Effects of 1,8-Dihydroxyanthraquinone on Different Segments of Rabbit Gastrointestinal Tract

THEODORE H. EICKHOLT, NOLAN D. COURVILLE*, LAWRENCE J. DANNA*, LYNN J. VERZWYVELT*, and GERALD P. WAGUESPACK*

Abstract □ Using an isolated muscle bath technique, five different segments of the rabbit gastrointestinal tract (duodenum, jejunum, ileum, ascending and descending colon) were used to record the effects of two doses of 1,8-dihydroxyanthraquinone (danthron). Transducer-recorded tracings were made of contractions per minute, interval between contractions, and amplitude of contractions. The results were evaluated and statistical tests among comparisons of the three parameters between the two drug doses and control values indicated the parameter of interval between contractions to be a statistically significant indication of activity.

Keyphrases □ 1,8-Dihydroxyanthraquinone effect—gastrointestinal segments □ Gastrointestinal segments, rabbit—1,8-dihydroxyanthraquinone effect □ Intestinal contractions—rate, interval, amplitude

Many reports on the analysis, standardization, and mode of action of senna, sennosides, and anthraquinones are in the literature (1–3). Cathartic pharmacology has been the subject of reviews and has involved the parasympathetic system, however, the basic mechanisms of peristaltic activity remain undefined (4–6).

Descriptions of clinical testing techniques, indications, contraindications, and side effects of cathartics are readily available (7–11).

Likewise, many different animal techniques have been reported along with untoward effects of cathartics in animals (7, 12). Most animals have been utilized in cathartic testing; the mouse bioassay and the mouse Fühner cathartic testing techniques are quite commonly used (13, 14). The monkey has been reported to be the most appropriate animal for *in vivo* cathartic testing (15, 16).

Although it has been stated that the rabbit is refractive to anthraquinone cathartics (12) and the value of the *in vitro* intestinal segment testing technique of cathartics has been questioned (15), it was the purpose of this study to observe anthraquinone effects on different segments of rabbit gastrointestinal tract and statistically compare these with previously delineated standards (17) in an attempt to develop a technique for drug-effect studies. The most appropriate parameter of measurement and the preferential intestinal segment of the drugs effect were also objectives.

EXPERIMENTAL

Adult, albino rabbits were anesthetized with ether, and 2–3-cm segments of duodenum, jejunum, ileum, ascending and descending colon were expediently removed. The anesthesia was accomplished in the manner used in the previously delineated standards (17) in order to make conditions as identical as possible. As light a stage of surgical anesthesia as possible was used. The segments were individually maintained in oxygenated Tyrode's solution in the constant-temperature muscle bath at 38° prior to their actual placement in the isolated muscle bath chambers. The Tyrode's solution contained sodium chloride 0.8%, potassium chloride 0.02%, calcium chloride 0.02%, magnesium chloride 0.01%, sodium bicarbonate 0.1%, sodium diphosphate 0.005%, and glucose 0.1% in glass-distilled water.

Tyrode's solution (15 ml.) was used to bathe the segment in the isolated muscle bath chamber; temperature was maintained at 38° and the preparation was oxygenated using an air flow slowly (2–3 bubbles/sec.) bubbled through the solution. The segment was attached to two muscle hooks, one stationary and one connected to a lever and a transducer (E & M Isotonic Myograph) so that the segment was completely submerged in the Tyrode's solution.

The intestinal activity was recorded on a physiograph (E & M). A timer was an integral part of the recorder and paper speed was 0.05 cm./sec. The only constant tension placed upon the segment was that of the weight of the movable armature (2.2g.) and of the lever which in turn was centered on its fulcrum and raised only until the attachment *via* muscle hooks was tight enough to transfer the segment's movements to the transducer and recorder. Minimal stretching on relaxation was attempted to best approximate normal conditions.

The myograph had amplitude and calibration controls and all pen deflections were equilibrated to the standard pen deflection displacements.

The segments were allowed to acclimate to the muscle bath environment as in the previous study. Acclimation was determined by consistency and/or rhythmicity of contractions and was accomplished within 20 min. or the segment was replaced. The light ether anesthesia did not appear to adversely affect acclimation and it was known from previous studies that once acclimation had been obtained it would continue for at least an hour with no apparent change. The preparation appeared quite stable.

