Influence of Dosage Form on Antipyretic Activity of Acetaminophen Administered Intraperitoneally in the Rat

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Abstract □ The relatively insoluble drug, acetaminophen, administered as a solution and injected intraperitoneally in rats, has been shown to produce an antipyretic response approximately twice that of the same dose administered as a suspension. This experiment indicates that ED_{50}, LD_{50}, and relative potency calculations may be inaccurately determined if dosage form influences on biological availability of insoluble drugs are not considered in the design and evaluation of animal drug testing procedures.

Keyphrases □ Acetaminophen—influence of dosage form on antipyretic activity, bioavailability, and potency calculations, intraperitoneal administration, rat □ Dosage forms—influence on antipyretic activity, bioavailability, and potency calculations, intraperitoneal acetaminophen administration, rat □ Antipyretic activity, acetaminophen—influence of dosage form, related potency calculations and bioavailability considerations, intraperitoneal administration, rat

Failure to maximize absorption of insoluble drugs by proper dosage form design may result in inaccurate determinations of drug potency and activity. The influence of dosage form design on drug activity has been extensively investigated in animals and man (1). The time course of a drug response has been shown to be influenced by factors other than the magnitude of the dose administered. Particle size, for example, has been demonstrated to have a striking effect on biological activity. Tablets prepared from micronized particles of spironolactone are approximately four times as potent as tablets made from coarser drug powders, illustrating that biological activity is generally inversely related to particle size (2).

Thus, it is apparent that improper dosage form design may produce significant alterations in the amount of drug available for transport to the site of action, *i.e.*, the bioavailability of the drug is altered. Drugs can only diffuse through membrane systems as single molecules[1]. A drug suspension consisting of an insoluble powder dispersed in a liquid vehicle (usually aqueous) represents not single molecular units but comparatively large aggregates of drug molecules held together by surface forces or crystalline bridges forming a particle. These particles are too large to diffuse through body membrane systems and must dissolve before absorption can occur. At a given time, the amount of drug in solution below the concentration at saturation is a function of the surface area of the particles, surface area being inversely related to particle size (3).

Such considerations are of importance primarily for drugs of limited aqueous solubility. Ingestion of a sugar cube would probably result in about the same blood level profile as a comparable dose of sugar syrup simply because the cube rapidly and completely dissolves in the GI fluids. With insoluble drugs, however, a significant amount of time is required for the particles to dissolve. The rate at which the primary drug particles dissolve, dissolution rate, is often the rate-limiting step for insoluble drugs in the absorption process. It is well established that the dissolution rate and, hence, the rate of absorption are directly related to particle surface area, which in turn is inversely related to particle size.

Administration of an insoluble drug as a solution rather than as a suspension increases, in effect, the surface area toward infinity and should theoretically produce faster absorption and higher blood levels of drug even though the total amount of drug ultimately absorbed may be the same for both dosage forms[2].

Investigations of the influence of dosage form in dose–response data have centered upon the oral and, to a lesser extent, the intramuscular and subcutaneous routes of administration. This is to be expected because these are the most common methods, along with the intravenous route, of administering drugs to humans. However, in many drug testing procedures involving animals, the preferred route of administration is the intraperitoneal route.

Inspection of the literature pertaining to drug screening procedures and bioassay shows that dosage form considerations are generally neglected. Typically, the testing of a group of drugs entails dissolving those drugs

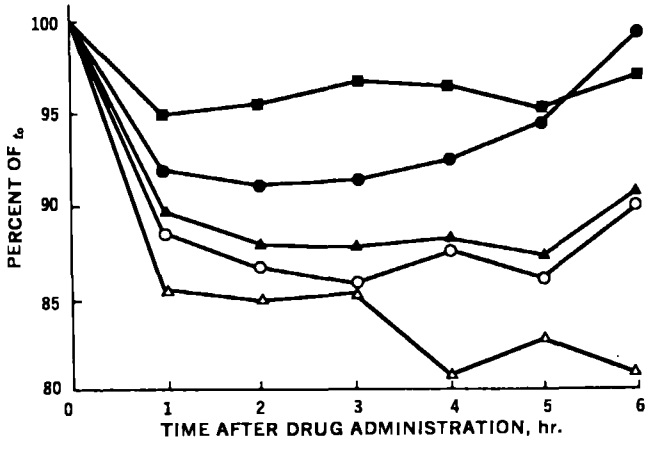

Figure 1—*Mean rectal temperature of rats expressed as percent of* t_0. *Key:* ○, *200 mg./kg. of solution;* △, *400 mg./kg. of solution;* ●, *200 mg./kg. of suspension;* ▲, *400 mg./kg. of suspension; and* ■, *control vehicle.*

[1] With the exception of special situations such as oral polio vaccine which is probably absorbed through the gut wall by a phagocytic process.

[2] Blood levels of drug are determined by a complex interrelationship between absorption, biotransformation, and excretion mechanisms; the level of drug in the body increases when the latter two are exceeded by the rate of absorption.

