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RESEARCH PAPERS

IN VITRO DISSOLUTION VERSUS *IN VIVO* EVALUATION OF FOUR DIFFERENT ASPIRIN PRODUCTS

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ABSTRACT

The single dose pharmacokinetic characteristics of four aspirin formulations in humans were compared with their *in vitro* dissolution properties. The pharmacokinetic parameters were determined by measuring salicylate concentrations in the plasma. Dissolution was measured by using the USP XX single time point dissolution test. The four aspirin products were a commercial tablet, a commercial capsule, a capsule filled with a commercial granulation, and a slow dissolving capsule formulation that failed the USP dissolution specification. It was found that there was a poor correlation between the *in vitro* dissolution results and the *in vivo* computed pharmacokinetic parameter statistics. *In vivo* testing in humans showed that all of the formulations were bioequivalent in terms of half-life and AUC, and the capsule that failed to pass the dissolution specification was

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bioequivalent to two of the three products that did pass the specification with respect to the maximum plasma concentration. None of the products could be demonstrated to be bioequivalent with regard to the time to maximum plasma concentration. However, none of the *in vivo* differences between the four aspirin formulations were judged to be clinically significant. Therefore, while there was a poor relationship between the *in vitro* USP XX dissolution test results and the bioequivalence test results, from a practical viewpoint the dissolution test is a satisfactory manufacturing quality control test, since it can detect differences in *in vitro* dissolution prior to their having a significant clinical impact.

INTRODUCTION

Aspirin is one of the most commonly used drugs, due to its usefulness as an analgesic, anti-inflammatory, antithrombotic, and antipyretic, and its ready commercial availability. The primary purpose of compendial *in vitro* aspirin product dissolution tests is to ensure lot-to-lot uniformity during manufacturing. However, it would be useful if this *in vitro* data could be correlated with results from *in vivo* absorption experiments, so that the dissolution data could be used to predict *in vivo* performance. It is difficult to obtain highly correlated *in vitro* versus *in vivo* results due to the use of a single dissolution test for different formulations, and due to compendial dissolution tests for immediate release products which typically employ only a single time point for analysis.

The objective of this study was to assess whether the *in vitro* USP XX single point dissolution test could be correlated with *in vivo* plasma pharmacokinetic parameters. To accomplish this objective, we investigated whether three aspirin products that readily passed the USP XX *in vitro* dissolution test specification would exhibit similar pharmacokinetic behavior, and also assessed whether a slow dissolving tablet that distinctly failed the

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in vitro specification would demonstrate pharmacokinetic characteristics that were significantly different from the other three products. The four aspirin formulations that were examined were a commercial tablet, a commercial capsule, a hard gelatin capsule shell filled with a commercially supplied aspirin granulation, and a slow dissolving aspirin capsule.

MATERIALS AND METHODS

Materials - The two commercial products were 5 grain (325 mg) aspirin tablets (Bayer Aspirin, lot # 1A007) and 5 grain aspirin capsules (Eli Lilly A.S.A. Pulvules, lot # 5RX51A). The third aspirin product was manufactured by filling the equivalent to 5 grains of aspirin of commercially supplied 90/10 aspirin/starch granulation (Monsanto, lot # QA-12329) into size 0 opaque white hard gelatin capsule shells (Elanco Qualicaps, lot # D1KY24) with a Haffliger-Karg capsule filling machine. The fourth aspirin product was manufactured by blending 216.66 kg of the commercially supplied 90/10 aspirin/starch granulation (Monsanto, lot # QA-12308) with 135.96 kg of spray dried lactose, USP (Foremost Whey Products, lot # 2RL006), 19.375 kg of regular lactose, USP (Foremost Whey Products, lot # SA125), and 18.6 kg of talc, USP (J. T. Baker Chemical Co., lot # 033338), in a Gemco double cone blender for twenty minutes. The material was then milled using a Tornado Mill (equipped with a 0.062 inch diameter perforated screen), mixed again in the Gemco double cone blender for twenty minutes, milled again using a Tornado Mill (equipped with a 0.033 inch diameter perforated screen), then mixed for 15 minutes in an AMF planetary blender, and filled into size 0 opaque white hard gelatin capsule shells (Elanco Qualicaps, lot # KOAJ33) using an Elanco capsule filling machine, to obtain a fill weight containing 5 grains of aspirin.

