Laboratory Studies with Nitrofurantoin

Relationship Between Crystal Size, Urinary Excretion in the Rat and Man, and Emesis in Dogs

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Studies were conducted relating the effect of varying the crystal size of orally administered nitrofurantoin on absorption and urinary excretion in the rat and man and emesis in dogs. It was discovered that there is an optimal average crystal size of about 150 mesh, or its equivalent surface area falling between 80 to 200 mesh limits, which reduces emesis while still permitting ample urinary excretion for efficacy.

THE USE OF nitrofurantoin[1] as a urinary tract antibacterial agent has become firmly established since initial reports of the first laboratory (1, 2) and clinical studies (3–5). During the years of successful use of this drug a limited number of patients have exhibited nausea or emesis to the extent that they were deprived of an effective medication. Little or no success attended attempts to overcome this defect by combining other substances with nitrofurantoin, although experience indicates administration of the drug with meals or with milk is helpful (6). A recent review article (7) provides general information on nitrofurantoin.

Since obvious means of controlling or reducing nausea and/or emesis associated with drug administration have not proved completely satisfactory in the case of nitrofurantoin, a thorough examination of the problem was undertaken. While studies in these laboratories have not clearly delineated the mechanism of nitrofurantoin-induced nausea or emesis, there is some evidence to indicate this undesirable side effect may be of neural origin. Rate of drug absorption could possibly be a factor. Accordingly, modification of the absorption pattern of individual nitrofurantoin doses by controlling release into the gastrointestinal tract might reduce this undesirable side effect.

Although there are several means whereby the absorption pattern of a drug may be modified or controlled (8, 9), one effective way with compounds having limited water solubility is the control of the surface area available for solution in the gut by varying the crystal size. Numerous reports (10–13) demonstrate the fact that the rate and amount of absorption of slightly soluble drugs can be markedly increased by reducing crystal size thereby providing increased surface area for a given dosage. Unfortunately, increased toxicity often accompanies this change (14, 15). Conversely, the use of larger crystals of drug having less surface area per dose, toxicity (as evidenced by nausea and vomiting) might be eliminated or greatly reduced. Such an approach, however, is beset with the likelihood of diminution of efficacy. Since nitrofurantoin has a water solubility of only about 200 to 400 mg./L. at 37° at the physiological pH values expected in application, larger crystals having considerably less surface area for a fixed dosage would dissolve much more slowly, thereby slowing the rate of absorption.

EXPERIMENTAL

Nitrofurantoin of different crystal sizes was made by recrystallization from nitromethane. These crystals of rhombic anhydrous nitrofurantoin were sieved into appropriate size ranges and some micronized in a Sturdevant mill for finest material. To explore emetic effect, very coarse (50–60 mesh) crystals, fine crystals (200–325 mesh), and micronized material (<10 μ) were orally administered in gelatin capsules to 20 fasted dogs in two independent comparative studies in single doses ranging from 10 to 20 mg./Kg. in the first and at a higher 25 mg./Kg. level in the second trial. An ample clearance time of 3 days between doses was established. Data from these studies are summarized in Table I. At these dosages emesis occurred in 40 to 75% of the dogs receiving either the fine 200–325 mesh or finer (micronized) material, but only in 5% of those receiving the coarse 50–60 mesh crystals—a great improvement. The crystal size ranges studied here actually measure from less than 10 μ up to about 300 μ in diameter, presenting almost a *thirtyfold* difference in available surface area for the *same weight* of drug.

While this control of emesis might be a result of the slower absorption rate produced by the decreased surface area of the larger crystals, it could

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[1] The registered trademark of the Norwich Pharmacal Co. for nitrofurantoin is Furadantin.

