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BIOAVAILABILITY AND IN VITRO DISSOLUTION OF TIOPINAC FROM SOLUTION AND CAPSULE FORMULATIONS

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SUMMARY

The absorption of a 75 mg dose of tiopinac, a non-steroidal anti-inflammatory agent, given orally as: (a) a solution; (b) capsules containing milled tiopinac; (c) capsules containing coarse topinac; and (d) capsules containing the sodium salt was evaluated in human volunteers. Also, in vitro dissolution studies were performed under different conditions on the dosage forms under study. The total absorption of tiopinac was complete from all dosage forms tested; however, the absorption rate was substantially and significantly affected by the dosage form. The order of the dosage forms according to decreasing absorption rate was: (1) solution; (2) sodium tiopinac capsules; (3) milled tiopinac capsules; (4) coarse tiopinac capsules. Using maximum plasma levels to reflect absorption rate these differences were statistically significant except for the milled vs coarse capsule comparison. Time to maximum plasma levels for each formulation were also compared. Some of the plasma profiles following the capsule doses exhibited 'double peaks', which was especially true in the case of the milled tiopinac formulation. This phenomenon was believed to be due to several factors: (a) the fact that 3 capsules (25 mg each) were administered; (b) 'gastric-emptying-rate' controlled absorption; and (c) the rapid distribution and elimination kinetics of tiopinac. In vitro dissolution conditions were established that correlated in vitro dissolution rate, tiopinac particle size (specific surface area) and in vivo absorption rate for the tiopinac acid capsule formulations. The in vitro dissolution conditions required modification to correlate the dissolution rate of the acid tiopinac capsules and the sodium salt tiopinac to correspond to their in vivo absorption rates. The effects of the dissolution medium and the dissolution apparatus on in vitro dissolution were also presented and discussed.

INTRODUCTION

As a part of Phase I development of a new therapeutic drug, it is important to ascertain the human bioavailability of the dosage form(s) intended for use in the clinical trials and what dosage form parameters alter bioavailability or characteristics thereof. In addition, the establishment of a relationship between a bioavailability parameter and a measurement of in vitro dissolution would be most helpful in establishing an in vitro dissolution specification for control of early clinical batches.

The physicochemical factors affecting drug absorption are generally well known and discussed in many publications and texts (for example, Lachman et al., 1976). Two physicochemical parameters, surface area and salt form, are assessed in the present study for their effect on bioavailability and in vitro dissolution.

Tiopinac (6,11-dihydro-11-oxidobenzo(b,e)thiepin-3-acetic acid) is a non-steroidal anti-inflammatory agent demonstrated to have potent activity in a number of animal models (Ackrell et a., 1978; Rooks et al., 1980). Its bioavailability in monkeys has been reported (Kent et al., 1977). This paper will describe the bioavailability of a 75 mg dose of tiopinac when given orally as: (a) a solution; (b) capsules containing milled (fine) tiopinac; (c) capsules containing coarse tiopinac; and (d) capsules containing the sodium salt. Also, the in vitro dissolution of the solid dosage forms and its relationship to the bioavailability parameters will be reported.

MATERIALS AND METHODS

Subjects. Twelve healthy male volunteers whose average age was 31 years (19–52 years) and whose average weight was 75.8 kg (61.8–86.4 kg) participated in the study. All subjects exhibited normal vital signs and selected laboratory parameters and were without evidence of renal, genito-urinary or hepatic disease. None had a history of medication allergies. The subjects were not allowed to use concomitant medications of any type during the course of the trial and were not permitted to consume alcoholic beverages between 48 h before and 24 h after administration of each dose. The subjects were required to abstain from any medication or other drugs for two weeks prior to the onset of the trial.

Formulations and dosing. Seventy-five mg of tiopinac (as the acid) was administered as a single dose under open-label conditions. The formulations are described in Table 1 and consisted of; (A) an oral solution, 200 ml administered; (B) capsules containing milled tiopinac, 3–25 mg capsules administered; (C) capsules containing coarse tiopinac, 3–25 mg capsules administered; and (D) capsules containing sodium tiopinac, 3–30.1 mg capsules administered (equivalent to 3–25 mg tiopinac capsules).

Each formulation was administered orally to each subject on one occasion between 19.00 and 20.00 h according to the randomization schedule (Table 2). The subjects fasted for 12 h prior to each administration. Heparinized venous blood specimens were obtained just prior to administration (baseline) and at 15, 30, 60 and 90 min and 2, 4, 8 and 12 h after administration of tiopinac. The study drug and all blood and plasma specimens were protected from direct light during all handling. The blood was centrifuged and the plasma frozen prior to assay for tiopinac by gas liquid chromatographic/mass spectrometric (GC/

TABLE 1

FORMULATIONS

Formulation A, an oral solution	mg/ml
Tiopinac	0.375
$NaH_2PO_4 \cdot H_2O$, USP	0.731
Na_2HPO_4, USP	13.4
Purified water, USP qs. ad	1.0 ml
Formulation B, a capsule Lot 150	mg/capsule
Tiopinac (milled (fine) material)	25.0
Lactose, spray dried, USP	258.5
Pregelatinized starch, USP	15.0
Magnesium stearate, USP	1.5
Total	300.0
Formulation C, a capsule Lot 156	mg/capsule
Tiopinac as a coarse material, amount and excipients same as B.	
Formulation D, a capsule Lot 157	mg/capsule
Tiopinac (the sodium salt, dihydrate equivalent to 25 mg of the acid)	30.1
Lactose, spray dried, USP	253.4
Pregelatinized starch, USP	15.0
Magnesium stearate, USP	1.5
Total	300.0

TABLE 2

RANDOMIZATION

Subject	Drug administration			
1	D	C	B	A
2	A	B	D	C
3	B	A	C	D
4	D	C	B	A
5	C	D	A	B
6	A	B	D	C
7	B	A	C	D
8	C	D	A	B
9	D	C	B	A
10	C	D	A	B
11	B	A	C	D
12	A	B	D	C

Formulations:
A = 75 mg, oral solution;
B = 75 mg, encapsulated milled material;
C = 75 mg, encapsulated coarse material;
D = 75 mg, encapsulated sodium salt.