Following acclimation, normal intestinal activity for the different intestinal segments was recorded. Six different rabbits were used to

Table I—Summary of Averages and Standard Deviations of Measurements of Rabbit Intestinal Activity Obtained with 15 mg. 1,8-Dihydroxyanthraquinone

	No. of Measurements	Contractions/min.	Interval Between Contractions, sec.	Amplitude of Contractions, mm.
Duodenum	18	14.4 ± 1.05	1.9 ± 0.3[a]	0.73 ± 0.36
Jejunum	18	12.4 ± 1.65	2.5 ± 0.4[a]	1.05 ± 0.46[a]
Ileum	18	9.8 ± 2.04	3.2 ± 0.6[a]	1.52 ± 1.35[a, b]
A. Colon	18	8.1 ± 2.75[a]	4.5 ± 2.5[a]	4.62 ± 5.79
D. Colon	18	7.2 ± 1.54	4.4 ± 0.9[a]	1.51 ± 1.63

[a] Significantly different from normal averages at p 0.05. [b] Significantly different from 30-mg. dose average at p 0.05.

Table II—Summary of Averages and Standard Deviations of Measurements of Rabbit Intestinal Activity Obtained with 30 mg. 1,8-Dihydroxyanthraquinone

	No. of Measurements	Contractions/min.	Interval Between Contractions, sec.	Amplitude of Contractions, mm.
Duodenum	18	15.2 ± 1.41	1.9 ± 0.2[a]	2.84 ± 1.92
Jejunum	18	13.4 ± 0.90	2.3 ± 0.2[a]	2.87 ± 2.44
Ileum	18	10.3 ± 1.10	2.7 ± 1.0[a]	7.04 ± 2.06[b]
A. Colon	18	7.7 ± 1.55[a]	3.5 ± 0.9[a]	0.18 ± 0.13
D. Colon	18	6.4 ± 1.69	4.3 ± 1.2[a]	0.35 ± 0.22

[a] Significantly different from normal averages at p 0.05. [b] Significantly different from 15-mg. dose average at p 0.05.

provide six different samples of duodenum, jejunum, ileum, ascending and descending colon for each of the two doses of the anthraquinone.

The anthraquinone was 1,8-dihydroxyanthraquinone (Matheson, Coleman & Bell, DX1440). The drug was dissolved in Tyrode's solution so as to provide 0.1 and 0.2% concentrations providing 15 and 30 mg./15 ml. in the isolated muscle bath chamber, respectively, for the low and high doses. Larger quantities of the drug were limited due to its poor solubility and there was a lack of measurable effects at lower doses.

After normal activity had been established and recorded, the surrounding Tyrode's solution was drained from the muscle chamber and quickly replaced with the drug containing Tyrode's solution at one dosage and the effects recorded for a minimum of 15 min. to provide recordings from which the three separate measurements on each parameter were made. This was done for each of the six different samples of the five different segments and was completely repeated at the second dosage for six different samples of the five different segments.

From each recording of each individual gut segment, amplitude of contractions, interval between contractions, and contractions per minute were measured. This procedure was identical to that reported earlier so as to be able to detect any quantitative changes in the type of contraction due to changes in strength or force (17). Speed of contraction and relaxation could change with no change in frequency. Three separate measurements of the three parameters above were made on each tracing providing an average of the three for that particular parameter of that single gut segment. These averages were in turn averaged for the six samples of each individual gut segment providing 18 actual measurements for the averages of the three parameters.

Standard deviations were calculated; analysis of variance with F test and Scheffe's S method tests were made between combinations of treated and control intestinal segments.

RESULTS

Table I summarizes the averages and standard deviations of the amplitude of contractions, interval between contractions, and contractions per minute obtained with the low dose (15 mg.) of 1,8-dihydroxyanthraquinone.

Table II summarizes the averages and standard deviations of the amplitude of contractions, interval between contractions, and contractions per minute obtained with the high dose (30 mg.) of 1,8-dihydroxyanthraquinone.

Table III summarizes the averages and standard deviations of the amplitude of contractions, interval between contractions, and contractions per minute obtained with the normal untreated intestinal segments.

Of the 45 possible comparisons of the three parameters between drug-treated and normal averages, 15 were significantly different statistically at p 0.05.

