOUS ADMINISTRATION OF DMCTC-METAL-ACID COMPLEX AND ASCORBIC ACID FORMULATION

DMCTC-Aluminum-Calcium Gluconate Complex in 56% Propylene Glycol, pH[b] 8.5	Hr. After Injection[d] 1	4	7	24	AUC, mcg./Hr./ml.	95% Confidence Limits
Molar ratio 1:3:1:6	51.7	29.7	18.2	2.67	407.4	345.5–451.4
Molar ratio 1:4:1:12	48.0	28.8	18.2	1.98	381.7	323.7–450.0
Molar ratio 1:4:2:9	57.0	36.0	22.0	4.65	492.0	417.3–580.1
DMCTC·HCl-ascorbic acid formulation[c]	7.95	7.65	6.80	1.54	117.7	99.8–138.8

[a] Based on microbiological assays expressed as micrograms per milliliter tetracycline·HCl. Average of three dogs per group. [b] pH adjusted with monoethanolamine after addition of 0.5% sodium formaldehyde sulfoxylate. [c] DMCTC·HCl, 960 mg.; ascorbic acid, 2.40 Gm. (natural pH in distilled water, 2.2 to 2.4). [d] Dose = 4 mg. DMCTC·HCl/lb. body weight.

TABLE IV.—STABILITY DATA FOR AQUEOUS PROPYLENE GLYCOL FORMULATIONS

DMCTC-Aluminum-Calcium-Gluconate (1:3:1:6) in 80% Propylene Glycol[a]	mg. DMCTC·HCl/ml. (Microbio Assay)	% of Initial		TC-Aluminum-Calcium-Gluconate (1:3:1:6) in 70% Propylene Glycol[a]	mg. TC·HCl/ml. (Microbio Assay)	% of Initial
Theoret.	20.0	...	Theoret.		35.0	...
Initial	20.8	100	Initial		38.2	100
37°, 1 wk.	20.1	97	42°, 2 mo.		34.3	90
37°, 2 wk.	20.0	96	23°, 2 mo.		37.7	99
37°, 3 wk.	21.2	102	23°, 4 mo.		35.6	93
37°, 5 wk.	19.9	96	23°, 8 mo.		36.3	95
37°, 6 wk.	18.9	91	23°, 12 mo.		36.5	95
37°, 7 wk.	20.4	98	23°, 18 mo.		38.6	101

[a] pH 8.5, adjusted with monoethanolamine after addition of 0.5% sodium formaldehyde sulfoxylate.

col formulation resulted in significantly higher blood levels than those obtained with the DMCTC·HCl-ascorbic acid formulation. Although the molar ratios of the DMCTC-aluminum-calcium-gluconate complexes varied somewhat, these differences were not sufficient to result in blood levels significantly different. Blood level studies after intramuscular administration of DMCTC complexes having the same molar ratios used in this study also did not show blood levels significantly different. Within the range studied, the molar ratio of the complex did not exert a significant effect on blood level by either intravenous or intramuscular routes of administration.

There were no gross signs of irritation following intravenous administration in the four formulations used.

Stability Studies.—Ampuls held at various temperatures and for various periods were submitted at regular intervals for microbiological assays. Table IV presents the results of stability studies on DMCTC and TC-aluminum-calcium gluconate complexes in the aqueous propylene glycol formulation. These data indicate that the formulations are stable

with respect to microbiological potency under the conditions studied.

Studies on modifications of the aqueous propylene glycol formulation indicate that pH and propylene glycol content are critical factors. If the propylene glycol content is at a level of 50% or greater at pH 8.0–8.5, there is little or no loss of microbiological potency after 9 weeks at 37°. If the pH ranges from 8.0 to 8.5, the formulation retains its potency after 5 weeks at 37°. Below this pH range, the formulation slowly loses activity, presumably through C.4 epimerization, the kinetics of which have recently been described (4).

SUMMARY

Stability studies indicated that stable preconstituted formulations suitable for parenteral administration can be prepared with DMCTC and TC-aluminum-calcium-gluconate complexes in 50–80% propylene glycol at pH 8.0–8.5. Both pH and propylene glycol content are important factors affecting the stability of the formulation. The degree of blood-level enhancement is not altered significantly by the percentage of propylene glycol in the formulation or by the molar ratios of the complexes within the range studied following intravenous and intramuscular administration. By either route, the DMCTC complexes in the aqueous propylene glycol formulation appeared to be well tolerated in dogs when administered at therapeutic levels.

