Antimicrobial Activity of Chlormerodrin

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Abstract □ A radiochemical study for antimicrobial activity of chlormerodrin was performed using some commonly occurring nonpathogenic and pathogenic microorganisms. Chlormerodrin concentrations of 15–35 and 20–45 μg/ml of the culture medium were microbiostatic and microbicidal, respectively. The microbiostatic effect was reversible in the presence of cysteine, an amino acid containing a sulfhydryl group.

Keyphrases □ Chlormerodrin—antimicrobial activity evaluated *in vitro* □ Antimicrobial activity—chlormerodrin evaluated *in vitro* □ Diuretics—chlormerodrin, antimicrobial activity evaluated *in vitro*

Chlormerodrin, an organomercurial compound, is classified as a diuretic and not as an antimicrobial agent like phenylmercuric nitrate and thimerosal (1).

When sterility testing is performed on solutions containing organomercurials such as thimerosal and phenylmercuric salts (0.01 and 0.002%, respectively) as preservatives, the inoculum is recommended to be diluted at least 10-fold with fluid thioglycolate medium (I), even though 5 ml of this medium can inactivate the mercurial preservatives in 1 ml of the solution (2). Medium I contains 0.03% (v/v) thioglycolic acid to neutralize the organomercurials.

This paper reports a study of the degree of antimicrobial activity of chlormerodrin using a variety of microorganisms and cysteine as a binding agent. To evaluate the microbiostatic and microbicidal doses of chlormerodrin, a radiochemical sensitivity test (3–5) against some commonly occurring nonpathogenic and pathogenic microorganisms was performed.

EXPERIMENTAL

Apparatus—A semiautomatic radioactive detection system[1] measured, in terms of the growth index reading (1 division = 0.25 μCi), $^{14}CO_2$ released from ^{14}C-glucose metabolized by microorganisms.

Preparation of Double-Strength Glucose-Free Medium with ^{14}C-Glucose—The medium consisted of 3.4% Bacto Tryptone[2], 0.6% Bacto Soytone[2], 1% NaCl, 0.5% K_2HPO_4, and 100 μCi of *U*-^{14}C-glucose[3] (specific activity of 1.39 μCi/mg)/liter. Aliquots of 10 ml of the medium were distributed into 50-ml culture vials, and the vials were autoclaved at 1.05 kg/cm² for 20 min.

Table I—Microbiostatic and Microbicidal Effects of Chlormerodrin

Test Culture	Hours of Incubation	Growth Index Reading at Chlormerodrin Concentration, μg/ml of medium							MMID, μg/ml	MLD, μg/ml
		Nil	10	15	20	25	30	35		
E. coli	24	100	35	15	10	No growth			25	35
	48	100	80	40	50					
	72	100	100	100	100					
	96	100	100	100	100					
S. typhosa	24	100	38	30	25	25	20	No growth	35	45
	48	100	85	80	80	83	80			
	72	100	100	100	100	100	100			
	96	100	100	100	100	100	100			
S. aureus	24	100	40	45	—	No growth			25	30
	48	100	90	50	15					
	72	100	100	100	40					
	96	100	100	100	70					
B. subtilis	24	100	40	40	35	30	No growth		25	25
	48	100	95	70	55	50				
	72	100	100	100	100	100				
	96	100	100	100	100	100				
B. cereus	24	100	35	30	25	No growth			25	40
	48	100	100	90	70					
	72	100	100	100	100					
	96	100	100	100	100					
Cl. sporogenes	24	100	35	30	35	25	25	No growth	35	40
	48	100	100	70	65	70	50			
	72	100	100	100	100	100	100			
	96	100	100	100	100	100	100			
C. albicans	24	100	40	40	35	20	20	No growth	35	40
	48	100	100	80	60	45	48			
	72	100	100	100	100	100	100			
	96	100	100	100	100	100	100			
Flavobacterium sp.	24	100	25	No growth					15	20
	48	100	60							
	72	100	80							
	96	100	100							

Sample Preparation—Chlormerodrin[4] (125 mg) was dissolved in 250 ml of freshly distilled water and sterilized by filtration[5] (pore size of 0.45 μm).

L-(−)-Cysteine[6] (1 g) was dissolved in 50 ml of freshly distilled water and sterilized by autoclaving at 1.05 kg/cm² for 15 min.

Preparation of Test Cultures—These cultures were standardized cell suspensions containing 3 × 10⁴ viable cells/ml and were prepared from a 24-hr-old culture of *Escherichia coli* (ATCC 9637), *Staphylococcus aureus* (ATCC 9144), *Bacillus cereus* (C. 1448), *Bacillus subtilis*

[1] Bactec-301, Johnston Laboratories, Cockeysville, Md.
[2] Difco Laboratories, Detroit, Mich.
[3] Isotope Division, BARC, Bombay, India.
[4] Neohydrin, Lakeside Laboratories, Milwaukee, Wis.
[5] Millipore Corp., Bedford, Mass.
[6] E. Merck, Darmstadt, West Germany.