MS) techniques. All subjects remained fasted until 4 h after test drug administration. Each capsule formulation was administered with 200 ml of water. A one-week interval separated each drug administration to allow plasma levels of tiopinac to attain baseline concentrations prior to subsequent doses.

GC/MS method for tiopinac in plasma. Since tiopinac is chemically unstable in the presence of light, blood and plasma samples were collected and stored in amber culture tubes (16 X 125 mm) and fitted with teflon-lined screw caps. The analysis procedure was carried out in the same tubes or in an area protected from direct light with glassware wrapped in aluminum foil.

To 1.0 ml of plasma, 100 ng of internal standard (2,5H-Dibenzo a, d cycloheptan-5-one-2-propionic acid) in 100 μl methanol was added. The plasma was diluted with water (1.0 ml) and acidified by the addition of 2 N HCl (0.5 ml). Tiopinac was removed from the acidified layer by extracting with 10 ml of hexane containing 30% ethyl acetate. The extracts were transferred to another tube and were evaporated at 60°C under nitrogen. The residue was dissolved in 3 ml acetonitrile and washed 4 times with 6 ml of hexane, centrifuging between each wash. The acetonitrile layer was then evaporated to dryness at 60°C under nitrogen.

After cooling, the residue was taken up in 0.4 ml of ethereal solution of diazomethane. The esterification reaction was carried out for 5 min at room temperature. Excess diazomethane and ether were evaporated under nitrogen. The residue was then taken up in 50 μl of ethyl acetate and 2–5 μl of ethyl acetate was injected onto the gas chromatograph connected to the mass spectrometer (a Finnigan Model 3200 gas chromatograph/mass spectrometer with a Model 6000 data system fitted with a chemical ionization source, methane as the reagent gas and a GC column, 61 cm X 2 mm i.d. glass column packed with 3% SP 2250 on 100/120 mesh Supelcoport) Selected ion monitoring at mass = 299 for the derivatized tiopinac and at mass = 293 for the derivatized internal standard provided the ion mass chromatograms. A blank plasma sample processed according to this procedure was clean. Calibration curves were constructed by adding 0–200 ng of tiopinac to plasma containing 100 ng of internal standard and processing according to the described procedure. The concentration of tiopinac in the unknown sample was determined from this calibration curve. The ratio of the peak area of the ion chromatogram for tiopinac over that for the internal standard was linear as a function of ng of tiopinac added per ml of plasma (10–200 ng/ml). The coefficient of variation over this concentration range varied from 0.8 to 4.1%.

Parameters evaluated. Comparisons among the 4 formulations were made with respect to the following parameters: (1) plasma concentrations at all sampling times (Cp 1 h, etc.); (2) time to maximum plasma concentration (T_{max}); (3) maximum plasma concentration (Cp_{max}) disregarding time of occurrence; (4) sequential areas under the plasma concentration curve (AUC), computed using the trapezoidal rule; and (5) total area under the plasma concentration curve, computed using the trapezoidal rule up to 12 h and thereafter by integration of an extrapolated exponential decay curve for each subject.

Data exclusions. The only anomaly occurring in the study was with respect to the 8 and 12 h plasma concentrations for subject 12, formulation A (oral solution); the 8 h tiopinac plasma concentration was slightly lower than the 12 h concentration. It was decided to exclude the 8 and 12 h plasma levels from the statistical analysis, along with

any parameters involving them. Specifically, the 8 and 12 h plasma concentrations, AUC 0–12 h, and total area under the plasma curve for subject 12 formulation A, were excluded from the statistical analysis.

Statistical methods. The statistical analysis of the data is that associated with a replicated Latin Square. The analysis is similar to that described as Plan 5 (Winer, 1971). Both univariate and multivariate analyses of variance were performed using the Statistical Analysis System (SAS) GLM procedure (Barr et al., 1976). Sums of squares identified as Type IV in SAS were used in constructing the F-ratios. Wilk's criterion was used in the multivariate analysis, which was done as an attempt to monitor the occurrence of spuriously significant results which is a concern when each of the computed parameters and plasma concentrations is analyzed individually. The multivariate analysis is a global test which controls the Type I error at the nominal level. Univariate analyses of variance were subsequently performed on each computed parameter and plasma concentrations at all sampling times. Where significant differences were shown to exist, as indicated by *P*-values less than 0.05, multiple comparisons were carried out using Duncan's new multiple range test (Miller, 1966). In view of the one-week interval between successive administrations, carry-over effects were not anticipated and were not provided for in the statistical analysis.

Surface area determination. The specific surface area of tiopinac contained in the capsule formulations was determined using the single-point BET procedure (Lowell, 1979; Quantasorb, Quantachrom, Syosset, N.Y. 11791). Each value represents the average of 3 determinations.