TABLE I—EFFECT OF NITROFURANTOIN CRYSTAL SIZE ON EMESIS IN DOGS

Study and Single Dosage	Crystal Size *vs.* No. of Dogs Vomiting Out of 20: 50–60 Mesh	200–325 Mesh	Micronized
First Study			
150 mg./dog (10–20 mg./Kg.)	1	8	9
Second Study			
25 mg./Kg./dog	1	14	15

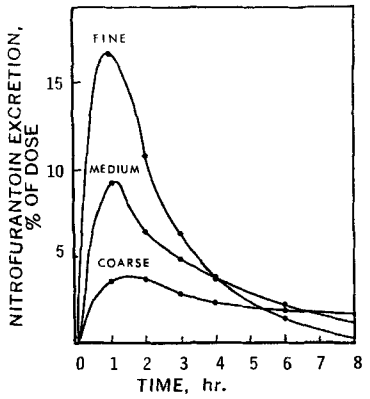

Fig. 1—Effect of crystal size of orally administered nitrofurantoin on urinary recovery rate in the rat. Key: coarse, 50–80 mesh (300–180 μ); medium, 80–200 mesh (180–75 μ); fine, 200 mesh to micronized (75–10 μ or less).

also have been the result of reduced total absorption, a possibility that must be considered when too large a crystal size of a slightly soluble drug is employed (16, 17). Since the effectiveness of nitrofurantoin is associated with its urinary concentration (18, 19), a reduction in toxicity (emesis) would have no medical practicality if excretion were also *proportionately* reduced.

Quantitative urinary excretion studies were initiated in rats, a physiologically suitable animal, to determine the effect of crystal size on both rate and total excretion of orally administered nitrofurantoin. This information would also provide an indication of the rate and amount of nitrofurantoin absorbed from the gut (13, 17, 20). Good correlation exists in the urinary excretion of nitrofurantoin by the rat, dog, and man (7).

In studying the effect of variation of nitrofurantoin crystal size on absorption, as measured by both urinary excretion rate and total excretion, several exploratory studies were conducted to establish size of oral dose, collection periods, crystal size ranges to study, *etc.* Urines were assayed by a spectrophotometric method (21). The nitrofurantoin crystals of selected size were administered as an aqueous suspension by gavage to adult rats—usually a group of four for each crystal size test. Following single dosage at the 10 mg./Kg. level, collection periods were established at 0–1, 1–2, 2–3, 3–4, 4–8, and 8–24 hr. to supply the required information. Of particular interest would be a substantial reduction of the absorption rate immediately following dosage. That this can be accomplished is clearly shown in the excretion curves covering the 0 to 8 hr. period as presented in Fig. 1. Each curve is based on data from 8 to 12 rats (2 or 3 groups of 4 animals each) receiving, respectively, relatively coarse (50–80 mesh), medium (80–200 mesh), and fine (200 mesh to micronized material). It is apparent that the particle size of nitrofurantoin alone can greatly influence the rate of intestinal absorption which is reflected in the measured urinary excretion (20). The rapidity with which urinary concentration rises and falls following administration of the finer crystal range shows that solution and absorption must have been rapid indeed. In fact, it has been shown that for micronized (<10 μ) material absorption can be so rapid that half of the total amount excreted can be recovered in the urine by the end of the first hour! Conversely, it will be noted that in the case of the medium and coarser crystals appreciable nitrofurantoin was still being excreted at 8 hr. In this study the 0 to 24 hr. total recovery of the dose given was 45% and 42% for the fine and medium crystals, but this had dropped to 33% for the coarser crystals. Not shown in this figure is an important further finding: in the last 8–24 hr. collection period the remaining portion excreted related to the total amount excreted (not to the total dose) was 3% for the fine, 20% for the medium, and 40% for the coarser material. The coarsest material alone resulted in prolonged excretion coupled with appreciably reduced total excretion.

Further studies with rats were conducted with particular emphasis on the relation of crystal size to total urinary excretion or drug recovery. Some variation in total recoveries was encountered between studies, but the over-all crystal size relationship to rate and total urinary excretion remained clear. It was found that sodium carboxymethylcellulose should not be used without proper precaution as a thickener for an aqueous suspension of nitrofurantoin. Unless this suspension is used *immediately*, needle-like nitrofurantoin hydrate crystals form on prolonged mixing or standing which appreciably affect nitrofurantoin-crystal size relationship studies. Methylcellulose or some other inert thickening agent could be used.

