Evaluation of Drug Formulations Using LD_{50} Testing in Mice

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Abstract □ The LD_{50} values were utilized to assess the relative rate of absorption of two very poorly soluble drugs. Formulations of these drugs were studied by micronization; addition of surfactant, alkaline or buffering agents, and/or bile salts; coprecipitation; melt or fusion techniques; or granulation with hydrophilic agents. Differences in toxicities were demonstrated from formulations compared to pure drugs by the LD_{50} method. This study shows that the LD_{50} is a practical, rapid method of achieving comparative evaluations of drug formulations.

Keyphrases □ Absorption, relative rate—poorly soluble drugs, various formulations evaluated using LD_{50} testing, mice □ Toxicity—poorly soluble drugs, various formulations evaluated using LD_{50} testing, mice □ LD_{50} testing—relative absorption rate and toxicity of various formulations of poorly soluble drugs evaluated, mice

The rate and extent of absorption of a drug are dependent on its solubility and transfer characteristics in the GI tract. Improvement of either the intrinsic solubility or the solubility rate of a poorly soluble drug invariably has a beneficial effect on GI absorption (1).

BACKGROUND

Enhancement of absorption by adjuvants and formulation parameters has been the topic of many previous investigations (2–5). In addition, the techniques in designing drug delivery systems can affect the absorption profile. At the early stages of drug development, the physical and chemical factors of the drug and the design of the dosage form should be evaluated carefully for enhancement of bioavailability to yield the desired pharmacological response.

The following steps are considered important for a new compound with very poor solubility (6): (*a*) improve the intrinsic solubility and/or solubility rate by physical or chemical means, (*b*) develop a drug delivery system, (*c*) design and study methods for the *in vitro* evaluation of the drug delivery system, (*d*) select the most efficient drug delivery system in terms of chemical and physical stability and the future manufacturing procedure, (*e*) evaluate the stability of the drug in GI fluid, (*f*) test the drug delivery system *in vivo* for drug bioavailability, and (*g*) correlate *in vitro* and *in vivo* data.

Steps *a* and *b* are important in the early stages of development. The relative *in vivo* efficiency of absorption of the various drug delivery systems can be measured by blood level or urinary excretion studies. However, these studies are expensive, time consuming, and frequently involve complicated analytical procedures. Several investigators reported that drug absorption may be assessed indirectly by determining quantitatively the oral toxicity of the drug (7–9). This method is based on the assumption that the toxicity of an orally administered drug is directly proportional to the amount of the drug in the body or its blood level, which, in turn, is directly proportional to its absorption rate from the GI tract (10). The two parameters most commonly employed to reflect the toxicity of a drug are its oral LD_{50} (mean lethal dose) and LT_{50} (median lethal time).

Several formulations were screened for their absorption rates by determining their LD_{50} values. This procedure is relatively rapid and inexpensive. Such toxicity studies are particularly useful when a limited amount of new drug is available.

Table I—Solubilities of Drugs A and B in Various Solvents

Solvent	Solubility, mg/ml Drug A	Drug B
Water	0.17	0.3
Ethanol	5.1	66
Propylene glycol	2.8	35
0.1 *N* HCl	0.05	0.82
Intestinal fluid	0.13	0.88
0.1 *N* NaOH	2.9	—
Chloroform	>150	>50

Table II—Design and Rationale for Different Formulations of Drug A

Excipient	Ratio, Drug–Excipient	Type of Preparation
—	—	Drug *per se*
Poloxamer 188	1:9	Surfactant addition
Polysorbate 80	1:1	Suspension of drug in surfactant solution
Meglumine	1:3.5	Inclusion of alkaline material
Dibasic potassium phosphate–monobasic potassium phosphate	1:2:1	Buffering agent
Sodium glycocholate	1:4	Addition of bile salt
Sodium glycocholate	1:4	Coprecipitate with bile salt
Povidone	1:4	Coprecipitate with hydrophilic polymer
Urea	1:4	Melt in soluble carrier
Methylcellulose–dibasic calcium phosphate–corn starch	1:0.3:9:1.5	Granulation by dispersing agent

EXPERIMENTAL

Materials—Drugs A[1] and B[1], povidone[2], polysorbate 80[3], mannitol[3], meglumine[4], monobasic potassium phosphate[5], dibasic potassium phosphate[5], poloxamer 188[6], sodium glycocholate[7], sodium taurocholate[7], urea[8], methylcellulose[9], dibasic calcium phosphate[10], corn starch[11], sodium lauryl sulfate[12], and polyethylene glycol 6000[13] were used as received.