All ten comparisons of the interval between contractions between both drug treatments and normal averages were significantly different statistically at the same probability level. Only the ileum *versus* ileum amplitude of contractions comparison between the 15 and 30-mg. doses was significantly different at this level. The remaining four significant comparisons were jejunum *versus* jejunum and ileum *versus* ileum amplitude of contractions comparisons between the 15-mg. doses and normal averages and the A. colon *versus* A. colon contractions per minute comparisons between both drug treatments and normal averages.

SUMMARY AND CONCLUSIONS

Five different segments of the rabbit intestinal tract, duodenum, jejunum, ileum, ascending and descending colon, were utilized in recording the activity of these segments in response to 15 and 30-mg. doses of 1,8-dihydroxyanthraquinone using an *in vitro* isolated muscle bath technique.

Contractions per minute, interval between contractions, and amplitude of contractions were recorded, determined, averaged, standard deviations calculated, and analysis of variance with F test and Scheffe's S Method tests performed on segment combinations between the drug-treated and normal segments.

Of the 45 comparisons of the three parameters between drug-treated and normal averages, 15 were significantly different statistically at p 0.05 and included all comparisons of interval between contractions between both drug treatments and normal averages.

No segment was consistently involved in all cases. Only the low dose *versus* the normal averages for the jejunum and ileum comparisons of amplitude of contractions were statistically different. No statistically significant difference could be obtained for effects on adjacent segments.

Comparing the actual figures (averages) of the three parameters for the five segments of the high dose and the normal averages, 5/5 values for interval between contractions for the high dose were less than the normal figures and all showed statistically significant differences.

Comparing the actual values of the three parameters for the five segments of the low dose and the normal averages, 5/5 values for interval between contractions for the low dose were less than the normal figures and showed again statistically significant differences.

The results from this *in vitro* study and their statistical comparisons show the parameter of interval between contractions to be a statistically significant measurement of activity. This is in contrast to the original observation indicating statistically significant differ-

Table III—Summary of Averages and Standard Deviations of Measurements of Normal, Untreated Rabbit Intestinal Activity

	No. of Measurements	Contractions/min.	Interval Between Contractions, sec.	Amplitude of Contractions, mm.
Duodenum	21	16.7 ± 1.68	3.9 ± 0.1	2.56 ± 2.50
Jejunum	21	12.4 ± 2.31	5.0 ± 1.5	6.53 ± 3.46
Ileum	21	10.5 ± 1.27	6.2 ± 1.1	9.17 ± 0.68
A. Colon	15	2.1 ± 0.83	15.0 ± 8.2	1.68 ± 0.57
D. Colon	15	4.3 ± 1.68	13.4 ± 5.0	3.91 ± 2.38

ences for all comparisons of contractions per minute and its potential in intestinal studies (17). The effect upon interval between contractions could be explained on the basis of increased contractions per minute along with the observed decrease in amplitude of contractions. This would partially agree with earlier statements indicating strength, force, or possibly tone, but not amplitude of the contractions was increased (5).

Comparisons of activity between the high and low doses on the three parameters indicate that the high dose is more effective but with only the ileum comparisons of amplitude of contractions statistically significant and with no other segment specifically indicating a preferential effect.

REFERENCES

(1) J. W. Fairbairn, *Planta Med.*, **12**, 260(1964).

(2) J. A. Rider and H. C. Moeller, *Clin. Med.*, **72**, 1645(1965).

(3) A. C. Greiner and W. E. Warwick, *Appl. Therap.*, **7**, 1096 (1965).

(4) R. S. Mechling, *Diseases Colon Rectum*, **1**, 356(1958).

(5) G. Vallette and H. Leboeuf, *Ann. Pharm. Franc.*, **5**, 89(1947).

(6) J. W. Fairbairn, *Pharm. Weekblad*, **100**, 1493(1965).

(7) "Animal and Clinical Pharmacologic Techniques in Drug Evaluations," J. H. Nodine and P. E. Siegler, Eds., Year Book Medical Publishers, Chicago, Ill., 1964.

(8) E. S. McCabe, *Penn. Med. J.*, **62**, 1662(1959).

(9) B. Schlegel, *Klin. Wochschr.*, **32**, 557(1954).