Study subjects - Six healthy male and six healthy female volunteers between 26 and 36 years of age (the average age was 32 $\pm$ 4 years, and the

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ıean weight was 64.2 ± 11.5 kg) were enrolled and completed the study. No ypnotics, sedatives, antihistamines, or other enzyme-inducing drugs were llowed one month prior to the study. No other drugs, including over-the-ounter medications and vitamins, were allowed 72 hours prior to and hroughout the study. Alcohol was not allowed from 24 hours before dosing ıntil 24 hours after dosing.

Study design and drug schedule - An open label, randomized, single lose, four way crossover study design, with a six or seven day washout ıeriod between doses, was employed. After an overnight fast, each subject eceived an oral dose of study medication which consisted of two 5 grain :apsules or tablets of aspirin, given with 200 cc of water. The order of drug .dministration was randomly selected according to a latin square design. ;erial blood samples were collected immediately prior to dosing and at 0.5, 1, ', 4, 7, 10, and 24 hours post-dose. The plasma was separated immediately rom each blood sample and frozen for subsequent salicylate level leterminations by HPLC. Subjects fasted until the 4-hour blood sample had ıeen collected. Thereafter, each subject ate a meal which included up to 16 ıunces of total fluids.

Pharmacokinetic parameters - The parameters for salicylate levels hat were compared statistically by using parametric analysis of variance ırocedures were 1) plasma concentration at each time point (C_p), 2) time to naximum plasma concentration (T_{max}), 3) peak plasma concentration C_{max}), 4) plasma half-life ($t_{1/2}$) determined by log linear regression ınalysis of the terminal linear portion of the log plasma concentration versus ime curve, 5) area under the plasma concentration-time curve from 0 to 10 ıours (10 hour AUC) and 0 to 24 hours (24 hour AUC) computed by using he linear trapezoidal rule, and 6) area under the plasma concentration-time :urve from 0 to infinity (total AUC) computed using the linear trapezoidal rule ıp to 24 hours and adding the term

$$C_{p(24\ hr)} \times (T_{1/2} / \ln 2)$$

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Parameters were analyzed according to an analysis of variance model appropriate for a crossover design (1). Terms for sequence, subject within sequence, formulation and visit were included in the model. Statistical analysis was performed using the GLM procedure of the Statistical Analysis System (2). Ninety percent classical confidence intervals (3) were computed for the ratio of means of all of the formulations.

***In vitro* dissolution** - Dissolution of the aspirin capsules and tablets was determined using USP XX methodology, which employs a basket rotating at 50 RPM in 500 ml of 0.05 M acetate buffer. The specification for tablets and capsules was Q=80% at 30 minutes.

RESULTS AND DISCUSSION

The *in vitro* dissolution results for the tablets and capsules are shown in Table 1. The two commercial products readily passed the USP Q value of 80% at 30 minutes, as did the 90/10 aspirin/starch capsule formulation. The slow dissolving capsule formulation failed the USP specification at all three stages of testing.

The mean salicylate plasma concentrations for the four formulations at each time point are illustrated in Figure 1. Table 2 demonstrates that from 30 minutes to 2 hours the usual order of the mean plasma salicylate concentrations was: commercial tablet>commercial capsule>90/10 aspirin/starch capsule>slow dissolving capsule. From 4 to 24 hours, the mean plasma concentrations were not significantly different. ($p>0.05$). A high degree of intersubject variability in plasma concentrations was seen at later time points. Plasma salicylate concentrations of the three capsule formulations were almost always lower than the commercial tablet.

The mean salicylate computed pharmacokinetic parameters are shown in Table 3. Plasma half-life ranged from 2.93 hours to 3.01 hours, and time

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TABLE 1
In Vitro Dissolution Results for the Aspirin Tablets and Capsules.

