Desoxycholic Acid Enhancement of Orally Administered Reserpine

By MARVIN H. MALONE, HOWARD I. HOCHMAN*, and KARL A. NIEFORTH

Quantities of 1:2, 1:4, 1:8, 1:16, and 1:32 molar amounts of reserpine : desoxycholic acid were dissolved in alcohol and/or chloroform, chilled to −5°, and solvents removed under reduced pressure. When administered orally to mice, the presence of desoxycholic acid hastened blepharoptotic activity and increased blepharoptotic potency of reserpine. Maximum enhancement was observed with 1:16 reserpine–desoxycholic acid where 1 mg. of reserpine as the combination was equivalent to 3.8–2.5 mg. of reserpine base using observations 2 and 24 hr. after dosage, respectively. An equivalent physical mixture prepared by trituration was significantly more potent than reserpine base but much less potent than the prepared combination. Desoxycholic acid alone was without blepharoptotic activity.

THE ENHANCED absorption of various medicinal agents upon concomitant administration with desoxycholic acid may be attributed to its ability to reduce interfacial tension and/or to form inclusion or clathrate compounds. Previous work concerning this increased absorption usually has been restricted to the effect of desoxycholic acid on the absorption of the fat-soluble vitamins and closely related analogs where the effect was thought to be due to the surface action of desoxycholic acid. This report is concerned with an increase in the blepharoptotic activity of reserpine when given in intimate combination with desoxycholic acid.

The general role of bile salts in absorption, the ability of desoxycholic acid to form inclusion compounds (choleic acids), and the relative inefficiency of reserpine absorption prompted this study of the effects of combining reserpine and this acid prior to oral administration. Evidence of an interaction between reserpine and desoxycholic acid was reported by Lach and Pauli (1) after the completion of the work reported here. The interaction was demonstrated by an increase in the solubility of reserpine in hydroalcoholic solutions of desoxycholic acid. Simple interfacial effects of desoxycholic acid were not thought to be the sole contributing factor for this increased solubility, and it was suggested that the interaction might result from a combination of micellar solubilization and inclusion formation.

EXPERIMENTAL

Preparation of Reserpine–Desoxycholic Acid Combinations.—The usual method for the formation of desoxycholic acid inclusion compounds (dissolving both desoxycholic acid and the guest component in ethanol and allowing the inclusion compound to crystallize) was not used in this instance due to the poor solubility of reserpine in anhydrous alcohol.

Method A.—The calculated quantity of purified desoxycholic acid (Nutritional Biochemicals Corp., lot 2185) was dissolved in 5 ml. of commercial grade absolute ethanol in a 20-ml. round-bottom flask. The desired amount of reserpine (C grade, Calbiochem, lot 502858) was added and dissolved with slight warming. The flask and its contents were chilled in an ice–salt bath to −5° and the solvent removed under reduced pressure and trapped in a cold finger cooled with a dry ice–acetone bath. The contents of the flask were protected from light as completely as possible. The residues were dried for 12 hr. in a vacuum to remove the last trace of alcohol.

Method B.—This method was essentially the same as above except that 5 ml. of chloroform was used to dissolve the reserpine, and this solution was added to the desoxycholic acid dissolved in 10 ml. of absolute alcohol. The resultant solution was then treated as described under *Method A*.

Method C.—A physical mixture of desoxycholic acid and reserpine was prepared by intimately triturating the two dry powders without previous dissolution in any solvent. Table I summarizes the physical properties of all the test mixtures.

Blepharoptotic Assay.—White mice were obtained from E. G. Steinhilber, Oshkosh, Wis., and maintained in this laboratory on Purina laboratory chow and water *ad libitum* for at least 4 days prior to test. All animals were taken off food 10 hr. prior to dosing and placed back on food after recording the +6 hr. observations. Free access to water was allowed throughout the entire test period. The mice were dosed orally using precision grade syringes and cut-off, blunted, polished 20-gauge hypodermic needles. A constant dosage volume of 30 ml./Kg. was maintained for all injections using 0.25% aqueous agar as the dosing vehicle. Test drugs and combinations were suspended by trituration. All test solution suspensions were coded and the animals dosed in a random test pattern using a 3 × 3 assay format with further randomization between the two sexes of mice and the two dosing technicians. Prior to ptotic scoring, each mouse was manually aroused and placed on a screen facing the scorer at the scorer's eye level. Ptosis for each eye was rated following the scale used by Rubin *et al.* (2): 4 = complete, 3 = 3/4, 2 = 1/2, 1 = 1/4 closure of the eyelids. Nonptotic responses were scored as 0. All scores of 4 were checked to insure that the lid was not encrusted shut by eye secretions. The individual metameter was the sum of the ptotic scores for both eyes so that the graded test response could vary from 0–8 per animal.