Solubility of Drugs A and B—The solubility studies of Drugs A and B were performed in various solvents using the method described in the USP (11) (Table I).

Preparation of Test Systems—The test preparations were made by one of the following methods.

Control—Drug *per se* was passed through a 100-mesh stainless steel screen.

Micronization—Drug was micronized[14] to approximate an average particle size of 3 μm.

Physical Mixture—The drug and the carrier (*e.g.*, surfactants, buffers, and/or bile salts) in the required amounts were geometrically mixed in a mortar with a pestle. No frictional pressure was applied that could significantly reduce size. The mixture was passed through a 100-mesh screen.

Coprecipitation—Requisite amounts of drug and soluble carrier were dissolved in a suitable solvent and evaporated to yield the coprecipitates. The coprecipitates were dried and passed through a 100-mesh screen.

Melts—Appropriate quantities of the drug and a carrier were mixed and heated to give a clear solution. The solution was chilled, and the resulting mass was reduced to fine particles by passing through a 100-mesh screen.

[1] Roche research compounds with very low solubility.
[2] General Aniline & Film Co., Linden, N.J.
[3] ICI America Inc., Wilmington, Del.
[4] Matheson, Coleman & Bell Co., East Rutherford, N.J.
[5] Mallinckrodt Chemical Works, St. Louis, Mo.
[6] Pluronic F68, BASF Wyandotte Corp., Wyandotte, Mich.
[7] National Biochemical Inc., Cleveland, Ohio.
[8] J. T. Baker Chemical Co., Phillipsburg, N.J.
[9] Dow Chemical, Midland, Mich.
[10] Stauffer Chemical Co., New York, N.Y.
[11] National Starch & Chemical, New York, N.Y.
[12] E. I. du Pont de Nemours, Wilmington, Del.
[13] Union Carbide Corp., New York, N.Y.
[14] Spex Industries, Metuchen, N.J.

Table III—Design and Rationale for Different Formulations of Drug B

Excipient	Ratio, Drug–Excipient	Type of Preparation
—	—	Drug *per se* nonmicronized
—	—	Micronized drug
Sodium lauryl sulfate	1:10	Surfactant addition
Sodium taurocholate	1:4	Bile salt addition
Sodium glycocholate	1:4	Coprecipitate with bile salt
Sodium glycocholate	1:4	Admixture of bile salt
Polyethylene glycol 6000–sodium glycocholate	1:5:4	Effect of soluble polymer and bile salt
Polyethylene glycol 6000–sodium glycocholate	1:5:4	Coprecipitate of soluble polymer and bile salt
Polyethylene glycol 6000	1:9	Melt with water-soluble polymer
Mannitol	1:9	Melt with water-soluble carrier
Povidone	1:9	Coprecipitate with water-soluble polymer
Urea	1:9	Melt in water-soluble carrier

Suspension/Solution—The requisite amount of nonmicronized drug was dispersed or dissolved in an aqueous polysorbate 80 solution. The particle-size ranges of suspended drug were 1–37.0 μm for Drug A, with more than 80% below 5 μm, and 10–150 μm for Drug B, with more than 80% below 50 μm.

Granulation—The drug and carriers were uniformly mixed in a mortar with a pestle. The mixture was granulated with water or an aqueous solution of polymer and dried overnight at 120°F. The dried mixture was passed through a 40-mesh screen.

LD_{50} Studies—*Drug A*—Charles River DCl mice, 17–25 g, were used. The powder mixtures were suspended in gum acacia solution as a 4% concentration of the active ingredient. The preparations were administered orally by intubation to six to 16 mice per dose level. Dosed animals were observed for 5 days, and the total mortality was reported. The LD_{50} values were calculated using a computer program based on the method of Finney (12).

Drug B—CF 1S mice, 17–25 g, were used. Six to 10 mice were used per dose level, and the dosed animals were observed for 5 days for mortality. All preparations were administered orally as a 5% concentration of the active ingredient in gum acacia solution. The LD_{50} values were calculated by the method of Finney (12) or Miller and Tainter (13).

Oral Toxicity of Excipients—The excipients added to the formulations were evaluated in the same manner as the drug.

RESULTS AND DISCUSSION

Tables II and III show the design of different formulations for an evaluation of the absorption rate of Drugs A and B, respectively. Tables IV and V show the resulting LD_{50} values. Drug A had an average particle size of about 4 μm as measured by a microscopic method. The LD_{50} values were considered as an index of relative absorption rates.