REFERENCES

(1) Remmers, E. G., *et al.*, THIS JOURNAL, 53, 1452(1964).
(2) Remmers, E. G., *et al.*, *ibid.*, 51, 86(1962).
(3) Dearborn, E. H., *et al.*, *Antibiot. Med. Clin. Therapy*, 4, 627(1957).
(4) Remmers, E. G., Sieger, G. M., and Doerschuk, A. P., THIS JOURNAL, 52, 752(1963).

Some Pharmacological Properties of Polymyxin B Sulfate

By DARRELL L. WITT and J. P. LONG

Polymyxin B sulfate produces diastolic standstill followed by contracture in the isolated perfused rabbit heart. No increase in cardiac output was noted in the perfused frog heart. Large doses inhibit splanchnic nerve innervation to the perfused superior mesenteric artery of the cat.

SYSTEMIC ADMINISTRATION of polymyxin B sulfate has a potent bactericidal effect on Gram-negative bacilli, particularly *Pseudomonas aeruginosa*. However, because of nephrotoxic and central nervous system effects, such as vertigo and paresthesias, it has generally been limited to topical application. Although it is generally realized that the systemic use of this antibiotic is hazardous, reports in the literature describe its use in this manner (1–4). Timmerman *et al.* (5) have demonstrated the neuromuscular blocking action of polymyxin in the rabbit.

The present work describes the effect of polymyxin B on the isolated rabbit heart, on cardiac output measured by the modified Howell and Clark (6) frog heart preparation, and on the vascular resistance of the perfused superior mesenteric artery of the cat.

METHODS

The effect of polymyxin B sulfate on the perfused isolated rabbit heart was studied using the preparation described by Langendorf (7). Dutch rabbits weighing 2–3 Kg. were sacrificed by a blow at the base of the skull, and the heart was removed *via* a midline chest incision. A glass cannula, with side arm tube, was placed in the aorta, and the coronary vessels were perfused with oxygenated Locke-Ringer's solution previously warmed to 37°. All drugs were administered through the side arm tube of the glass cannula. Isotonic recordings were made using an ink recorder on kymograph paper. The action of potassium chloride, calcium chloride, and magnesium chloride on the isolated rabbit heart was evaluated before and after the administration of polymyxin B sulfate. The results are expressed as per cent changes of the systolic contraction.

The effect of polymyxin B sulfate on cardiac output in the frog *in situ* was evaluated by a modification of the Howell and Clark technique (6). *Rana pipiens*, weighing 200–250 Gm., were double pithed and the hearts exposed. After ligation of the right aortic arch cannulae were placed in the inferior vena cava and cardiac output measured by collecting the perfusate in graduated cylinders for 1-minute periods. The heart rate was also recorded.

The action of polymyxin B sulfate on the perfusion of the cat gut was evaluated as follows. Anesthesia was induced by pentobarbital sodium (35 mg./Kg.), and a tracheotomy was performed. For intravenous administration of drugs, the inferior vena cava was cannulated below the renal vein. Heparin sodium (5 mg./Kg.) was administered intravenously. The abdominal aorta was cannulated with Tygon tubing and a Sigmamotor pump (model T-8) maintained a constant volume flow into the superior mesenteric artery. To measure changes in perfusion pressure, a pressure transducer was connected between the pump and the artery. The superior mesenteric plexus was isolated, and bipolar silver electrodes were placed on these nerves. Supramaximal stimulation with a frequency of 20/second for 30 seconds was employed. Systemic blood pressure was measured by cannulation of the common carotid and recorded using an Offner dynograph (type 542). The effect of intra-arterial doses of polymyxin B sulfate varying from 1 to 50 mg. (total dose) was evaluated in this preparation.

The data were evaluated statistically using a Student *t* test (8).

Received June 18, 1964, from the Department of Pharmacology, College of Medicine, State University of Iowa, Iowa City.
Accepted for publication July 16, 1964.
This investigation was supported in part by grant RG-B-1396 from the U. S. Public Health Service, Bethesda, Md.