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Table II—Influence of Cysteine on the Antimicrobial Effect of Chlormerodrin

	Vial					
	1	2	3	4	5	6
Volume of double-strength medium, ml	10	10	10	10	10	10
Chlormerodrin solution, ml	2.4	2.4	1.2	1.2	1.2	1.2
Test column of *E. coli* cell, ml	0.2	0.2	0.1	0.1	0.1	0.1
Mixed well and allowed to stand at room temperature for 4 hr						
Cysteine solution, ml	0.0	3.0	0.0	0.5	1.0	3.0
Sterile water, ml	7.4	4.4	8.7	8.2	7.7	5.7
Mixed well and incubated at 30–34°						
Incubation period, hr	Growth index reading					
24	Nil	Nil	Nil	Nil	Nil	70
48	Nil	Nil	Nil	Nil	25	90
72	Nil	Nil	Nil	10	60	100
96	Nil	Nil	Nil	35	100	100

(ATCC 6633), *Clostridium sporogenes* (ATCC 19404), *Salmonella typhosa*, *Candida albicans*, and *Flavobacterium* sp. isolated and identified according to a literature method (6).

Antimicrobial Activity of Chlormerodrin—All operations were carried out on a laminar flow bench. Twelve culture vials, each containing 10 ml of double-strength medium, were arranged in duplicate for each test organism. Then 0.4, 0.6, 0.8, . . . , 2.2, and 2.4 ml of chlormerodrin solution were added to the vials serially marked 2–12. Sterile water was added to each vial to a volume of 20 ml so that the concentration of ^{14}C-glucose was 0.05 μCi/ml of the culture medium.

The test cultures, 50 μl, were added to each vial, and the vials were capped and incubated at 30–34°. The growth index reading was measured at the end of every 24 hr for 4 days. After the last observation, a 0.1-ml aliquot from each culture vial (showing no growth index reading) was transferred to soybean–casein digest medium (15 ml) and incubated for 5 days at 30–34° to confirm the microbiostatic and microbicidal doses of chlormerodrin for each test culture (Table I).

Antimicrobial Activity of Chlormerodrin in Presence of Cysteine—Six culture vials, each containing 10 ml of double-strength medium, were taken. Chlormerodrin solution was added to the vials as follows: 2.4 ml to vials 1 and 2 and 1.2 ml to vials 3–6. Then 0.1–0.2 ml of test culture of *E. coli* was added to each of the six vials (Table II). The vials stood at room temperature for 4 hr to allow sufficient time for inactivation of the microbial cells.

Vials 1 and 3 were kept aside as controls. To vials 2, 4, 5, and 6, 3.0, 0.5, 1.0, and 3.0 ml of cysteine solution were added, respectively. The mixture was diluted to 20 ml with sterile distilled water and then incubated at 30–34°. The growth index reading was measured at the end of every 24 hr for 4 days (Table II).

RESULTS AND DISCUSSION

Preliminary tests for sterility on aqueous solutions of chlormerodrin showed that visible growth did not occur in the control tube containing 1–2.5 mg of chlormerodrin in 15 ml of fluid thioglycolate and soybean–casein digest media (7) with *B. subtilis* or *C. albicans* as the challenging microorganism, even after 10 days of incubation. Therefore, eight different microorganisms were tested for their sensitivity to chlormerodrin.

The microbiostatic or minimum metabolic inhibition dose (MMID), *i.e.*, the minimum dose at which there is no growth index reading but growth occurs on subculturing, is in the range of 15–30 μg/ml of the culture medium. The microbicidal dose or minimum lethal dose (MLD), *i.e.*, the minimum dose at which there is neither a growth index reading nor growth on subculturing, is in the range of 20–45 μg/ml (Table I). The microbiostatic effect was reversed by the addition of cysteine (0.3% w/v final concentration) but not the microbicidal effect (Table II). This result indicates that the microbiostatic and microbicidal effects of chlormerodrin, like other organomercurials, may result from its strong binding capacity with the sulfhydryl group of microbial enzymes involved in the cellular metabolic cycle.

The chlormerodrin concentration in chlormerodrin Hg 203 injection may sometimes be in the range of 1–2.5 mg/ml, which would be 20–125 times higher than the microbicidal dose. Hence, the chances of survival of viable cells are almost negligible in the radiopharmaceutical preparation. If 1 ml of such a solution is diluted to 20 ml as described earlier, the final concentration of chlormerodrin becomes 50–150 μg/ml, which would still be 1–8 times higher than the microbicidal dose. If any surviving microorganisms are in a "shock-state," the chances of their growth would be very poor.

In view of this finding, the sterility test on this radiopharmaceutical preparation should be carried out either by dilution of the injection with sufficient sterility test medium to bring the final concentration in the medium to not more than 10 μg/ml, which is below the range of its microbiostatic effect, or by aseptic filtration through a bacteria-retaining filter and then performance of the sterility test on the filter.

REFERENCES

(1) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, p. 622.

(2) "Difco Manual," 9th ed., Difco Laboratories, Detroit, Mich., 1971, pp. 196, 197.

(3) F. H. Deland and H. N. Wagner, Jr., *J. Lab. Clin. Med.*, **75**, 529 (1970).

(4) F. H. Deland and H. N. Wagner, Jr., *Radiology*, **92**, 154 (1969).

(5) F. H. Deland, *J. Nucl. Med.*, **13**, 424 (1972).

(6) R. S. Breed, E. G. D. Murray, and N. R. Smith, "Bergey's Manual of Determinative Bacteriology," Williams & Wilkins, Baltimore, Md., 1957.

(7) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, pp. 592, 593.

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