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Benoxaprofen, a New Anti-Inflammatory Agent: Particle-Size Effect on Dissolution Rate and Oral Absorption in Humans

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Abstract □ The particle-size effect of benoxaprofen, a new nonsteroidal anti-inflammatory agent, on the *in vitro* dissolution rate and oral absorption in humans was evaluated. Ten normal subjects participated in a randomized crossover-designed absorption study with two sieved particle-size formulations: one with crystals larger than 60 mesh (mean equivalent spherical diameter = 640 μm) and the other with crystals smaller than 100 mesh (mean equivalent spherical diameter = 67 μm). Plasma drug concentrations and urinary drug excretion were used to determine the relative absorption of the two formulations. The standard USP procedure was used for the dissolution study. Particle size had a dramatic effect on both the *in vitro* drug dissolution and its oral absorption in humans. *In vitro*, the smaller crystals dissolved more rapidly and more efficiently than the larger crystals. *In vivo*, the smaller crystals produced higher plasma concentrations, more rapid peak concentration attainment, and more drug excreted in the urine.

Keyphrases □ Benoxaprofen—effect of particle size on dissolution rate and oral absorption in humans □ Dissolution rate—benoxaprofen, effect of particle size □ Absorption, oral—benoxaprofen, effect of particle size □ Anti-inflammatory agents—benoxaprofen, effect of particle size on dissolution rate and oral absorption in humans

The particle size of sparingly soluble drugs (drugs that are practically insoluble in aqueous fluids at physiological pH) can affect their dissolution rate *in vitro* (1–7). Similarly, the rate and extent of drug absorption can be reduced *in vivo* when dissolution in GI fluids is limited by particle size (2, 8–11). In several cases, rank correlations were obtained between sparingly soluble drug dissolution *in vitro* and absorption *in vivo* (12). In several instances, drug dissolution improved when particle size was reduced, probably because an increased surface area was available for dissolution.

Certain physicochemical properties of the experimental anti-inflammatory compound benoxaprofen, *dl*-2-(4-chlorophenyl)-α-methyl-5-benzoxazoleacetic acid, indicate that its dissolution *in vitro* and its absorption *in vivo* might be affected by altering its crystal particle size. It is a crystalline solid at room temperature. The pKa in 66% dimethylformamide is 6.9, and its solubilities at 25° in phosphate buffer at pH 5.0, 6.0, 7.0, and 7.6 are 4.4, 21, 207, and 835 μg/ml, respectively[1].

Because of benoxaprofen's low aqueous solubility and its largely unionized, lipoid-soluble form in the GI tract, a rate-limiting step in oral absorption could be drug dissolution in the GI fluids. This report describes the relationship between drug particle sizes and dissolution rates *in vitro* and the effect of particle sizes on oral bioavailability in humans.

EXPERIMENTAL

Dosage Preparation—GLC showed benoxaprofen[2] to be 99.9% pure. Two particle-size fractions were isolated using U.S. standard mesh sieves. One fraction contained particles larger than 60 mesh (mean diameter = 640 μm), and one contained particles less than 100 mesh (mean diameter = 67 μm). The individual fractions were mixed with corn starch USP in a laboratory blender[3]. The blends were hand filled into size 2 gelatin capsules, each containing 100 mg of benoxaprofen and 180 mg of starch.

Dissolution Study—The assembly and conditions used to study the dissolution rate and extent of the two drug particle sizes are described in USP XIX (13). The dissolution assembly consisted of four variable-speed stirring motors attached to four basket and shaft assemblies with a three-blade stainless steel propeller mounted on each basket shaft immediately above each basket, four 3-liter beakers, and a water bath at 37 ± 0.5°. The dissolution medium for each determination was 2 liters of pH 7.6 phosphate buffer. One capsule was placed in each basket and rotated at 100 rpm. Four capsules from each formulation were tested.

