Drug-Test Interaction in the Submaximum-Effort Tourniquet Technique[1,2] (34934)

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In recent years the submaximum-effort tourniquet technique of producing ischemic pain has been proposed as a reliable test for the evaluation of new analgesics in man. Smith and his associates have reported that it differentiated intravenous morphine (1, 2) as well as 600 mg of aspirin (3) from placebos. Thus far, however, it has not been used to compare a new to a known analgesic agent. The present report describes the results of such an effort and some problems encountered in the use of the method for this purpose.

The original purpose of the study was to compare the relative effect of flufenisal and aspirin in relieving tourniquet pain. Flufenisal, 4′-fluoro-4-hydroxy-3-biphenylcarboxylic acid acetate, is a new nonsteroidal agent with analgesic, antipyretic, and anti-inflammatory properties. The serum half-life of flufenisal was 7 hr compared to 3 hr after equivalent doses of aspirin. Moreover, preliminary clinical studies (4) indicated that flufenisal was several times more potent than aspirin on a weight basis. Hence, flufenisal appeared both more potent and longer lasting than aspirin after oral ingestion.

Methods and Materials. Subjects. Ten men between the ages of 21 and 29 were chosen among volunteers from the Iowa State Men's Reformatory, Anamosa, Iowa. Inmates with a history of drug abuse and those who required medications were excluded. Written, informed consent was obtained and each subject was instructed not to take any medications, including aspirin, during the study.

Study design. A double-blind crossover study was planned to obtain a potency ratio for flufenisal and aspirin at 2 and at 8 hr after ingestion. Doses of 300 and 600 mg of flufenisal and 600 and 1200 mg of aspirin were selected for comparison. All medications were prepared by pharmacists in identical capsules to be given in a Latin-square sequence. An interval of at least 1 day separated treatments. Ten days after completion of the first study, 9 of the 10 men were tested in the same manner after a placebo and 900 mg of aspirin. Two days intervened between the two treatments which were given in a balanced crossover design.

Test procedure. All studies were carried out in the Hospital of the Men's Reformatory. The testing procedure was previously described (5). A hand exerciser of coiled spring construction was employed in all experiments. A force of approximately 8 kg was necessary to close the handles. The subject to be tested entered a room and reclined on a padded examining table. He extended his nondominant arm toward the ceiling while it was wrapped to the elbow with an elastic bandage to drain the venous blood. A 5-in. blood pressure cuff (Propper Mftg. Co.) was connected to a Baumanometer stand sphygmomanometer. The cuff was inflated around the subject's upper arm until the pressure read 250 mm Hg when the bandage

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TABLE I. Minutes to Report Each of Four Pain Ratings After Flufenisal and Aspirin.[a]

Pain level[b]	Hours after drug	Flufenisal 300 mg	Flufenisal 600 mg	Aspirin 600 mg	Aspirin 1200 mg
Mild	2	10.2 ± 2.1	9.2 ± 1.6	7.7 ± 1.5	10.3 ± 1.5
	8	12.7 ± 2.4	9.5 ± 1.6	10.9 ± 2.0	9.9 ± 1.9
Moderate	2	17.4 ± 2.0	18.2 ± 1.6	14.6 ± 1.3	18.5 ± 1.5
	8	19.5 ± 2.4	16.5 ± 1.5	17.8 ± 1.5	18.5 ± 1.5
Severe	2	23.9 ± 2.4	23.5 ± 1.8	20.0 ± 1.7	24.1 ± 1.6
	8	25.1 ± 2.3	23.7 ± 1.9	22.2 ± 1.7	23.8 ± 1.6
Unbearable	2	30.2 ± 2.8	29.6 ± 1.4	26.3 ± 1.6	29.1 ± 2.1
	8	30.2 ± 2.2	30.6 ± 1.7	27.7 ± 1.6	30.0 ± 1.9

[a] Values represent the mean ± standard error for 10 subjects.

[b] Mean pretreatment values were as follows: mild = 7.1 ± 1.7; moderate = 12.2 ± 1.7; severe = 18.8 ± 1.9; unbearable = 27.0 ± 2.1.

was removed. The arm was then lowered to the subject's side to rest on the table and the subject completed 20 full squeezes of the hand exerciser. At the moment the last squeeze was completed, a stop watch was started. The subject was asked to avoid moving his arm until the pressure was released.