In vitro dissolution. The in vitro dissolution apparatus has been described previously (Kent et al., 1977). Early dissolution experiments utilized a polyethylene propeller stirrer (PPS) (Kent et al., 1977). The later studies utilized the USP paddle method as described in the USP XIX, fifth supplement. The stirring rate for the PPS method was 75 rpm while the USP paddle method employed 50 rpm. The dissolution medium volume was 0.5 liters with the PPS method (unless indicated otherwise) and 0.9 liters with the USP paddle method. Temperature of the dissolution medium was maintained at 37°C. The buffer systems used were pH 7.0, 0.1 M phosphate; pH 5.9, 0.1 M phosphate; pH 5.1, 0.1 M citrate/phosphate; pH 4.1, 0.1 M citrate/phosphate. Each capsule was located at the bottom of the dissolution vessel by use of a stainless steel wire, coiled loosely around the capsule. The UV detector of the automated system (Kent et al., 1977) monitored the dissolution at 254 nm. Per cent dissolved was determined from the Beer's law plot generated from a set of standards. In addition to the capsule formulations (B, C and D) utilized in the bioavailability study, capsule formulations (E and F), equivalent to B and C except for tiopinac of different surface area, were included in the in vitro dissolution portion of the study.

RESULTS

Clinical observations

Tiopinac when administered in 75 mg oral doses (one solution and 3 capsule formulations) was well tolerated by all of the 12 healthy male subjects who participated in the trial. None expressed subjective complaints nor manifested objective side-effects. None

demonstrated clinically important laboratory abnormalities which could be related to test drug administration.

Plasma level data

Mean plasma levels (μg/ml) for each formulation are presented in Tables 3 and 4 with statistical analysis. A plot of mean plasma levels vs. time for each formulation is also presented in Fig. 1. Statistically significant differences were observed at all sample times except 1.5 and 12 h (Table 3). Individual comparisons among the 4 formulations are presented in Table 4. The solution (A) provided significantly higher plasma levels than all other formulations at 15 and 30 min. At 1 h the solution (A) and sodium salt capsule (D) were comparable and significantly higher than the other two formulations. In general, over the early part of the plasma concentration vs. time curve (0–1.5 h) the solution and sodium salt capsule were comparable and presented the highest plasma levels while the milled (B) and coarse (C) capsule were comparable and presented the lowest plasma levels.

At 2 h and beyond considerable overlap was observed (Table 4). Generally, a reversal of the above trend occurred such that the solution (A) presented the lowest levels and the coarse capsule (C) the highest levels. The differences between the solution and coarse capsule were statistically significant at 4, 8 and 12 h. The milled (B) and sodium salt (D) capsule were quite similar through the terminal phase of the plasma concentration vs. time curve (2–12 h).

The plot of mean plasma levels vs. time (Fig. 1) graphically depicts the more rapid

TABLE 3

AVERAGED TIOPINAC PLASMA CONCENTRATIONS (μg/ml) FOLLOWING SINGLE ORAL DOSES

Time (h)	Formulations [a]				*P*-value
	A	B	C	D	
0.25	8.96	0.37	0.05	1.42	0.0001 [c]
0.50	9.08	2.50	0.81	6.56	0.0001 [c]
1.00	4.71	3.05	1.91	5.40	0.0001 [c]
1.50	3.08	2.68	2.16	3.29	0.1611
2.00	1.93	3.22	2.41	2.95	0.0184 [c]
4.00	0.76	1.40	2.13	1.04	0.0001 [c]
8.00	0.27 [b]	0.28	0.36	0.29	0.0075 [c]
12.00	0.16 [b]	0.18	0.20	0.18	0.0644

[a] Formulations:
A = 75 mg, oral solution;
B = 75 mg, encapsulated milled;
C = 75 mg, encapsulated coarse;
D = 75 mg, encapsulated sodium salt.

[b] Data from subject 12, formulation A, excluded.

[c] Statistically significant difference ($P < 0.05$).

TABLE 4

MULTIPLE COMPARISONS [a] AMONG MEAN TIOPINAC PLASMA CONCENTRATIONS (μg/ml)

Time	Formulations [b]			
15 min	C : 0.05	B : 0.37	D : 1.42	A : 8.96
30 min	C : 0.81	B : 2.50	D : 6.56	A : 9.08
1 h	C : 1.91	B : 3.05	A : 4.71	D : 5.40
1.5 h	C : 2.16	B : 2.68	A : 3.08	D : 3.29
2 h	A : 1.93	C : 2.41	D : 2.95	B : 3.22
4 h	A : 0.76	D : 1.04	B : 1.40	C : 2.13
8 h	A : 0.27 [c]	B : 0.28	D : 0.29	C : 0.36
12 h	A : 0.16 [c]	B : 0.18	D : 0.18	C : 0.20

[a] Duncan's new multiple range test – means underlined by the sample line are *not* significantly different at $P < 0.05$.

[b] Formulations:

A = 75 mg, oral solution;

B = 75 mg, encapsulated milled;

C = 75 mg, encapsulated coarse;

D = 75 mg, encapsulated sodium salt.

[c] Data from subject 12, formulation A, excluded.

absorption of tiopinac from the solution and encapsulated sodium salt dosage forms as compared to the milled and coarse capsule formulations. Another interesting observation is the 'double peak' character of the plasma profile for the milled capsule formulation and the 'sustained absorption' character of the plasma profile for the coarse capsule formulation. These curves will be commented on further in the Discussion.

