Antipyretic Testing of Commercial Aspirin Formulations in Rats

J. J. LOUX *x, P. D. DePALMA *, R. Z. EBY ‡, and S. L. YANKELL *§

Abstract □ Four commercial aspirin formulations and aspirin powder USP were assayed in yeast-fevered rats for antipyretic activity. Tablets allowed to disintegrate spontaneously prior to dosing yielded aggregates of various sizes which failed to produce uniform patterns of antipyresis. When tested at smaller, more uniform particle sizes of total product, consistent, statistically significant antipyresis was observed with no significant variation among formulations. The ED_{50} values and parallel line assays were homogeneous.

Keyphrases □ Aspirin—four commercial formulations evaluated for antipyretic activity, compared to powder, effect of particle size, rats □ Antipyretic activity—aspirin, four commercial formulations evaluated and compared to powder, effect of particle size, rats □ Particle size—effect on antipyretic activity of aspirin, four commercial formulations evaluated and compared to powder, rats

Previously, this laboratory reported a method for obtaining statistically significant antipyresis in yeast-fevered rats utilizing low doses of aspirin (1). Since the aspirin tested was the pure chemical (white, crystalline powder, USP), those results provided little information on the pharmacological effects of commercial formulations. Thus, experiments were conducted, utilizing the same basic methodology, in which several commercially available products were assayed for their comparative antipyretic activity. Since it was anticipated that differences in activity might be realized with the various products, an attempt also was made to determine what factor(s) might be responsible for such variances.

EXPERIMENTAL

Male albino Charles River rats, 200–250 g, were used. Fever was induced by 20 ml/kg of a 20% aqueous suspension of brewer's yeast[1], which was injected subcutaneously in the back below the nape of the neck. The animals were then fasted for the duration of the experiment (approximately 24 hr). Water was available *ad libitum*.

Temperatures[2] were taken with a lubricated[3] thermistor probe[4] inserted approximately 4 cm into the rectum for 45 sec. Rectal temperatures were taken 24 hr after the yeast injection to determine the pyretic response to yeast. This response ranged between 1.25 and 2.0° above the normal nonfevered control value of 36.40°. No marked variability was encountered. These temperatures, taken 24 hr after yeast administration and 30 min prior to drug administration on fevered animals, served as the predrug controls.

Study 1—Eight animals were used per dose for each compound studied, with eight animals serving as controls. The following were tested: Formulation 1, an aspirin tablet[5]; Formulation 2, a generic aspirin tablet[6]; Formulation 3, a buffered aspirin tablet[7]; Formulation 4, an effervescent aspirin tablet[8]; and Formulation 5, aspirin powder USP[9] as the positive control.

All preparations were tested at equal doses in terms of aspirin content, determined from the "Handbook of Non-Prescription Drugs" (2), label statements, and multiple weighings of groups of 10 tablets.

All preparations except the effervescent one were allowed to disintegrate spontaneously in 0.5% tragacanth; water was employed for the effervescent preparation. Disintegration was considered complete when the respective test preparations had fallen apart into small particles, usually in 5 min or less. After the stock bottles were manually shaken, a stirring bar[10] was added to each. The bottles remained on magnetic stirring plates throughout the animal dosing. Doses employed were 18.75, 37.50, 75, and 150 mg/kg po at a dose volume of 10 ml/kg. Control animals received 0.5% tragacanth at 10 ml/kg. Antipyretic activity was measured at 15, 30, 60, 120, 180, and 240 min after administration.

Study 2—The data from Study 1 indicated variable antipyretic activity among the test formulations. Thus, in this study the following variables were controlled carefully.

Differences in Aspirin Content, Tablet to Tablet—Groups of 10 tablets were ground, and an aliquot was taken of the resulting powder.

Particle Size—All preparations were further extensively ground to a uniform fine particle size in a mortar prior to suspension in the vehicle. With standard USP XVIII sieves (3), it was determined that all products assayed in Study 2, prior to suspension in a vehicle, had a particle-size composition of which 60% passed a 60-mesh, 20% passed an 80-mesh, and 20% passed a 100-mesh sieve.

Dilutions—All doses of a given formulation were prepared by serial dilutions of the respective formulation.

Pyretic Response—The pyretic response to yeast injections was controlled by not using any animal that did not demonstrate a minimum 1.5° elevation in rectal temperature.

Experimenters—The entire study was performed by the same experimenter to eliminate any variability between technical personnel.