	% Dissolved (Individual Units) at 30 Minutes	Mean % Dissolved ± S.D. at 30 Minutes
Commercial Tablets	102, 98, 101, 102, 100, 104	101 ± 2
Commercial Capsules	101, 100, 101, 97, 101, 101,	101 ± 2
90/10 Aspirin/Starch Capsules	99, 100, 106, 104, 101, 103	102 ± 3
Slow Dissolving Capsules	71, 95, 72, 63, 85, 86 (Failed USP Stage 1)	79 ± 12
	79, 96, 90, 69, 67, 82 (Failed USP Stage 2)	80 ± 11[a]
	79, 86, 84, 81, 59, 84	
	51, 91, 93, 94, 94, 85 (Failed USP Stage 3)	81 ± 12[b]

[a] Mean ± standard deviation of USP Stage 1 and Stage 2 test results
[b] Mean ± standard deviation of USP Stage 1, Stage 2, and Stage 3 test results

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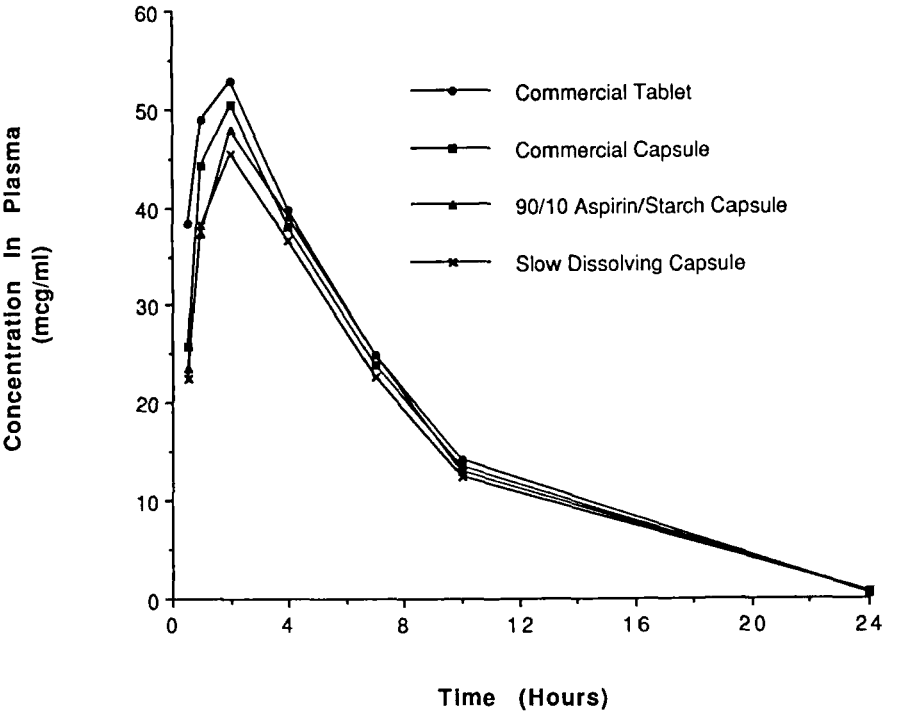

FIGURE 1
Plot of Mean Salicylic Acid Plasma Concentrations Following Dosing With Four Different Aspirin Formulations.

to maximum concentration (T_{max}) from 1.71 to 1.92 hours. The highest maximum plasma concentration was achieved with the commercial tablet (56.0 mcg/ml) and the values for C_{max} were significantly different ($p<0.05$) among the four formulations. For sequential areas under the curve, the early superiority of the commercial tablet observed in the plasma concentrations carried over to each of the later times, including the total area under the curve.

The ratios of the various mean computed pharmacokinetic parameters for each of the formulations are listed in Table 4. Using the "80% to 120% rule" for bioequivalence (based on 90% confidence interval analysis), all of the formulations were bioequivalent with respect to half-life and area under the curve (if rounding off figures to the nearest percent is employed). All of

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TABLE 2

Mean Salicylate Plasma Concentrations[a] (± Standard Deviations) for Aspirin Tablets and Capsules.