Various computed bioavailability parameters are presented and statistically analyzed in Tables 5 and 6. The mean Cp_{max} was greatest following the solution dose (10.6 μg/ml) followed by the sodium salt capsule (7.10 μg/ml) and these values were significantly different from each other and the two acid capsules. Mean Cp_{max} following the milled capsule (4.25 μg/ml) and the coarse capsule (3.78 μg/ml) was not significantly different (Table 6).

T_{max} was earliest following the solution (22.5 min) followed by the sodium salt capsule (51.3 min) but these differences were not statistically significant. T_{max} following the milled capsule (90.0 min) was significantly different from the solution but not the sodium salt capsule. The coarse capsule provided the most prolonged T_{max} (158 min) and this was significantly longer than all of the other formulations (Table 6). It should be pointed out that in plasma profiles exhibiting a double peak the T_{max} corresponding to the highest C_{max} was selected for computer analysis.

Sequential AUC was evaluated over the 0–1-, 2-, 4- and 12-h intervals. The solution (A) AUC was always the largest followed by the sodium salt capsule (D), followed by the

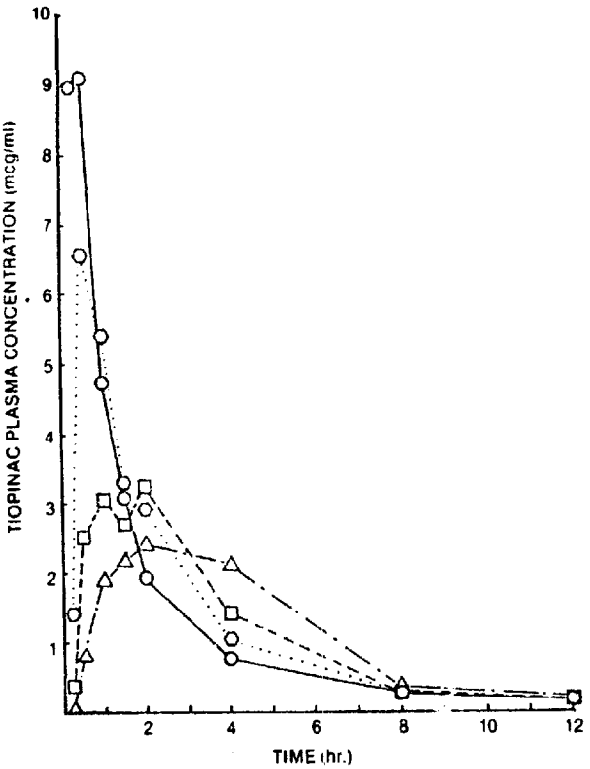

Fig. 1. Mean tiopinac plasma levels vs. time following a 75 mg oral dose of: (A) a solution (○); (B) 3–25-mg capsules containing milled tiopinac (□); (C) 3–25-mg capsules containing coarse tiopinac (△); and (D) 3–25-mg (acid equivalent) capsules containing sodium tiopinac (○).

TABLE 5

VARIOUS BIOAVAILABILITY PARAMETERS CALCULATED FOLLOWING SINGLE ORAL DOSES OF TIOPINAC

Parameter	Formulations [a]				*P*-value
	A	B	C	D	
T_{max} (min)	22.50	90.00	157.50	51.25	0.0001 [d]
Cp_{max} (μg/ml)	10.56	4.25	3.78	7.10	0.0001 [d]
Area, total [b]	17.41 [c]	15.91	15.86	17.28	0.1103
Area, 0–1 h	6.82	1.79	0.79	4.17	0.0001 [d]
Area, 0–2 h	10.02	4.70	2.96	7.90	0.0001 [d]
Area, 0–4 h	12.71	9.31	7.49	11.89	0.0001 [d]
Area, 0–12 h	15.62 [c]	13.58	13.60	15.49	0.0120 [d]

[a] Formulations:
A = 75 mg, oral solution;
B = 75 mg, encapsulated milled;
C = 75 mg, encapsulated coarse;
D = 75 mg, encapsulated sodium salt.
[b] Areas are in units of (μg/ml) · h.
[c] Data from subject 12, formulation A, excluded.
[d] Statistically significant difference ($P \leq 0.05$).

TABLE 6

MULTIPLE COMPARISONS [a] AMONG MEANS OF COMPUTED PARAMETERS

	Formulations [b]			
T_{max} (min)	A : 22.50	D : 51.25	B : 90.00	C : 157.50
Cp_{max} (μg/ml)	C : 378	B : 4.25	D : 7.10	A : 10.56
Area, Total [c]	C : 15.86	B : 15.91	D : 17.28	A : 17.41 [d]
Area, 0–1 h	C : 0.79	B : 1.79	D : 4.17	A : 6.82
Area, 0–2 h	C : 2.96	B : 4.70	D : 7.90	A : 10.02
Area, 0–4 h	C : 7.49	B : 9.31	D : 11.89	A : 12.71
Area, 0 –12 h	B : 13.58	C : 13.60	D : 15.49	A : 15.62 [d]

[a] Duncan's new multiple range test – means underlined by the sample line are *not* significantly different at $P < 0.05$.

[b] Formulations:
A = 75 mg, oral solution;
B = 75 mg, encapsulated milled;
C = 75 mg, encapsulated coarse;
D = 75 mg, encapsulated sodium salt.