Table I—Analysis of Variance Table

Variation	*df*	*SS*	*MS*	*F*	*p*
Between formulations	4	0.05605	0.01401	55.4	<0.001
Group A *versus* Group C	1	0.00775	0.00775	30.6	<0.001
Group B *versus* Group D	1	0.00634	0.00634	25.1	<0.001
Between times	5	0.00265	0.000530	2.10	0.10
Interaction (formulation × time)	20	0.00586	0.000293	1.15	—
Between rats (error)	90	0.02276	0.000253	—	—
Total	119	0.08732	—	—	—

that are soluble and preparing simple tragacanth or methylcellulose suspensions of the insoluble drugs. Thus, the possibility exists that intraperitoneal injection of slightly soluble drugs as suspensions rather than solutions will result in reduced pharmacological activity by virtue of the fact that the rate of absorption and, therefore, the amount of drug absorbed during the test period is quite low, even though the peritoneal membranes present a large absorbing surface. This is a result of the particle-size considerations already discussed. It is conceivable then that a drug might be discarded as inactive due to the dosage form used in its evaluation rather than because of any inherent lack of biological activity. At the very least, the determination of parameters such as LD_{50} and ED_{50} will be in error. In this report, data are presented demonstrating the misleading results that may be obtained from a bioassay procedure if precautions to ensure optimum drug absorption are not observed.

EXPERIMENTAL

Acetaminophen[3] was used as a test drug since it is of limited aqueous solubility and possesses good antipyretic activity, a convenient response to measure. Twenty female, albino rats (approximately 300 g.) were marked and randomly divided (*via* a table of random numbers) into five groups of four animals each. Fever was produced in the rats by the subcutaneous injection of 15% brewer's yeast suspension at a dose of 1 ml./100 g. body weight (4). Three test formulations were prepared: a solution of acetaminophen in 75% propylene glycol, a 0.5% tragacanth suspension of acetaminophen in saline, and a control formulation containing the solution vehicle plus tragacanth but without acetaminophen.

Ten hours after the yeast injection, intraperitoneal injections were given to the rats as follows: Group A, acetaminophen solution, 200 mg./kg.; Group B, acetaminophen solution, 400 mg./kg.; Group C, acetaminophen suspension, 200 mg./kg.; Group D, acetaminophen suspension, 400 mg./kg.; and Group E, control vehicle at dosage volume equivalent to that of the 400-mg./kg. groups on a body weight basis.

Rectal temperatures of the animals were recorded[4] at 1-hr. intervals for 6 hr. Room temperature during the experiment was 24 ± 0.5°.

RESULTS AND DISCUSSION

Analysis of variance was used to analyze the results. The experimental differences tested were: (*a*) differences in response between all drug formulations and between Groups A and C and Groups B and D specifically (the primary objective of the experiment was to determine whether a dose of an insoluble drug administered intraperitoneally as a suspension would produce the same response when administered intraperitoneally as a solution), (*b*) differences in response between times after administration, *i.e.*, the change in antipyretic effect with time, and (*c*) interaction of formulation and time, *i.e.*, to determine if the differences in response between formulations would remain constant over the test period. If the interaction term was found to be significant, the specific interaction for formulations A *versus* C and B *versus* D would be tested.

Analysis of variance methods assume that the data are normally distributed and that the variances are homogeneous. Since dose–response data generally follow a log–normal curve, the dosage data were transformed to $\log_{10}$ values. Bartlett's test was used to test the data for homogeneity of variances. Chi-square was calculated to be 0.0718, a value that is not statistically significant ($\chi^2_{0.99(4)} = 0.297$). The variances are thus assumed to be equally distributed.

The temperature measurements were converted to percent of t_0, where t_0 = mean temperature of the rats at the time of drug injection. This transformation was corrected for the variation in fevers produced by the yeast. Figure 1 shows that injection of acetaminophen as a solution produces a much larger percent reduction in temperature than the corresponding dose administered as a suspension. A dose of 200 mg./kg. of solution produced approximately the same response as 400 mg./kg. given as a suspension.

The analysis of variance data are shown in Table I. The interaction term, although suggestive, is not statistically significant. Therefore, the variation between rats was used as the error term to test the other differences. Differences in antipyretic response between times after administration were found to be significant at the 0.10 level. This was to be expected, although the differences were somewhat less than anticipated. Formulation differences (the primary interest of this study) were highly significant at less than the 0.001 level.

The results demonstrate that an insoluble drug, upon intraperitoneal injection, will produce a greater biological response if administered as a solution rather than as a simple aqueous suspension. One may speculate that this is due to the large particle size of the suspended drug powder acting to decrease the amount of drug available for absorption during the test period. Calculation of ED_{50}, LD_{50}, and relative potency values may be adversely affected by neglect of dosage form design. Drug testing procedures involving the intraperitoneal injection of insoluble or slightly soluble drugs may thus be significantly improved by the simple procedure of altering the formulation vehicle.

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[3] Lot 702-1, Eastman.

[4] Using a Yellow Springs Telerecorder 43TD with a thermistor probe 402.