Three formulations, each containing an additive (*e.g.*, meglumine, sodium glycocholate, or urea), showed a decrease in the LD_{50} with an assumed increase in the *in vivo* absorption rate. The solubility data for Drug A (Table I) showed that the solubility was pH dependent; absolute drug solubility was increased considerably from 0.05 mg/ml in 0.1 *N* HCl to 0.17 mg/ml in water and to 2.9 mg/ml in 0.1 *N* NaOH. This result clearly explains why the compositions containing alkaline materials showed the highest toxicity.

Formulations containing povidone coprecipitate, polysorbate 80, and monobasic and dibasic potassium phosphates showed a considerable increase in toxicity when evaluated by the LD_{50} test. The fine dispersion of the drug may be the possible explanation (14, 15). The granulation technique employed with methylcellulose as a wet binder, dibasic calcium phosphate as the filler, and corn starch as the disintegrant may lower LD_{50} values due to the increased hydrophilicity of the drug.

The use of meglumine, sodium glycocholate, or urea was restricted because of instability in the alkaline environment. This finding might explain why the admixture of the drug and sodium glycocholate gave a

Table IV—Acute Toxicity (in Mice) of Drug A

Formulation	Dose, mg/kg po	Number Dead/ Number Dosed	$LD_{50} \pm SE$, mg/kg po	Relative Potency[a]
100% active (control)	800	2/10	1271 ± 111	1.0
	1000	4/16		
	1250	2/6		
	1600	8/10		
	2000	5/6		
Drug and poloxamer 188	600	0/6	934 ± 59	1.17
	800	1/6		
	1000	4/6		
Drug and polysorbate 80	300	0/10	497 ± 39[b]	2.53
	400	3/10		
	600	7/10		
	1000	10/10		
Admixture of drug and meglumine	100	0/10	351 ± 56[b]	3.65
	200	2/10		
	300	6/10		
	600	7/10		
	1000	9/10		
Admixture of drug with monobasic potassium phosphate and dibasic potassium phosphate	300	2/10	707 ± 137[c]	1.91
	600	2/10		
	1000	8/10		
Admixture of drug and sodium glycocholate	300	1/10	454 ± 42[b]	2.93
	500	8/10		
	600	6/10		
	1000	10/10		
Drug and sodium glycocholate (coprecipitate)	600	2/10	1033 ± 172	1.27
	1000	5/10		
	1250	6/10		
Coprecipitates of drug and povidone	300	0/10	660 ± 77[b]	1.95
	600	5/10		
	1000	8/10		
Drug in urea	100	0/6	423 ± 104[b]	3.24
	200	1/6		
	300	2/6		
	600	4/6		
Drug granulation with methylcellulose, dibasic calcium phosphate, and corn starch	300	0/6	518 ± 62[b]	2.48
	400	1/6		
	600	6/6		
	1000	5/6		

[a] Compared to Drug A alone. [b] The *t* test *versus* controls, $p < 0.01$. [c] The *t* test *versus* control, $p < 0.05$.

Table V—Acute Toxicity in Mice of Drug B

Preparation	Dose, mg/kg	Number Dead/ Number Dosed	$LD_{50} \pm SE$, mg/kg	Relative Potency[a]
Nonmicronized (control)	2000	4/10	2150 ± 196	—
	2500	7/10		
	3200	9/10		
Micronized	1250	0/6	1600 ± 126	1.34
	1600	3/6		
	2000	6/6		
Drug and sodium lauryl sulfate	1250	0/6	1550	1.39
	1400	2/6		
	1600	6/6		
	2000	6/6		
Drug and sodium taurocholate (coprecipitate)	800	5/6	700[b]	3.07
	1250	4/6		
	2000	6/6		
Drug and sodium glycocholate (coprecipitate)	200	0/6	310 ± 26[b]	7.0
	320	4/6		
	400	5/6		
Admixture of drug and sodium glycocholate	500	0/6	615 ± 2[b]	3.49
	560	2/6		
	630	3/6		
	700	5/6		
	800	6/6		
Drug and polyethylene glycol 6000 and sodium glycocholate (coprecipitate)	100	0/6	425 ± 101[b]	5.07
	400	3/6		
	800	5/6		
Drug in polyethylene glycol 6000	2000	1/6	2250 ± 86	1.0
	2250	0/3		
	2500	6/6		
Drug in mannitol	1250	0/6	1615 ± 129	1.33
	1400	2/4		
	1600	4/6		
	2000	4/6		
Coprecipitate of drug with povidone	1250	1/6	1490	1.44
	1600	2/2		
	2000	6/6		
Drug in urea	1250	0/6	1440 ● 47[b]	1.49
	1400	3/6		
	1600	5/6		
	2000	6/6		

[a] Compared to Drug B alone. [b] The t test *versus* controls, $p < 0.01$.

lower LD_{50} value than a coprecipitate of the same combination. In the preparation of the coprecipitate, the solvent and heat might have accelerated the degradation of Drug A in the alkaline environment. The formulation containing the povidone coprecipitate was completely stable.