(10) A. G. MacGregor, *Brit. Med. J.*, **5183**, 1422(1960).

(11) M. F. Kossover, M. E. Beckham, and S. A. Threefoot, *Am. J. Med. Sci.*, **247**, 694(1964).

(12) T. Sollmann, "A Manual of Pharmacology and Its Applications to Therapeutics and Toxicology," 8th ed., Saunders, Philadelphia, Pa., 1957.

(13) R. T. Brittain, P. F. D'Arcy, and J. J. Grimshaw, *J. Pharm. Pharmacol.*, **14**, 715 (1962).

(14) "Modern Pharmacognosy," E. Ramstad, McGraw-Hill, New York, N. Y., 1959.

(15) M. H. Hubacher, S. Doernberg, and A. Horner, *J. Am. Pharm. Assoc., Sci. Ed.*, **42**, 23(1953).

(16) M. H. Hubacher and S. Doernberg, *J. Pharm. Sci.*, **53**, 1067 (1964).

(17) T. H. Eickholt, R. H. Box, and N. D. Courville, *ibid.*, **56**, 1328(1967).

ACKNOWLEDGMENTS AND ADDRESSES

Received July 18, 1968 from the *Department of Pharmacology and Toxicology, School of Pharmacy, Northeast Louisiana State College, Monroe, LA 71201*

Accepted for publication June 5, 1969.

The authors wish to acknowledge Dr. L. T. Bennett and Dr. H. L. Dockery, Director, Academic Computer Center, for their assistance with the statistical analyses.

* Special Problems undergraduate student in pharmacology.

Reactivity of the Hydroxyl Groups in Selected Derivatives of Lincomycin

W. E. HAMLIN

Abstract□ Reactivity of the hydroxyl groups in tris-2,3,4-*O*-trimethylsilyl lincomycin [$(TMS)_3$-L], lincomycin-2,7-diacetate·HCl (L-Ac_2) and 7-*O*-trityl-3,4-*O*-anisylidene lincomycin (TAL) as measured by the rate of acylation with valeric anhydride at room temperature was found to be in the order of 3-OH > 2-OH > 7-OH > 4-OH with estimated half-lives of 8, 32 and 160 min., and 23 hr., respectively. Gas chromatographic procedures were utilized to monitor the acylation of $(TMS)_3$-L and L-Ac_2. In the latter reaction silylation was necessary for measurement of unreacted starting material and the intermediate monovalerate. The acylation of TAL was monitored polarimetrically.

Keyphrases □ Lincomycin derivatives—hydroxyl groups, reactivity □ Acylation rate—lincomycin derivatives □ Polarimetry—reaction monitoring □ GLC—reaction monitoring

A consideration of a molecular model of lincomycin indicates that the steric and electronic environment of the hydroxyl groups at Positions 2, 3, 4, and 7 (Fig. 1) would differ and produce differences in reactivity. A knowledge of the relative reactivity of these hydroxyl groups can be useful in the synthesis of lincomycin derivatives. In order to obtain the desired information, the rates of acylation of tris-2,3,4-*O*-trimethylsilyl lincomycin, lincomycin-2,7-diacetate HCl, and 7-*O*-trityl-3,4-*O*-anisylidene lincomycin by valeric anhydride in pyridine were determined at room temperature. The acylating agent was used in such excess (20:1 mole ratio) that a pseudo-first-order reaction would apply.

EXPERIMENTAL

Acylation of Tris-O-2,3,4-trimethylsilyl Lincomycin [$(TMS)_3$-L]—This was an adaptation of a procedure for preparing lincomycin-7-acylates (1). To a solution of 1.428 g. of $(TMS)_3$-L (0.0025 mole) in 10 ml. of dry pyridine was added 9.31 g. (0.05 mole) of valeric anhydride (VA) at time zero. The solution was thoroughly mixed after adjusting to 25 ml. with dry pyridine and stored at room temperature. Sample aliquots (2 μl.) were removed by syringe and injected into the gas chromatograph at designated time intervals.

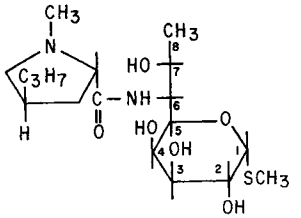

Figure 1—*Structure of lincomycin.*