RESULTS AND DISCUSSION

As shown in Table II, eight balanced log dose-response blepharoptotic assays were conducted assaying reserpine (in combination with desoxycholic acid) against reserpine base as a standard. For the assay calculations the reserpine content of

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* Present address: Department of Pharmacology, Yale University, New Haven, Conn.

TABLE I.—PREPARATION OF RESERPINE–DESOXYCHOLIC ACID AND RESERPINE PLUS DESOXYCHOLIC ACID TEST MATERIALS

Identification	Exact molar Ratios	Prepn., Method	Quantities, mg. Reserpine	Quantities, mg. Acid	Melting Range, ° C.
1:2	1:2.06	*B*	59.0	80.0	80–110
1:4	1:4.02	*B*	62.0	161.5	98–125
1:8	1:7.44	*A*	16.2	77.9	114–125
1:16	1:16.31	*A*	29.7	312.6	114–145
1:32	1:31.89	*A*	15.2	311.7	145–148
1 + 16	1 + 17.16	*C*	28.6	316.4	...

TABLE II.—APPARENT ORAL POTENCIES OF RESERPINE IN VARIOUS DESOXYCHOLIC ACID COMBINATIONS AS COMPARED TO RESERPINE BASE ALONE

Identification	hr. After Dosage 2	4	6	10	24
0:0	...	...	...	...	...
1:0	0.781 (0.536–1.138)[a]	1.000 (0.836–1.196)	0.957 (0.793–1.155)	0.928 (0.741–1.162)	1.078 (0.776–1.496)
1:2	2.293 (1.460–3.602)	1.924 (1.482–2.499)	1.658 (1.326–2.073)	1.587 (1.282–1.966)	2.144 (1.672–2.747)
1:4	2.642 (2.182–3.199)	2.542 (1.885–3.428)	2.428 (1.959–3.010)	2.2811 (1.859–2.799)	2.692 (2.135–3.394)
1:8	2.828 (1.858–4.305)	2.349 (1.607–3.433)	2.732 (1.739–4.291)	1.948 (1.393–2.724)	2.056 (1.084–3.901)
1:16	3.779 (2.870–4.974)	3.201 (2.221–4.615)	3.262 (2.645–4.022)	2.594 (2.072–3.247)	2.487 (1.767–3.499)
1:32	3.352 (2.403–4.674)	2.650 (2.075–3.384)	2.471 (1.930–3.165)	2.650 (2.074–3.386)	1.966[b] (1.143–3.382)
1 + 16	1.382 (0.990–1.928)	1.882 (1.393–2.542)	1.876 (1.355–2.596)	1.719 (1.157–2.553)	...[c]

[a] Range of figures within parentheses indicate the calculated 95% confidence limits for the potency. [b] Significant departure from parallelism (P = 0.01–0.05) in 3 × 3 assay; best graphical estimate of potency = 2.49 × reserpine base. [c] Recovery was sufficient so that neither statistical nor graphical estimates of potency could be determined with validity.

the combinations was handled using the "exact" ratios shown in Table I, although the approximate molar ratios are used in this text to identify the various combinations. Doses of 3, 6, and 12 mg./Kg. were used for the reserpine standard (C grade, Calbiochem, lot 502858) and for the reserpine of the 1:2, 1:4, and 1:8 reserpine–desoxycholic acid combinations. Doses of 1.5, 3, and 6 mg./Kg. and 0.75, 1.5, and 3 mg./Kg. were used for the reserpine of the 1:16 and 1:32 combinations, respectively. Where enhancement of the reserpine-like activity did not allow 3 × 3 calculations, a 2 × 2 assay was calculated matching two dosages of the standard with two doses of the combination in the same linear portion of the dose-response curve. Significant departure from parallelism was noted in only one calculation. The statistical treatment for the 3 × 3 (120 mice, 20 animals/dosage level) and 2 × 2 (80 mice) assays involved analysis of variance, factorial analysis, and calculation of potency and its 95% confidence limits using the techniques of Bliss and Calhoun (3). An average λ value (s/b) of 0.27 was obtained for the 32 valid assay calculations reported in Table II, which value agrees well with a λ of 0.29 reported earlier for this assay technique (4) using a different mouse stock, reserpine acetate as the standard, and water as the dosage vehicle.

As shown in Fig. 1, the 1:2 to 1:16 reserpine–desoxycholic acid combinations show a progressive increase in the apparent potency of reserpine with the 1:16 reserpine–desoxycholic acid combination

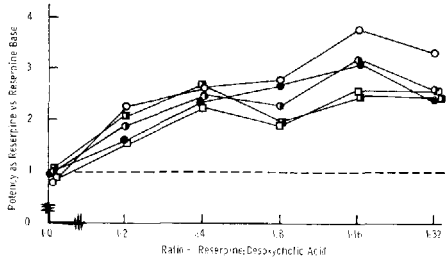

Fig. 1.—Variation of ptotic potency of reserpine when administered orally in the form of various molar ratio coprecipitates with desoxycholic acid. Key: ○, +2 hr.; ◑, +4 hr.; ●, +6 hr.; □, +10 hr.; ◧, +24 hr.

producing the maximum enhancement of activity. The decrease in activity shown by the 1:32 combination would appear to indicate that the maximum attainable activity (1:16) has been physically diluted by excess desoxycholic acid.