At irregular intervals the subject was asked to rate his pain on a scale of 0–4. The ratings were as follows: 0 = none, 1 = mild, 2 = moderate, 3 = severe, 4 = unbearable. A response of "4" at any time signified unbearable pain, and the experimenter released the cuff and stopped the watch. The pain ratings and time intervals used were essentially the same as those in the study of Smith and Beecher (3).

Subjects were allowed to eat only after the 2-hr test. A venous blood sample was drawn before ingestion of each medication and midway during each test for the determination of serum concentrations of total flufenisal and salicylate (6). Flufenisal, present in the serum primarily in the deacetylated form, was measured by a fluorometric method (unpublished). Pretreatment responses were determined once on 1 day before any medication was given.

Calculations. Means and standard error were computed for each treatment, and comparisons were made by analysis of variance (7). Yates' formula was used to compute missing values for one subject at one time period after 600 mg of flufenisal. Paired t tests were used to compare pretreatment and postdrug means. In all cases $p < .05$ was considered significant.

Results. Mean times to report each of four pain ratings after each of the two doses of flufenisal and aspirin are shown in Table I. For the 2-hr test the response times after 300 and 600 mg of flufenisal were not significantly different at any pain level. At all pain levels a dose-response relationship was obtained with the two doses of aspirin. The times at each pain level after 1200 mg of aspirin were nearly identical to those after both 300 and 600 mg of flufenisal.

At 8 hr, the mean times to report each pain level remained essentially the same as those at 2 hr for the high doses of flufenisal and aspirin despite a fall in serum level of the drugs (Table II). At nearly every level the mean response times after the low doses of flufenisal and aspirin increased over those of the 2-hr tests. The increases occurred in spite of the fact that the serum levels of each drug fell to half or less of that measured at 2 hr. Moreover, the mean response times after 600 mg of aspirin approached those after 1200 mg even though the concurrent salicylate level was 9 as compared to 39 μg/ml. Thus, at 8 hr, there were no differences among drugs or doses at any level.

Comparison of the values of the pretreatment trial with those of each corresponding

TABLE II. Serum Concentrations of Flufenisal and Aspirin.[a]

Hours after drug[b]	Flufenisal		Aspirin	
	300 mg	600 mg	600 mg	1200 mg
0	2 ± 0.2	1 ± 0.1	2 ± 0.2	2 ± 0.4
2	48 ± 8.8	98 ±15	47 ± 3.3	88 ± 6.0
8	20 ± 2.7	47 ± 9.3	9 ± 1.4	39 ± 5.7

[a] Values represent the mean serum concentration in micrograms per milliliter ± standard error for 10 subjects.

[b] Blood samples were drawn before ingestion of each medication and midway during each test.

pain level in Table I showed, that, with one exception, significant increases in response times followed drug administration. The differences between pretreatment values and those 2 hr after 600 mg of aspirin were not statistically significant. At 8 hr, however, the differences were significant at all but the highest pain level.

In the second study which began 10 days after the completion of the first, a placebo and 900 mg of aspirin were given to 9 of the 10 original subjects. The results are shown in Table III. At 2 hr, the differences between aspirin and placebo were significant only for the moderate and severe pain levels. After placebo, the response times of the 8-hr test did not change at the two lower levels, but tended to decrease at the two higher levels from those of the 2-hr test. After aspirin, the response times at 8 hr remained essentially the same as those at 2 hr for all pain levels.

When compared, the response times 2 hr after placebo were nearly equal to those after 1200 mg of aspirin in the first study (Table I). They also were significantly longer than the pretreatment times recorded at all except the highest level. In the second study the serum levels of total salicylate decreased between 2 and 8 hr from 58 ± 3.1 to 18 ± 1.3 μg/ml for the nine subjects.

Discussion. Interested investigators continue the search for an experimental method to test analgesic drugs in man. Difficulties have been recognized in most of the varied approaches (8). The method developed by Smith and co-workers (1–3) appeared to provide considerable improvement in reproducibility of pain. In addition to responding to morphine (1, 2), the pain induced by this method was sensitive to the analgesic effect of 600 mg of aspirin (3). The purpose of the present study was to compare the relative efficacy of aspirin and flufenisal, both at 2 and at 8 hr after oral administration, in relieving this type of pain.