[c] Areas are in units of μg-h/ml.

[d] Data from subject 12, formulation A, excluded.

milled capsule (B) and lastly the coarse capsule (C) – except for the 0–12-h interval. These differences were generally statistically significant except for 0–12-h AUC comparisons (Table 6).

Mean total AUC did not show statistically significant differences among any of the formulations. However, the same general trend noted in the sequential AUC analysis was observed for total AUC such that the solution total AUC was greatest (17.4 μg/ml $\times$h), followed by the sodium salt capsule (17.3 μg/ml $\times$ h) followed by the milled and coarse capsule (15.9 μg/ml $\times$ h). In a previous metabolism study (Mroszczak et al., 1980) it was shown that an oral solution dose was completely absorbed as evidenced by equivalent total AUCs following intravenous and oral administrations. Hence, one could conclude that since all formulations in the present study are equivalent (in total AUC) to the oral solution dose, all formulations were completely absorbed.

Specific surface area

The mean values and standard deviation for the specific surface area determinations for the different lots of tiopinac and the one lot of tiopinac sodium salt are reported in Table 7. The tiopinac utilized in formulation C was representative of unmilled material, while that used in formulation B was finely milled. That in both formulations E and F was between the two extremes. The specific surface area of tiopinac sodium salt (D) was extremely high, which was probably due to the precipitation method used in its preparation.

TABLE 7

SPECIFIC SURFACE AREAS OF TIOPINAC AND ITS SODIUM SALT USED IN THE CAPSULE FORMULATIONS STUDIED

Formulation code	Lot	Specific surface area Mean ± S.D. (m^2/g, n = 3)
B (fine)	150	0.803 ± 0.004
C (coarse)	156	0.147 ± 0.002
D (sodium salt)	157	7.91 ± 0.02
E	51a	0.346 ± 0.0
F	46	0.64 ± 0.01

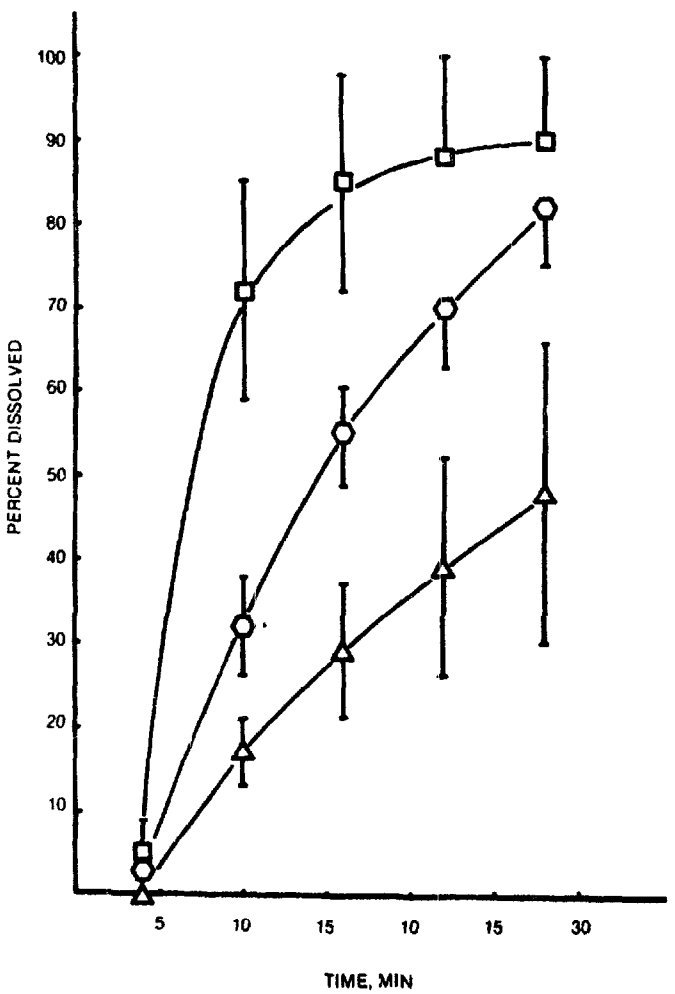

Fig. 2. In vitro capsule dissolution profiles, PPS method, 37°C, 75 rpm, 0.5 liters, pH 5.9. Formulations: B, □; C, △; D, ○; error bars are S.D.

In vitro dissolution

In vitro dissolution results from the PPS method are depicted in Fig. 2. This figure includes the complete in vitro dissolution profile at pH 5.9 of the capsule formulations used in the bioavailability section. It should be noted that under these conditions the dissolution rate of formulation D, the sodium salt, was less than that of tiopinac formulation B containing finely milled drug. This would not be expected due to the high solubility of the sodium salt. A substantial difference in the dissolution rate of formulations B and C was observed and expected if the dissolution conditions were to be discriminatory.