Drug A with polysorbate 80, even though stable and having a low LD_{50} value, was not a formulation of choice because of encapsulation and tableting problems generally associated with the high amounts of polysorbate 80 in the formula. Thus, formulations containing a povidone coprecipitate or the granulation with methylcellulose were considered formulations of choice for future drug delivery systems.

The value of this LD_{50} procedure can be appreciated readily if one considers a hydrophobic drug like Drug A, where passive absorption was limited by the rate at which solution was effected in the GI fluids. In this type of absorption pattern, any change in the rate of solution of drug in the GI fluids produces a corresponding change in its absorption rate. Due to the intricate assay procedure, the dissolution rates of the formulations were not determined. However, enhanced aqueous solubility with an increasing pH suggests that the higher solubility of Drug A in an alkaline environment can account for the lower LD_{50} values with meglumine, sodium glycocholate, and urea formulations.

Micronization has been known to improve absorption of poorly soluble drugs. Nonmicronized Drug B had an average particle size of approximately 50 μm by microscopic examination, which was reduced to an average of approximately 3 μm by micronization. Drug B in micronized form, as urea or mannitol melts, and as a povidone coprecipitate exhibited LD_{50} values in the same approximate range and considerably lower than the control nonmicronized drug. The results are in agreement with previously published reports (2, 3, 8).

The polyethylene glycol melt of Drug B surprisingly did not show any change from the control. This result may be due to interaction of the drug with polyethylene glycol. Polyethylene glycols are known to form insoluble complexes with drug moieties, thereby impeding drug absorption (16). In other instances, improvement in dissolution and absorption rates may be obtained when polyethylene glycol is used as a carrier to form glass solutions (5).

Table VI—Acute Toxicity of Excipients Used in Drug A Formulations

Formulation Excipient	Dose, mg/kg		Number Dead/Number Dosed	
	Drug A	Excipients[a]	Excipient and Drug A	Excipient Alone
Poloxamer 188	1000	4000	4/6	0/10
Polysorbate 80	1000	1000	10/10	1/10
Meglumine	1000	3500	9/10	0/10
Monobasic potassium phosphate and dibasic potassium phosphate	— + 1000	2000 + 1000	8/10	1/10
Sodium glycocholate	1000	4000	16/20	0/10
Povidone	1000	4000	8/10	0/10
Urea	1000	4000	4/6	0/10
Methylcellulose plus dibasic calcium phosphate and corn starch	1000	300 + 9000 + 1500	5/6	0/10

[a] Quantity of excipients contained in each formulation when 1000 mg/kg of Drug A was administered (ratio of excipients to Drug A is the same as in Table II).

The coprecipitates of Drug B with the salt of cholic acid increased toxicity, while coprecipitates with sodium glycocholate were the most toxic. Cholic acid and its salts may have increased the drug solubility by micellar effects and thus showed better absorption. This type of effect is well documented (17).

For Drug B, which was stable in alkaline medium, the coprecipitate with sodium glycocholate was more toxic than the physical admixture. This result indicates that a molecular dispersion was obtained and was absorbed more readily than the physical admixture. Thus, the coprecipitate formulation containing sodium glycocholate would be considered a formula of choice for further development work.

The excipients used in the formulations of both drugs administered individually had little effect on the LD_{50} values in the maximum amount used in the formulation and, therefore, did not contribute to the toxicity (Table VI).

CONCLUSION

The LD_{50} procedure can estimate relative absorption rate differences between formulations of drugs with very low solubility. The most pharmaceutically acceptable formulations in this study were the povidone coprecipitate with Drug A and the sodium glycocholate coprecipitate with Drug B.

In the design of future drug delivery systems, a formulation with an optimum availability rate would be necessary for an acceptable pharmaceutical product. The relatively inexpensive LD_{50} studies can be a practical rapid method of achieving comparative ratings of drug formulations.

REFERENCES

(1) M. Gibaldi, "Introduction to Biopharmaceutics," Lea & Febiger, Philadelphia, Pa., 1971, pp. 6–8.

(2) G. Levy, *Am. J. Pharm.*, **135**, 78 (1963).