At 2 hr there was little difference in response times after 300 and 600 mg of flufenisal. This lack of dose-related response with flufenisal may have been due in part to an order effect since, as a result of a mix-up in the Latin-square sequence, four subjects received the 600-mg dose on the first day of testing with drug. Since the dose-response curves were not parallel, it was not possible to compute a potency ratio at 2 hr.

The most notable observation in the first study was the lack of correlation between the mean response times and the drug serum levels at 8 hr. After the high doses of flufenisal and aspirin, the times to report each pain level remained the same despite a decrease in serum concentration of each drug. Furthermore, the mean response times increased at nearly every pain level after the low doses of the two drugs. The increases occurred in spite of the fact that the serum levels of the drugs fell to less than half those measured at 2 hr. In the case of 600 mg of aspirin, the in-

TABLE III. Minutes to Report Each of Four Pain Ratings After Placebo and Aspirin.[a]

Pain level	Hours after drug	Placebo	Aspirin 900 mg
Mild	2	10.3 ± 2.1	11.3 ± 2.2
	8	9.9 ± 2.1	12.5 ± 2.1
Moderate	2	16.2 ± 1.8	19.8 ± 1.5[b]
	8	16.5 ± 1.7	19.4 ± 1.7[b]
Severe	2	23.1 ± 2.1	25.0 ± 1.8[b]
	8	22.2 ± 2.0	25.2 ± 2.0[b]
Unbearable	2	28.4 ± 2.4	29.6 ± 2.4
	8	26.4 ± 2.4	29.8 ± 2.0

[a] Values represent the mean ± standard error for nine subjects.

[b] Indicates significant ($p < .05$) difference from placebo by paired t tests.

creases had the effect of producing significant differences from pretreatment values at all but the unbearable pain level. The similar response times at 8 hr after all treatments undoubtedly resulted from carryover effects from the 2-hr tests.

Although pretreatment responses were determined at the beginning of the study, no placebo medication was given. It was of interest, therefore, to find out if the increases would occur after a placebo or were related to a pharmacological effect. Accordingly, on two separate days, 9 of the 10 original subjects were tested at 2 and at 8 hr after placebo and after 900 mg of aspirin. The later dose of aspirin was chosen to compare it with the two doses given in the first study. Mean responses at 8 hr after placebo remained the same as those at 2 hr for the two lower pain levels, and decreased slightly for the two higher pain levels. After aspirin, 900 mg, the values at 2 and 8 hr were the same for all levels despite the fact that the serum levels declined markedly. These differences, although not statistically significant, suggested that prior testing with aspirin was more effective in increasing pain tolerance in the 8-hr test than was testing with placebo.

The times to report each pain level 2 hr after placebo were nearly as long as those after 1200 mg of aspirin in the initial study. Since previous work (5) had shown little difference between response with no treatment and those after a placebo, enhanced pain tolerance also appeared to be the explanation for the improvement in performance between studies. How much of the improvement was due to carryover from the first study and how much occurred during the second study could not be determined exactly. In those five subjects who received placebo followed by aspirin in the second study, response times after placebo were considerably increased over those in the pretreatment session, especially at the lower pain levels. In spite of the improvement, hoewver, significant differences between aspirin and placebo were still obtainable at two pain levels (Table III).

Augmented tolerance to pain after a placebo repeated after a pharmacologically active agent has been observed by other workers (9–11). In general, this effect was not considered to be a significant interfering factor in the crossover design. In the present study, however, carryover effects erased anticipated treatment differences at 8 hr after administration of non-narcotic drugs. Moreover, similar effects appeared to persist 10 days later when most of the same subjects were tested after a placebo.

Summary. A double-blind crossover comparison of flufenisal and aspirin was made using the submaximum-effort tourniquet technique to test analgesic effect. Each of 10 men was tested at 2 and at 8 hr after ingestion of each of two doses of each drug. Four pain ratings were recorded: mild, moderate, severe, and unbearable. Serum levels of each drug were measured before ingestion and midway in each test. Comparison of the responses in both tests indicated enhanced pain tolerance in the 8-hr as compared to the 2-hr test after drug ingestion. The carryover effects occurred despite a reduction in serum levels of drug between the two tests. A second study suggested that carryover effects were greater after aspirin than after placebo. The studies indicate that a drug-test interaction can occurr in the submaximum-effort tourniquet technique and may obscure expected treatment differences. The findings also suggest that investigators should consider the possibility of such interactions in the design of experiments employing this method.

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