The time action study in Study 1 showed that the peak antipyretic activity of aspirin occurred between 1 and 2 hr. The tight, within-group variability in Study 2 along with the steep slope seen in a preliminary dose range study suggested that only four animals per dose were needed. This modification was introduced based on complete statistical analysis of the data. Previous unreported work in this laboratory has repeatedly demonstrated that control yeast responding animals maintain a long pyretic response (up to 4 or 5 hr).

Each of the five preparations was tested at the following doses in terms of aspirin content: 18.75, 37.50, 75, and 150 mg/kg po. Two animals were tested at each dose of each preparation on 2 consecutive days. Thus, any day-to-day variability effect was balanced over all preparations to yield unbiased comparisons. The antipyretic activity assay was measured at 60 and 120 min.

The data were analyzed for the presence of statistically significant antipyretic activity, and ED_{50} values with 95% confidence limits were calculated by paired analysis (4). The ED_{50} refers to the dose of test compound (milligrams per kilogram) that reduced the rectal temperature in treated fevered rats to 50% of the response observed in the fevered nontreated control groups. "Significant antipyretic activity" indicates statistical significance at p values of <0.05. Parallel-line bioassay was performed to test deviation from linearity and parallelism (5).

RESULTS

Study 1—The data from Study 1 indicated that all compounds tested produced effective antipyresis for the duration of the study (4 hr). The time to peak effect was between 2 and 3 hr. Controls did not vary significantly over the 4-hr test period.

Since all preparations were tested at equivalent doses of aspirin, an attempt was made statistically to prepare comparative dose–response

[1] Mead Johnson, Evansville, Ind.
[2] Tele-Thermometer, model 44DT, Yellow Springs Instrument Co., Yellow Springs, Ohio.
[3] K-Y Jelly, Johnson & Johnson, New Brunswick, N.J.
[4] Series 400, Yellow Springs Instrument Co., Yellow Springs, Ohio.
[5] Bayer aspirin, Bayer Co., Glenbrook Laboratories, Division of Sterling Drug, New York, NY 10016.
[6] Rite Aid aspirin, Rite Aid Corp., Shiremanstown, PA 17091.
[7] Bufferin, Bristol-Myers Co., New York, NY 10022.
[8] Alka-Seltzer, Miles Laboratories, Elkhart, IN 46514.
[9] Pure aspirin crystalline powder from bulk lot used in Ecotrin, Smith Kline and French Laboratories, Philadelphia, PA 19101.
[10] Coated with Teflon (du Pont).

Table I—Mean Antipyretic Responses of Test Formulations in Yeast-Fevered Rats Given Spontaneously Disintegrated Tablets

Formulation	Dose, mg/kg	Predrug Rectal Temperature	Time after Administration, min: 15	30	60	120	180	240
			Mean Decrease in Temperature					
1	150	37.94°	0.32°	0.96°	2.02°	2.46°	2.73°	2.42°
	75	37.88°	0.61°	0.86°	1.34°	1.92°	1.94°	1.77°
	37.5	37.80°	0.32°	0.82°	0.94°	1.01°	1.04°	0.95°
	18.75	37.52°	0.01°	0.28°	0.27°	0.51°	0.60°	0.42°
2	150	37.82°	0.33°	0.98°	1.56°	2.12°	2.03°	1.92°
	75	37.74°	0.50°	0.91°	1.53°	2.01°	2.11°	1.79°
	37.5	37.69°	0.05°	0.36°	0.37°	0.67°	0.88°	0.69°
	18.75	37.85°	0.15°	0.20°	0.51°	0.51°	0.46°	0.47°
3	150	37.94°	0.17°	0.73°	1.47°	1.93°	1.99°	2.13°
	75	37.72°	0.02°	0.44°	0.88°	1.68°	1.63°	1.46°
	37.5	37.75°	0.13°	0.49°	0.98°	0.72°	0.78°	0.75°
	18.75	37.92°	0.16°	0.36°	0.49°	0.68°	0.38°	0.46°
4	150	37.78°	0.25°	0.73°	1.08°	1.74°	1.69°	1.33°
	75	37.95°	0.51°	1.02°	1.32°	1.81°	1.81°	1.62°
	37.5	37.79°	0.37°	0.23°	0.48°	0.55°	0.52°	0.32°
	18.75	37.71°	0.10°	0.10°	0.48°	0.59°	0.80°	0.52°
5	150	37.92°	0.38°	1.29°	2.27°	2.78°	3.08°	2.82°
	75	37.62°	0.18°	0.51°	0.96°	1.59°	1.63°	1.47°
	37.5	37.74°	0.22°	0.51°	0.78°	1.16°	0.78°	0.86°
	18.75	37.80°	0.48°	0.37°	0.64°	0.73°	0.59°	0.68°
Controls	—	37.85°	0.13°	0.05°	0.04°	0.06°	0.16°	0.15°

curves. However, significant variations were present that precluded fitting linear and parallel lines to the data. As an alternative, the 1- and 2-hr postdrug responses to the highest dose tested were compared and ranked according to effectiveness. This approach showed that Formulation 5 was the most effective, closely followed by Formulation 1. These agents did not differ from each other. The remaining formulations were statistically less effective, with Formulation 2 better than Formulation 3. Formulation 4 was the least effective. Table I presents the mean antipyretic responses for each dose of the five test formulations at each time period.