The in vitro dissolution profiles of formulations B, C, E and F are described in Fig. 3. This figure depicts the dissolution profiles utilizing USP paddle method at 50 rpm 0.9 liters and pH 5.9. In comparison with the PPS method, Fig. 2, the USP method indicates a higher dissolution rate for the two formulations included in both procedures. Also, a

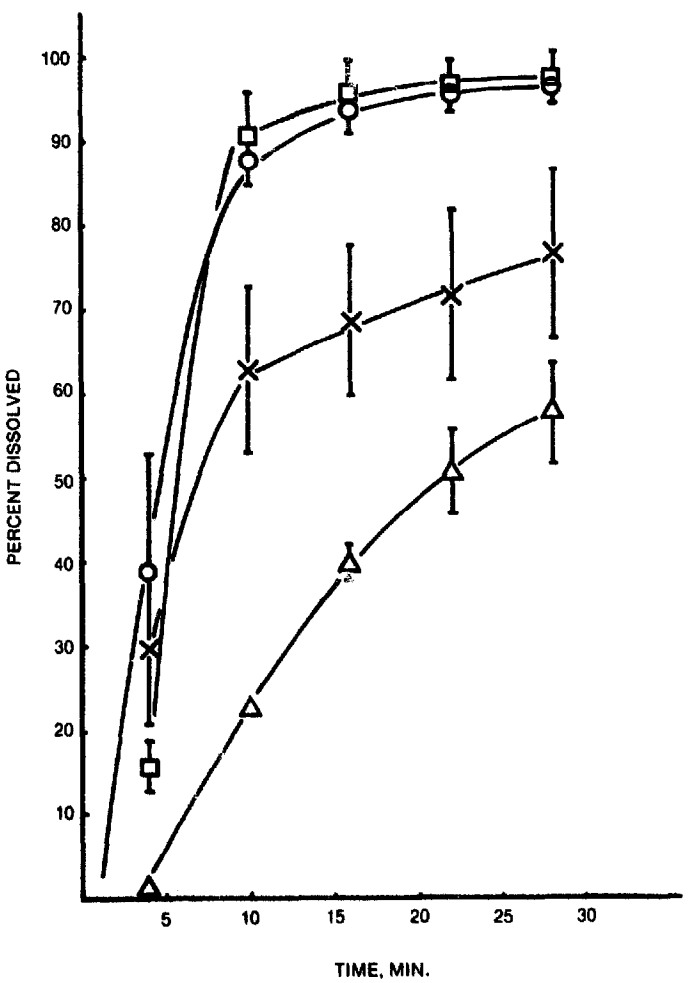

Fig. 3. In vitro capsule dissolution profiles, USP paddle method, 37°C, 50 rpm, 0.9 liters, pH 5.9. Formulations: B, □; C, △; E, X; F, ○; error bars are S.D.

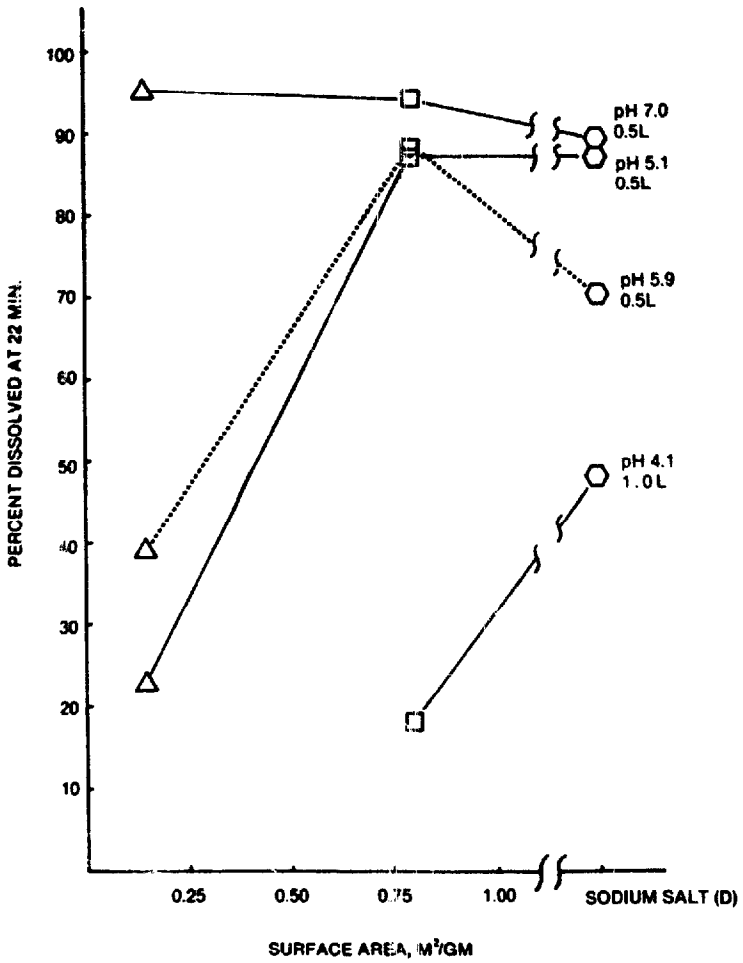

Fig. 4. In vitro capsule dissolution at one time point as a function of pH, sodium salt, and surface area of the acid form. PPS method, 37°C, 75 rpm, pH and volume as indicated. Formulations: B, □; C, △; D,○.

comparison of the variability between methods suggests that the USP method has slightly less variability.

The effect of the dissolution medium pH and the surface area of the formulated tiopinac on in vitro dissolution is shown in Fig. 4. This figure indicates, as would be predicted for dissolution of weak organic acids, an increase in per cent dissolved as the pH is increased. Also, an increase in specific surface area of the free acid tiopinac resulted in an increase in the per cent dissolved following dissolution theory. The formulation containing the tiopinac sodium salt (D) was included in this comparison to demonstrate that a dissolution rate greater than that of the fine tiopinac acid formulation (B) was not shown for the sodium salt (D) until a dissolution medium pH of 4.1 was utilized. This difference in dissolution rate at the low pH can be understood based on the solubility at the micro-

environment of the dissolving particles. However, the rationale for the lower dissolution rate of the sodium salt formulation relative to the acid formulation at pHs above this is not apparent.