Time (Hours)	Commercial Tablet	Commercial Capsule	90/10 Aspirin/Starch Capsule	Slow Dissolving Capsule	F-test P-values[b]		
					Formula	Period	Sequence
0.5	38.38 ± 19.54	25.73 ± 14.52	23.52 ± 8.78	22.51 ± 15.38	0.013	0.121	0.619
1.0	48.98 ± 13.08	44.27 ± 11.15	37.41 ± 9.69	38.26 ± 10.09	<0.001	0.478	0.662
2.0	52.94 ± 8.66	50.43 ± 10.69	47.88 ± 9.13	45.40 ± 9.14	0.002	0.386	0.247
4.0	39.76 ± 9.92	38.09 ± 9.44	39.13 ± 9.96	36.74 ± 8.07	0.254	0.082	0.486
7.0	24.87 ± 10.13	23.78 ± 9.80	24.96 ± 9.86	22.72 ± 8.27	0.207	0.034	0.547
10.0	14.20 ± 7.84	13.45 ± 8.87	13.01 ± 6.72	12.46 ± 7.09	0.653	0.149	0.665
24.0	0.60 ± 0.83	0.54 ± 0.52	0.62 ± 0.80	0.64 ± 1.10	0.885	0.127	0.607

[a] n = 12

[b] Crossover analysis of variance testing for formulation, period, and sequence effect.

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TABLE 3

Computed Mean Human Pharmacokinetic Parameters (± the Standard Deviations)[a] for Aspirin Tablets and Capsules.

	Commercial Tablet	Commercial Capsule	90/10 Aspirin/Starch Capsule	Slow Dissolving Capsule	F-test P-values[b]		
					Formula	Period	Sequence
Half-life (hours)	2.93 ± 0.56	2.96 ± 0.38	2.98 ± 0.61	3.01 ± 0.76	0.800	0.203	0.748
Range	(2.44 - 4.52)	(2.48 - 3.93)	(2.40 - 4.53)	(2.40 - 5.28)			
T_{max} (hours)	1.71 ± 0.92	1.75 ± 0.45	1.92 ± 0.29	1.92 ± 0.79	0.750	0.304	0.975
Range	(0.5 - 4.0)	(1.0 - 2.0)	(1.0 - 2.0)	(1.0 - 4.0)			
C_{max} (mcg/ml)	56.0 ± 10.3	51.4 ± 10.2	48.2 ± 9.0	45.6 ± 9.0	<0.001	0.260	0.283
Range	(44.3 - 77.9)	(39.6 - 75.9)	(32.3 - 62.5)	(33.5 - 62.4)			
10 hour AUC (mcg hr/ml)	330.7 ± 80.2	308.5 ± 91.0	303.8 ± 81.4	286.7 ± 73.0	0.002	0.184	0.467
Range	(247.8 - 513.2)	(277.9 - 540.6)	(195.4 - 416.5)	(201.5 - 431.2)			
24 hour AUC (mcg hr/ml)	434.3 ± 135.5	406.4 ± 155.2	399.2 ± 125.8	378.4 ± 125.6	0.050	0.116	0.542
Range	(289.4 - 707.8)	(275.4 - 800.3)	(241.7 - 593.4)	(235.5 - 610.6)			
Total AUC (mcg hr/ml)	437.4 ± 139.1	408.9 ± 157.4	402.5 ± 129.2	382.2 ± 131.1	0.060	0.101	0.543
Range	(290.5 - 712.7)	(276.5 - 807.4)	(242.7 - 612.3)	(236.6 - 641.8)			

[a] n = 12 [b] Crossover analysis of variance testing for formulation, period, and sequence effect.

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TABLE 4

Ninety Percent Confidence Intervals[a] on Ratios of Formulations for Computed Parameters for Aspirin Formulations[b].