Study 2—The mean antipyretic responses for each dose of the five test formulations at each time period are presented in Table II. The ED_{50} values with 95% confidence limits are presented in Table III for both the 2- and 3-hr data. Statistically significant antipyresis was observed for all formulations. The ED_{50} values ranged between 17.2 and 22.7 mg/kg. All responses were linear and parallel. No significant variation was observed between any two formulations. The statistical evaluation of the 2-hr data is presented in Table IV.

The test of validity showed that deviations from parallelism and from linearity were not significant. Thus, relative potencies and 95% confidence limits were calculated. The relative potencies were very near 1.0 (*i.e.*, equality) with 95% confidence limits that bracket 1.0. There was no significant difference in potency between any of these preparations.

Table II—Mean Antipyretic Responses of Test Formulations in Yeast-Fevered Rats Given Finely Ground Tablets

Formulation	Dose, mg/kg	Predrug Rectal Temperature	Time after Administration, min: 120	180
			Mean Decrease in Temperature	
1	150	37.88°	2.10°	2.00°
	75	37.95°	1.80°	1.78°
	37.5	37.55°	1.35°	1.78°
	18.75	37.88°	0.95°	1.25°
2	150	37.85°	2.40°	2.08°
	75	37.78°	1.78°	1.95°
	37.5	37.95°	1.60°	1.40°
	18.75	38.00°	0.88°	0.77°
3	150	37.78°	1.95°	1.98°
	75	37.78°	1.72°	1.52°
	37.5	37.78°	1.60°	1.52°
	18.75	37.80°	0.80°	1.10°
4	150	37.92°	2.52°	2.20°
	75	37.82°	1.78°	1.58°
	37.5	37.92°	1.17°	1.35°
	18.75	37.80°	0.65°	0.50°
5	150	37.85°	2.10°	2.05°
	75	37.98°	2.08°	1.80°
	37.5	37.88°	1.60°	1.65°
	18.75	37.72°	0.85°	0.75°
Controls	—	37.95°	0.20°	0.25°

DISCUSSION

The initial study (1) described the criteria for significant antipyresis; *i.e.*, the test substance must reduce the pyretic response induced by yeast to 50% of the value obtained for the controls. The pyretic control value in this study was 1.86° above the value before yeast administration, which indicated a satisfactory response to the pyretic agent. All test formulations allowed to disintegrate spontaneously prior to testing exhibited effective antipyretic responses (Table I). However, the study indicated wide variability; some preparations exhibited poor activity at doses as high as 75 mg/kg while another demonstrated good activity at half that dose.

Examination of these data for the time course of activity also demonstrated variability in that one preparation effected favorable activity at 30 min with 75 mg/kg whereas another preparation required 120 min to exhibit similar activity at the same dose. Since it had previously been determined that the ED_{50} for aspirin was 10.3 (1.8–23) mg/kg, the lack of significant antipyresis at doses up to 75 mg/kg in this portion of the study was questioned. However, these results were not entirely unexpected. Apparently, one may not assume that equal doses of the same compounds (in terms of active ingredient) will produce the same degree of pharmacological effect. The results clearly indicate that the behavior of several commercial preparations of aspirin was variable when tested under similar experimental conditions.

Some factors contributing to the variability in drug response include nonuniformity of dose among the test preparations (6), disintegration

Table III—ED_{50} and Relative Potency of Test Formulations

Formulation	ED_{50} (95% Confidence Limits), mg/kg	Relative Potency (95% Confidence Limits)
	120-min Data	
1	20.6 (14.4–28.0)	0.85 (0.57–1.27)
2	17.4 (11.9–23.8)	1.01 (0.68–1.51)
3	21.6 (15.2–29.3)	0.81 (0.54–1.21)
4	21.2 (14.9–28.7)	0.83 (0.55–1.23)
5	17.5 (12.0–24.0)	Standard
	180-min Data	
1	17.9 (11.1–26.4)	0.96 (0.57–1.59)
2	17.5 (10.8–25.9)	0.98 (0.59–1.63)
3	18.1 (11.2–26.7)	0.95 (0.57–1.57)
4	22.7 (14.5–32.9)	0.76 (0.45–1.25)
5	17.2 (10.5–25.3)	Standard