Five-milliliter aliquots were withdrawn at 20, 60, and 120 min using a pipet fitted with a suitable filter. Aliquots withdrawn were not replaced with corresponding volumes of the dissolution medium[4]. After the 120-min sample withdrawal, the basket contents were quantitatively transferred to the remaining dissolution medium, a stirring bar was added, and the mixture was magnetically stirred (at >500 rpm) for ~1 hr. A final aliquot was then taken. The benoxaprofen content in each specimen was measured spectrophotometrically.

Clinical Study—In humans, the benoxaprofen availability from the dosage forms containing the two different particle sizes was compared on the basis of plasma unchanged drug concentration and of urinary excretion of the unchanged drug and its glucuronide conjugate.

Twelve healthy males participated in the study after being informed of the objectives, potential risks, and procedures. The subjects were 21–33 years and within an acceptable weight range (14). According to a randomized crossover design, each subject received a single oral 100-mg drug capsule. Two weeks elapsed between the single doses of the two parti-

[1] The pKa and solubilities of benoxaprofen were determined at the Lilly Research Laboratories, Indianapolis, Ind., by Dr. R. F. Childers, Jr. (unpublished data).

[2] Synthesized at the Lilly Research Centre in England.
[3] Twin-Shell.
[4] Corrections were made for changes in dissolution volume in calculations of the percent of drug released at each period.

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Table I—Analyses of Variance (Mean Squares)

Source	*df*	Plasma Concentrations 0.5 hr	1 hr	1.5 hr	2 hr	3 hr	6 hr	9 hr	12 hr
Subject	9	0.67	2.80	4.40	5.58	2.65	3.50	3.50	2.66
Day	1	0.04	0.90	0.02	5.33	0.08	0.56	0.13	0.11
Mesh	1	6.44[a]	62.92[b]	156.75[b]	349.39[b]	342.09[b]	80.10[b]	44.00[b]	47.63[b]
Residual	8	0.95	3.90	6.15	4.09	1.39	1.23 (7 *df*)	2.75 (7 *df*)	0.97

Source	*df*	Plasma Concentrations 24 hr	48 hr	72 hr	96 hr	Peak	Time of Peak	Area	Urine
Subject	9	1.20	0.60	0.36	0.22	4.47	4.87	5,842.13	85.16
Day	1	0.04	0.04	0.33	0.07	1.28	1.52	726.19	0.02
Mesh	1	16.28[b]	2.86[b]	0.60[a]	0.13	208.03[b]	41.42[b]	95,631.95[b]	938.56
Residual	8	0.45	0.14	0.11	0.04	5.21	2.70	1,931.58	14.49

[a] $p < 0.05$. [b] $p < 0.01$.

cle-size formulations. This period represents about 10–12 plasma disappearance half-lives of the unchanged drug.

All subjects fasted overnight (at least 8 hr) before dosing. No food or liquid, except water, was permitted for at least 2 hr after the study dose, when a standard light meal was offered. Water, *ad libitum*, was permitted during this period. All doses were taken with 180 ml of water. After dosing, subjects remained ambulatory or sitting for at least 2 hr.

Blood samples (10 ml) were obtained by venipuncture at 0, 0.5, 1, 1.5, 2, 3, 6, 9, 12, 24, 48, 72, and 96 hr after dosing. The samples were heparinized and centrifuged; the plasma was collected and frozen until assayed. Total urine samples were collected every 6 hr for 24 hr and then daily through Day 4; the volume was measured and aliquots were frozen until assayed.

The plasma benoxaprofen concentration was determined by GLC after extraction and derivatization. Fifty micrograms of the mass internal standard, 2-(3,5-dichlorophenyl)-α-methyl-5-benzoxazoleacetic acid, in 1 ml of 0.01 *M* NaOH was added to 1 ml of plasma. The plasma proteins were precipitated with 2 ml of 10% trichloroacetic acid, and the mixture was extracted with 9 ml of 20% (v/v) hexane in ethyl acetate. The organic phase containing the drug and the internal standard was back-extracted into 5 ml of 0.3 *M* Na_3PO_4. The aqueous phase was recovered, made acidic with 2 ml of 1 *M* HCl, and reextracted with 5 ml of methylene chloride.