As illustrated in Fig. 2, the potency of 1:0 reserpine–desoxycholic acid does not significantly deviate from the theoretical value of 1.0, while maximum distortion of the apparent potency of the 1:2–1:32 combinations was apparent at +2 hr. after oral administration. This appears to indicate that desoxycholic acid increases the speed of absorp-

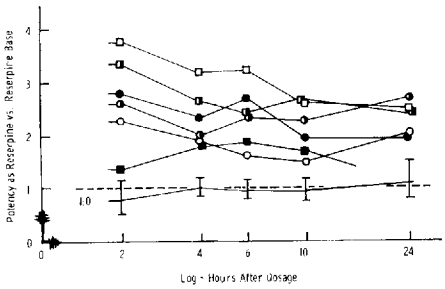

Fig. 2.—Variation with time of the oral potencies of various reserpine–desoxycholic acid molar coprecipitates as compared to reserpine base as a standard. Key: □, 1:16; ◨, 1:32; ●, 1:8; ◑, 1:4; ○, 1:2; ■, 1 + 16; I, 1:0.

tion of reserpine as well as increasing the potency. Considering the 95% confidence limits of potency reported in Table II, reserpine administered as the 1:16 combination behaves equivalent to at least 1.8 mg. of reserpine base (+24 hr.) and possibly equivalent to as much as 5.0 mg. of reserpine (+2 hr.).

A molar equivalent physical mixture of reserpine and desoxycholic acid (1 + 16) produced a slower onset of the period of maximum enhancement (+4–6 hr.), and the potencies were significantly less ($P \leq 0.001$) than those reported for the 1:16 intimate combination. In all cases where calculation was possible, the 1 + 16 mixture was significantly more potent than reserpine base alone (observed P: 0.025–0.05 at +2 hr. and ≤ 0.001 at +4, 6, and 10 hr.).

While there is debate as to whether palpebral ptosis is a peripheral or central manifestation of reserpine-like activity (4–6), this characteristic symptom does indicate absorption of reserpine from the gastrointestinal tract. Coprecipitates of reserpine and desoxycholic acid both increase the potency of reserpine and produce a more rapid onset of reserpine-like activity when administered orally. No attempt has been made here to define the exact physical/chemical nature of the reserpine–desoxycholic acid combination.

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Utilization of Hydrophilic Gums for the Control of Drug Release from Tablet Formulations I. Disintegration and Dissolution Behavior

By H. E. HUBER, L. B. DALE*, and G. L. CHRISTENSON

Tablet formulations and data to illustrate rate of drug dissolution and tablet volume decay during *in vitro* disintegration tests are presented. It was found that tablets prepared by compression of hydrophilic gums, excipients, and drug in specified ratios result in prolonged release of drug. Assay of simulated gastric and intestinal fluids from *in vitro* tests show the drug to be released at essentially a uniform rate after an initial hydration phase. The mechanism of prolonged release is proposed as a combination of drug diffusion from, and attrition of, a dynamically changing gel barrier at the tablet periphery.

ORAL CONTROLLED release dosage forms have been recognized as a therapeutically significant advance in dosage form design, whereby a more uniform and prolonged tissue concentration of drug substance may be achieved. The methods used for obtaining prolonged action have been reviewed by Ballard and Nelson (1) and Parrott (2) and various systems are described, whereby an initial therapeutic dose is released followed by a continual release of additional drug substance over a prolonged period of time.

In 1962, a system was developed by The Wm. S. Merrell Co. (3) describing a novel approach for the control of drug substance release rate from tablet formulations. The method described involves mixing a medicinal agent or agents with certain nondigestible, hydrophilic gums and compressing the mixture into tablets. When such a tablet is exposed to water or digestive fluids, a rapid release of drug substance from the dosage form to the dissolution medium is initially observed. However, hydration and gelation of gum at the tablet–liquid interface also occurs to form a viscous gel barrier. The remaining drug substance is then released at a much slower rate that apparently depends on diffusion from and/or attrition of the gel barrier. The nature of the phenomenon is illustrated by Fig. 1, which shows, in cross section, the appearance of such a tablet after exposure to solvent. It can be seen that the intact tablet core is surrounded by a gel barrier layer of significant size.

The present communication is concerned with studies that were conducted to obtain preliminary

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* Present address: 8607 Bradmoor Drive, Bethesda, Md.