	Half-life	T_{max}	C_{max}	10 hr AUC	24 hr AUC	Total AUC
Ratio of B/A x 100	101.0	102.4	91.7	93.3	93.6	93.5
90% Limits	96.1, 105.9	78.3, 126.6	85.5, 97.7	88.1, 98.4	86.1, 101.0	85.9, 101.0
Ratio of C/A x 100	101.9	112.2	86.0	91.9	91.9	92.0
90% Limits	97.0, 106.8	88.0, 136.4	80.0, 92.0	86.7, 97.1	84.5, 99.4	84.5, 99.6
Ratio of D/A x 100	102.8	112.2	81.5	86.7	87.1	87.4
90% Limits	97.8, 107.6	88.0, 136.4	75.5, 87.5	81.6, 91.9	79.7, 94.6	79.8, 94.9
Ratio of C/B x 100	100.9	109.5	93.8	98.5	98.2	98.4
90% Limits	96.1, 105.8	85.9, 133.1	87.2, 100.3	93.0, 104.0	90.3, 106.2	90.4, 106.5
Ratio of D/B x 100	101.7	109.5	88.9	93.0	93.1	93.5
90% Limits	96.9, 106.6	85.9, 133.1	82.3, 95.4	87.4, 98.5	85.1, 101.1	85.4, 101.5
Ratio of C/D x 100	99.2	100.0	105.5	106.0	105.5	105.3
90% Limits	94.4, 104.0	78.5, 121.5	98.2, 112.8	100.0, 111.9	96.9, 114.1	96.7, 113.9

[a] Computed by using t-distribution

[b] Formulations: A - Commercial tablet
B - Commercial capsule
C - 90/10 aspirin/starch capsule
D - Slow dissolving capsule

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the formulations were also bioequivalent with regards to the maximum plasma concentration, except that the slow dissolving capsule was not bioequivalent to the commercial tablet. However, none of the formulations could be demonstrated to be equivalent for the time to maximum plasma concentration, due to large variability leading to high statistical uncertainty.

These data indicate that it is difficult to correlate *in vivo* results with the single time point USP XX *in vitro* dissolution test results for aspirin products. Capsules that failed the dissolution test specification were bioequivalent to products that passed the specification, in terms of half life and area under the curve. On the other hand, the three products that readily passed the dissolution test specification could not be demonstrated to be bioequivalent with respect to the time to maximum plasma concentration, although a study with a larger number of subjects might be able to demonstrate bioequivalence for T_{max} for some of the formulations (particularly with respect to the two commercial products). For the maximum plasma concentration the slow dissolving capsule was bioequivalent to two of three articles that passed the dissolution specification.

However, it is important to note that despite the statistical differences, none of the variations in the pharmacokinetic parameters are likely to be clinically significant (although, since efficacy was not examined in this study, this cannot be definitively stated). Review of the data shows that clinically effective plasma salicylate levels were probably obtained with all the formulations, including the capsule that failed dissolution testing. If this is the case, then the single time point USP XX *in vitro* dissolution test for aspirin is, in a practical sense, effective in acting as a manufacturing quality control test, in that it can detect differences *in vitro* that are *in vivo* statistically but not clinically significant. The test can therefore appropriately lead to the rejection of batches before their dissolution characteristics create a problem in terms of clinical efficacy.

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CONCLUSIONS

This study demonstrated that three aspirin products that readily passed the USP XX in vitro dissolution specification were bioequivalent with respect to half-life, area under the curve, and maximum plasma concentration, but not with regard to time to maximum plasma concentration. On the other hand, the slow dissolving capsule, which failed the dissolution specification, was also bioequivalent to the other products in terms of half-life and area under the curve, and for maximum plasma concentration the slow dissolving capsule was bioequivalent to two of the three formulations that passed dissolution testing. The slow dissolving capsule also was not bioequivalent with respect to time to maximum plasma concentration. This study indicates that there is a poor correlation between the single point USP XX *in vitro* aspirin dissolution test results and *in vivo* pharmacokinetic behavior. However, none of the statistical differences were likely to be clinically significant. Therefore, while this dissolution test cannot be used to predict absorption characteristics, it can satisfactorily accomplish the goal it was designed for, that is, to ensure lot-to-lot manufacturing consistency.

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