The organic phase was recovered and evaporated to dryness at 50° under a nitrogen stream, and the residue was treated with diazomethane in methylene chloride containing 10% (v/v) methanol. After evaporation, the derivatized material was dissolved in a small volume of methanol and analyzed by GLC using a 0.61-m × 3-mm i.d. glass column of 80–100-mesh Chromosorb W-HP coated with 2% OV-7. Chromatographic conditions were: injection temperature, 250°; column oven temperature, 235°; and flame-ionization detector temperature, 275°. Quantitation was by electronic integration.

The urine benoxaprofen concentration was measured as the sum of the unchanged free form plus the amount resulting from glucuronide conjugate hydrolysis (15). The analytical technique was the same as for plasma, except that the urine plus the added internal standard was treated with β-glucuronidase[5] before extraction to hydrolyze the glucuronide metabolite to the free drug and glucuronic acid.

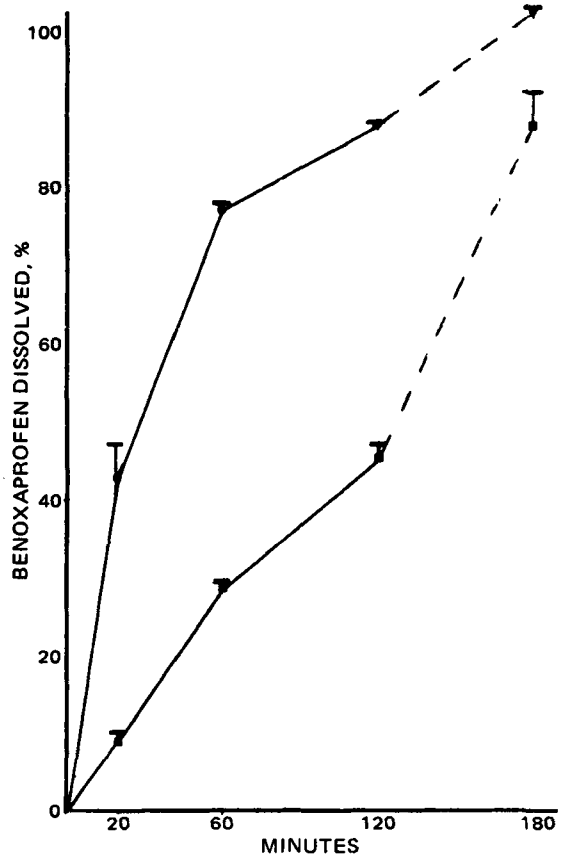

Figure 1—*Dissolution of two benoxaprofen particle-size formulations using the standard (USP XIX) 2-hr* in vitro *procedure. Key:* ■, *crystals larger than 60 mesh;* ●, *crystals smaller than 100 mesh; and* - - -, *results of vigorous magnetic stirring for an additional 1 hr. Each data point represents the mean of four assays with standard error.*

RESULTS AND DISCUSSION

The *in vitro* dissolution of the two benoxaprofen particle sizes is illustrated in Fig. 1. Twice as much benoxaprofen dissolved from the small particle formulation at each sampling time as from the large particle formulation, except the final sampling after an additional hour of vigorous stirring (>500 rpm with stirring bar). Since the medium would be only 6% of saturation if the 100 mg of drug dissolved, it was expected that both particle sizes would dissolve completely during this additional period of vigorous stirring. Instead, even under these relatively extreme conditions, the large particles dissolved relatively poorly.

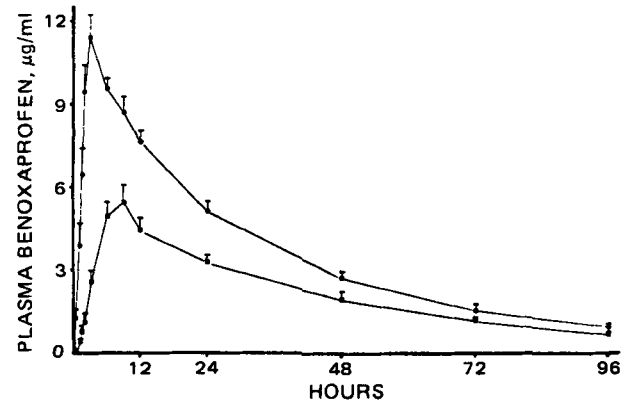

Figure 2—*Plasma unchanged benoxaprofen concentrations after oral dosing with 100 mg of two particle-size formulations. Key:* ■, *crystals larger than 60 mesh; and* ●, *crystals smaller than 100 mesh. Each data point represents the mean of 10 subjects with standard error.*