(3) G. Levy, *J. Mod. Pharm.*, **10**, 237 (1967).

(4) A. H. Goldberg, M. Gibaldi, and J. L. Kanig, *J. Pharm. Sci.*, **54**, 1145 (1965).

(5) W. L. Chiou and S. Riegelman, *ibid.*, **58**, 1505 (1969).

(6) J. Swarbrick, "Current Concepts in Pharmaceutical Sciences, Dosage Form Design and Bioavailability," Lea & Febiger, Philadelphia, Pa., 1973, pp. 1, 31, 181.

(7) T. R. Bates and M. Gibaldi, in "Current Concepts in the Pharmaceutical Sciences, Biopharmaceutics," J. Swarbrick, Ed., Lea & Febiger, Philadelphia, Pa., 1970, p. 95.

(8) E. Stupak and T. Bates, *J. Pharm. Sci.*, **62**, 1806 (1973).

(9) W. F. White and O. Grisvold, *J. Am. Pharm. Assoc., Sci. Ed.*, **41**, 42 (1952).

(10) L. W. Dittert, H. J. Adams, F. Alexander, C. W. Chong, T. Ellison, and J. Swintosky, *J. Pharm. Sci.*, **57**, 1146 (1968).

(11) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970, p. 942.

(12) D. J. Finney, "Probit Analysis," Cambridge University Press, Cambridge, England, 1971.

(13) L. C. Miller and M. L. Tainter, *Proc. Soc. Exp. Biol. Med.*, **57**, 261 (1944).

(14) M. Gibaldi and T. Bates, *J. Pharm. Sci.*, **57**, 708 (1968).

(15) D. E. Wurster and P. Taylor, *ibid.*, **54**, 1654 (1965).

(16) P. Singh, S. Guillory, T. Sokolosky, L. Benet, and V. Bhatia, *ibid.*, **55**, 63 (1966).

(17) R. Stoll, T. Bates, K. Nieforth, and J. Swarbrick, *ibid.*, **58**, 1457 (1969).

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Bunolol Metabolism by Dogs: Identification of Basic Metabolites and Their Conjugates

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Abstract □ Female beagles dosed once with encapsulated ^{14}C-bunolol (10 mg/kg) excreted 61% of the isotope in urine in 24 hr. The pooled urine contained a minimum of 18 labeled compounds. Two previously unknown metabolites were purified and were identified by UV and mass spectral data; they were hydroxybunolol (10.1% of urinary radioactivity) and hydroxydihydrobunolol (9.8%). The urine also contained bunolol (0.7% of urinary carbon-14), dihydrobunolol (0.5%), conjugated dihydrobunolol (2.8%), β-(5-oxytetralonyl)lactic acid (16.3%), and (5-oxytetralonyl)acetic acid (7.1%).

Keyphrases □ Bunolol—metabolites and conjugates identified, dog urine □ Metabolism—bunolol in dogs, metabolites and conjugates identified in urine □ Antiadrenergic agents—bunolol, metabolism in dogs, metabolites and conjugates identified in urine

Bunolol [*dl*-5-[3-(*tert*-butylamino)-2-hydroxypropoxy]-3,4-dihydro-1(2*H*)-naphthalenone hydrochloride] (I) is a potent β-adrenoceptor blocking agent (1–3). Previous studies showed that I was absorbed rapidly by dogs (4) and biotransformed extensively to acidic metabolites (5). Additionally, I was reduced to a secondary alcohol by human cadaver liver (6), human and rat erythrocytes, and liver and extrahepatic tissues (7).

The secondary alcohol, dihydrobunolol (II), is an interesting metabolite because it is a β-adrenergic blocking agent with approximately the same potency as I (6). The present report describes the identification and quantification of II and previously unidentified basic metabolites in the urine of dogs dosed with ^{14}C-labeled bunolol.

EXPERIMENTAL

^{14}C-Labeled Bunolol—Bunolol labeled on the 1-carbonyl was synthesized (8). The preparation was 99.0% pure, both chemically and radiochemically, as judged by TLC; it had a specific activity of 4.90 mCi/g.

Radioactivity Counting—Quantitative assays for carbon-14 were performed using a liquid scintillation spectrometer[1]. The external standardization method was used for quench corrections.

TLC—Chromatograms for analytical purposes were run on 5 × 20-cm glass plates coated with 250 μm of silica gel G bound with calcium sulfate[2]. For preparative efforts, 20 × 20-cm plates were used; they were prewashed by one development in methanol, dried in air, and heated for 1 hr at 100°.

[1] Packard Tri-Carb model 3320.

[2] Analtech.