Table IV—Analysis of Variance for 2-hr Data, Study II, Antipyresis in Rats

Source	*df*	*MS*	*F*
Treatments	20	1.310	9.51
Control *versus* preparations	1	3.197	23.2
Among preparations	4	0.078	0.57
Regression	1	20.566	149.3
Deviation from parallelism	4	0.216	1.57 N.S.
Deviation from linearity	10	0.126	0.92 N.S.
Error	143	0.138	
Total	163		

and dissolution characteristics of the various preparations (7), additives that alter the absorptive processes in the GI tract (8), particle size (9, 10), fasted *versus* nonfasted subjects (7), and normal *versus* febrile (disease) states (11). In these studies, nonuniformity of dose was controlled by testing all preparations at equivalent aspirin doses. The disintegration parameter was controlled by allowing complete disintegration of tablets prior to dosing the animal. Since it has been pointed out that the amounts of buffering agents contained in analgesic products are probably insufficient in quantity to affect drug action or gastric pH (8, 12), this parameter was considered insignificant. Finally, all animals were fasted and febrile. However, one parameter was not controlled: particle size.

The particle size of a drug influences dissolution or disintegration rates of tablets (9) and absorption from the GI tract (10, 13). Study 2 attempted, in part, to control particle size more closely. In this second study, in which testing parameters were more tightly controlled, the data obtained indicated statistically significant antipyresis for all test preparations, and no statistical or pharmacological differences could be determined among the various test preparations.

Relatively few reports have been published wherein an attempt was made to ascertain the variability of pharmacological response relative to controlled particle sizes. One report (11) detailed the variability of responses not only due to different particle sizes but also due to differences in dissolution rates and absorption rates, all of which are interdependent. An interesting factor was noted, *i.e.*, that the plasma concentration and time course activity of aspirin not only differs with the particle size of aspirin crystals but also differs between febrile and normal rabbits; febrile rabbits obtain faster, higher, and longer blood levels than the nonfevered controls.

Additionally, data reported (11) for the antipyretic activity of various aspirin preparations of different particle sizes in fevered rabbits correlate with the present data for fevered rats dosed with random particle-size aspirin. Itami *et al.* (11) concluded that the data supported the theory that as the particle size of aspirin decreases, antipyretic activity is more pronounced and more rapid in onset. Study 2 results also supported this theory, particularly at low dose levels where the antipyretic activity (Table II) was markedly different than the activity of the larger sized aggregates (Table I).

On the other hand, the favorable improvement in the physical properties of aspirin crystals may be reversed when subjected to the tableting process (9), a factor that also could compromise much of the pharmacological benefits derived from smaller crystal size. Thus, the recent suggestion (14) that aspirin tablets be crushed or chewed to a fine powder prior to being swallowed merits some attention.

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§ Present address: University of Pennsylvania School of Dental Medicine, Philadelphia, PA 19174.

ˣ To whom inquiries should be directed. Present address: Department of Pharmacology, Cooper Laboratories, Cedar Knolls, NJ 07927.

Effect of Temperature and Relative Humidity on Nitrazepam Stability in Solid State

D. GENTON and U. W. KESSELRING ˣ

Abstract □ The decomposition of a 1% dilution of nitrazepam in microcrystalline cellulose was established by quantitative determination of the two main breakdown products, 2-amino-5-nitrobenzophenone and 3-amino-6-nitro-4-phenyl-2(1*H*)-quinolone, using *in situ* diffuse reflectance measurements on thin-layer chromatograms. The decomposition and formation rate constants of nitrazepam and of the breakdown products, respectively, were determined at four temperatures and six relative humidities. By means of a three-parameter regression equation, it was possible to correlate quantitatively the decomposition constant of nitrazepam to both temperature and relative humidity.

Keyphrases □ Nitrazepam, solid—decomposition in microcrystalline cellulose, effect of temperature and humidity □ Decomposition—nitrazepam in solid state in microcrystalline cellulose, effect of temperature and humidity □ Stability—nitrazepam in solid state in microcrystalline cellulose, effect of temperature and humidity □ Anticonvulsants—nitrazepam in solid state in microcrystalline cellulose, decomposition, effect of temperature and humidity □ Solid state—decomposition, nitrazepam in microcrystalline cellulose, effect of temperature and humidity

Stability studies of drugs in pure form and in solid dosage forms were reviewed and summarized previously (1, 2). Apparently, no workers have attempted to combine the influences of both temperature and humidity (or water content) in a unique mathematical expression.

The purpose of this work was to assess quantitatively