[5] Ketodase, General Diagnostics, Morris Plains, N.J.

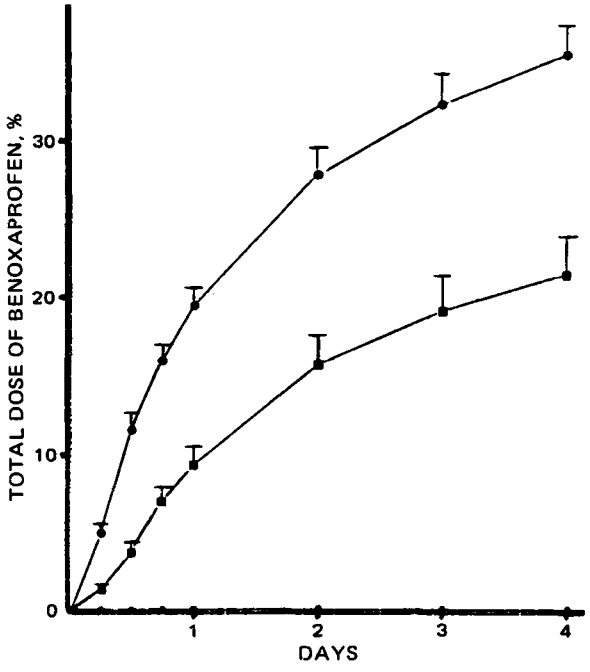

Figure 3—*Cumulative urinary benoxaprofen excretion (unchanged plus glucuronide metabolite) after oral dosing with 100 mg of two particle-size formulations. Key: ■, crystals larger than 60 mesh; and ●, crystals smaller than 100 mesh. Each data point represents the mean of 10 subjects with standard error.*

The average large particle dissolution after vigorous stirring was 87% of the drug in the capsules; the corresponding value for the small particles was 102%. Large particle dissolution also was more variable than dissolution of the small particles. Relative standard deviations for the average 20-, 60-, and 120-min large particle dissolution values were 26.7, 9.0, and 9.4, respectively; the corresponding determinations for the small particles were 25.6, 4.1, and 1.8.

These *in vitro* studies show that the dissolution of the two sieved benoxaprofen fractions containing particles with an ~10-fold difference in mean diameter was significantly different.

In the clinical study, two subjects withdrew after one dose for reasons other than those relating to drug dosing. The plasma data from the 10 subjects who completed the study are depicted in Fig. 2. Particle size dramatically affected both the rate and extent of GI benoxaprofen absorption. The calculated mean peak plasma concentration (12.0 μg/ml) for the small particle sizes was twice that for the large ones (5.5 μg/ml). Differences were also reflected in the calculated mean time to peak, 3.2 *versus* 6.2 hr, and the area under the mean plasma concentration–time curve, 357 *versus* 213 μg/hr/ml (0–96 hr).

The difference in benoxaprofen absorption from the two formulations also was reflected in the urinary drug and metabolite excretion (Fig. 3). An average of ~20% of the drug in the small particle formulation was excreted in the first 24 hr as the free plus glucuronide form compared to about 9% with the large particle formulation. Total drug excreted in the urine during the 96-hr collection averaged 35 and 21 mg, respectively.

That the differences in absorption noted were due to particle size is supported by analyses of variance (Table I) on the timed plasma levels, peak values, time to peak, area under plasma concentration–time curve, and total urinary drug excretion. The p value was <0.01 for all variables except the 0.5-, 72-, and 96-hr plasma levels.

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