DISCUSSION

The plasma level curves for the fine and coarse capsules (as compared to the solution or sodium salt capsule) exhibited unusual characteristics (Fig. 1). Following the solution dose (Fig. 1A) none of the subjects exhibited a double peak and T_{max} occurred at 0.25 or 0.5 h in all subjects. Hence, in the solution control group, rapid absorption and a smooth decline in plasma levels following Cp_{max} was observed. This eliminates unusual disposition characteristics of the drug, such as enterohepatic circulation, for example, as an explanation of the double peak. Six of the 12 subjects receiving the milled tiopinac capsules (B) exhibited a double peak and T_{max} ranged from 0.5 to 4 h. Therefore, this clearly must be the result of the formulation and method of dosing. In this study, tiopinac was formulated into capsules and three 25-mg capsules were administered (as a single dose) to each subject. Four of the 12 subjects receiving the coarse tiopinac capsules (C) exhibited a double peak but 6 of the subjects exhibited a single delayed peak which occurred from 2 to 4 h post-dosing. Each subject received three 25-mg capsules as above.

It is suggested that the double peak in some of the tiopinac plasma profiles following tiopinac (acid) capsules results from 'stomach-emptying-rate' control of absorption (Clements et al., 1978). The 'stomach-emptying-rate' of a solution is normally continuous because the dose is homogeneous. This appears to be the case with the sodium salt formulation which may rapidly dissolve in the stomach. However, the gastric emptying rate of the acid formulations given in 3 capsules may be discontinuous. For example, the contents of 2 of the 3 capsules may be emptied into the small intestine within 10 min after ingestion but emptying of the third capsule may be delayed for an hour (or longer) following ingestion. Hence, two absorption surges can occur and double peaking may be observed. Relative to other drugs the effect is amplified in the case of tiopinac because of the very steep decline of plasma concentrations immediately after peak plasma levels (Fig. 1) and because of the pronounced solubility increase that occurs when the contents of the acid capsules transit from the stomach (low pH) to the small intestine (high pH) as demonstrated by in vitro dissolution in Fig. 4. It should be noted that this model is consistent with negligible absorption from the stomach. The coarse tiopinac capsules exhibited more of a 'sustained absorption' character and this may be explained by the above considerations along with a much slower dissolution rate (and consequently absorption rate) superimposed (as demonstrated in Figs. 2 and 3).

It is the goal of an in vitro dissolution method to differentiate between dosage forms that exhibit different bioavailability characteristics. As discussed previously, the extent of bioavailability of the 3 capsule formulations (B, C and D) and the solution (A) were equivalent. However, the overall plasma profile and absorption rate characteristics (Fig. 1 and Table 6) were different in each case. Hence, the AUC for early times (e.g. 0–2 h) T_{max} or Cp_{max} may be used as a comparative measure of absorption rate. These parameters are correlated to the in vitro dissolution response for the tiopinac acid capsule formulations (B and C) in Table 8. Admittedly there are only two formulations for this comparison

TABLE 8

IN VITRO DISSOLUTION RESULTS (USP) AT 22 MIN FOR FORMULATIONS B AND C COMPARED TO IN VIVO ABSORPTION RATES AS MEASURED BY AUC, Co_{max} AND T_{max}

Formulation	% dissolved at 22 min (USP) (Mean ± S.D.)	AUC (0–2 h) (μg-h/ml)	T_{max} (min)	Cp_{max} (μg/ml)
B	97 ± 3	4.70	90.0	4.25
C	51 ± 5	2.96	157.5	3.78

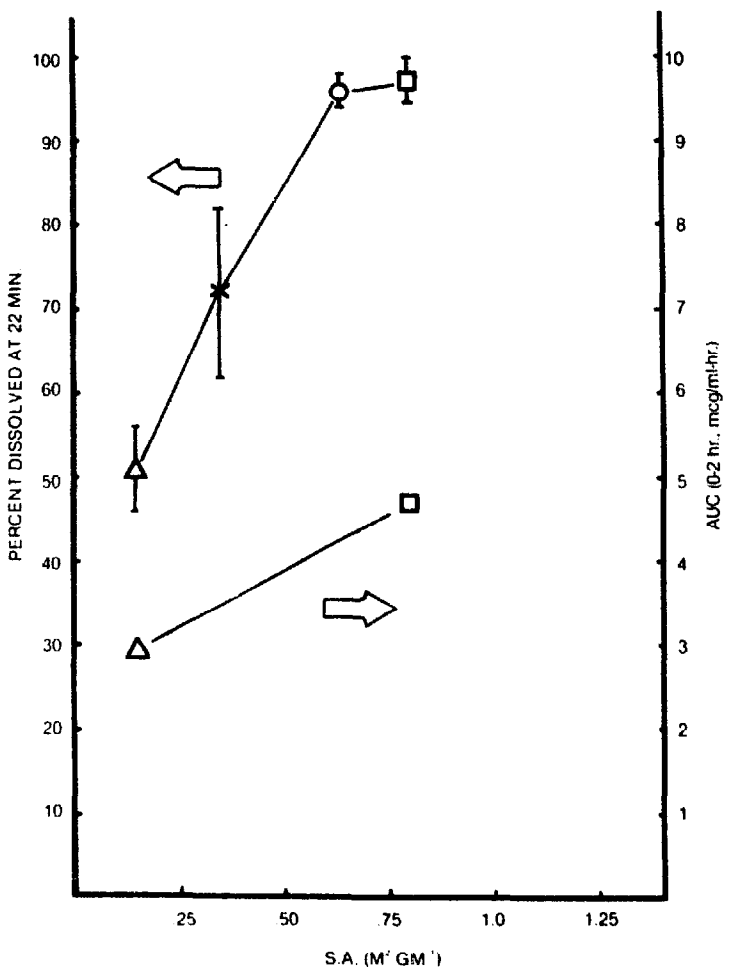

Fig. 5. Relationship between in vitro dissolution (USP paddle, 50 rpm, pH 5.9) of tiopinac capsules and surface area of tiopinac and the absorption rate parameter, AUC (0–2 h). Formulations: B; □; C, △; E, X; F, ○.

which certainly places limitations on its value. However, in the early stages of a drug development program this yields valuable information regarding the meaning of the in vitro dissolution results and their corresponding relation to in vivo absorption rates.

Additional information is obtained when the specific surface area of the tiopinac is included in this comparison (Fig. 5). This figure describes visually the relationship of per cent dissolved (in vitro dissolution) to surface area to AUC (0–2 h). AUC (0–2 h) was used in this comparison; however, Cp_{max} or T_{max} could also have been utilized as the measure of absorption rate. The information now relates surface area of tiopinac acid, a physical parameter, to an in vivo absorption parameter, AUC. This suggests that control of tiopinac surface area will provide appropriate in vitro dissolution values and the corresponding in vivo absorption characteristic. Overall, this data indicates that the in vivo absorption rate of tiopinac is dissolution rate-controlled.

The fact that the in vitro dissolution rate (Fig. 4) of capsules of tiopinac sodium salt (D) was not greater than the tiopinac acid capsule (B) until an acidic pH of 4.1 was reached leads to speculation as to the mechanism by which the salt form yields a more rapid absorption rate versus the acid formulation (B). This indicates that tiopinac sodium salt formulation (D) dissolves rapidly in the gastric environment providing a solution for ready transport to and absorption from the upper intestine or possibly finely precipitated particles of the acid which move into the intestinal tract to undergo rapid dissolution and absorption. This is in agreement with the general theory on the rationale for the use of a sodium salt of a weak acid for improved in vivo absorption (Lachman et al., 1976). It is important to note, however, that an in vitro dissolution method that distinguishes among formulations of a weak acid, such as tiopinac, may provide misleading information on the predicted in vivo absorption rate of a formulation containing the salt of the weak acid, eg. tiopinac sodium salt. In vitro dissolution conditions must be modified such that in vitro dissolution results will correspond to in vivo absorption parameters if a meaningful in vitro dissolution test (and specification) is desired.

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REFERENCES

Ackrell, J., Antonio, Y., Franco, F., Landeros, R., Leon, A., Muchowski, J.M., Maddox, M.L., Nelson, P.H., Rooks, W.H., Roszkowski, A.P. and Wallach, M.B., Synthesis and antiinflammatory activity of 6,11-dihydro-11-oxodibenzo (b, e) thiepinalkanoic acids and related compounds. J. Med. Chem., 21 (1978) 1035–1044.

Barr, A.J., Goodnight, J.H., Sall, J.P. and Helwig, J.T., A Users Guide to SAS 76, SAS Institute, Raleigh, N.C. 1976, pp. 127–144.

Clements, J.A., Heading, R.C., Nimmo, W.S. and Prescott, L.F., Kinetics of acetaminophen absorption and gastric emptying in man. Clin. Pharmacol. Ther., 24 (1978) 420–431.

Kent, J.S., Mroszczak, E. and Yost, M., The use of radio-labeled drug in early dosage form development to provide a relation between physical dosage form characteristics and bioavailability. Drug Develop. Ind. Pharm., 3 (1977) 507–522.

Kent, J.S., Wong, P.P. and Hegde, G.P., Design and evaluation of an automated system for in vitro dissolution testing utilizing a high-pressure liquid chromatographic multiport switching value. J. Pharm. Sci., 66 (1977) 1665–1670.

Lachman, L., Lieberman, H.A. and Kanig, J.L., The Theory and Practice of Industrial Pharmacy, 2nd Edn., Lea and Febiger, Philadelphia, 1976, pp. 93–104.

Lowell, S., Introduction to Powder Surface Area, John Wiley, New York, 1979, p. 34.

Miller, R.G., Jr., Simultaneous Statistical Inference, McGraw-Hill, New York, 1966, pp. 81–89.

Mroszczak, E.J. and Lee, F.W., Tiopinac absorption, distribution, excretion and pharmacokinetics in man and animals. Drug Metab. Dispos., (1980) in press.

Rooks, W.H., II, Tomolonis, A.J., Maloney, P.J., Roszkowski, A. and Wallach, M.B., The antiinflammatory and analgesic profile of 6,11-dihydrodibenzo-(b,e)-thiepin-11-one-3-acetic acid (Tiopinac). Agents and Actions, 10 (1980) 266–273.

Winer, B.J., Statistical Principles in Experimental Design, McGraw-Hill, New York, 1971, Chapter 9.