More refined and extensive comparative tests were conducted with humans and dogs to study the relationship of crystal size to total urinary excretion in humans and nitrofurantoin-induced emesis in dogs. In these tests 32 purebred beagles and a population of 15 humans were used. Wherever possible, each test animal or human received every crystal size for comparison. A previously established sensitive dose of 10 mg./Kg./dog was used. Humans were standardized at 100 mg./subject. Ample clearance time was allowed—3 days for dogs and 7 days for humans. Crystals were admixed with lactose and administered in capsules. To demonstrate the relationship between crystal size, drug excretion, and emesis, data from representative studies are plotted in Fig. 2. Contrary to expectation there is a crystal size range in which there is a marked reduction of nausea and minimal reduction of total nitrofurantoin excretion.

A crystal size of about 150 mesh nitrofurantoin (about 100 μ), or its surface area equivalent, was selected as an optimal desired size from a potentially

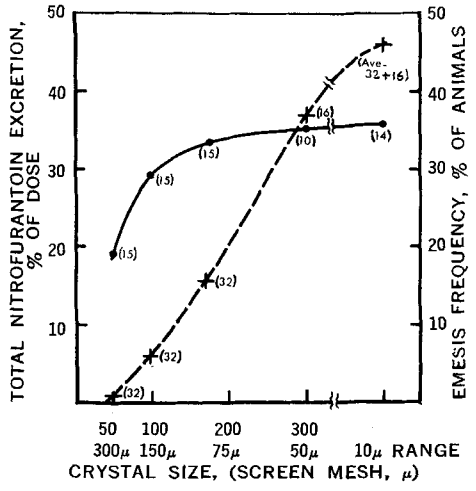

Fig. 2—Relation between total urinary recovery in humans and emesis frequency in dogs following oral administration of nitrofurantoin of different crystal sizes. Values in parentheses are numbers of subjects used. Points are plotted essentially at median values for nonoverlapping crystal size ranges used. Key: ●, urinary excretion (humans); ×, emesis (dogs).

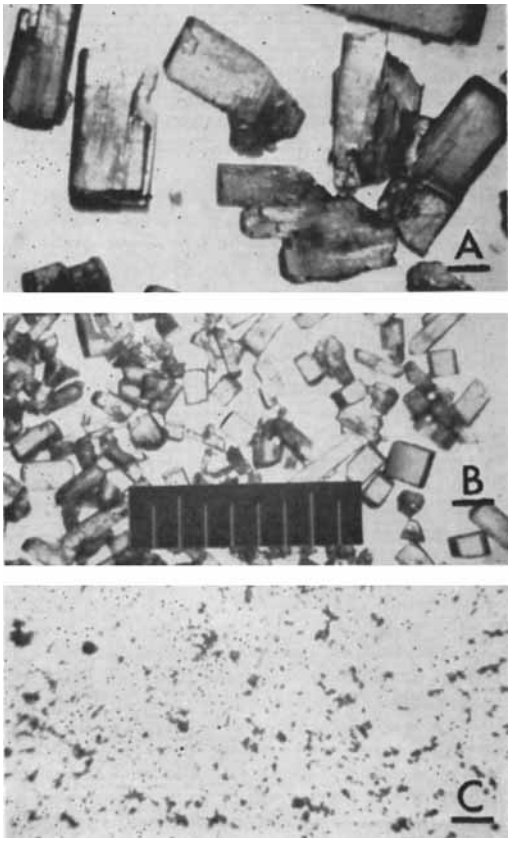

Fig. 3—Nitrofurantoin crystals. Key. A, coarse, 50 to 80 mesh or 300 to 180 μ; B, medium, 80 to 200 mesh or 180 to 75 μ; reference scale 100 μ/division; C, fine, micronized crystals. 25× magnification.

useful range of 50 to 400 mesh. For practical reasons a narrower working range was chosen of 80–200 mesh (180–75 μ size), or its surface area equivalent of about 200–500 cm.²/Gm. Figure 3 illustrates crystals of this median size range compared to coarse (50–60 mesh) and fine material (micronized or marketed tablet—as providing crystals in the 10 μ range, equivalent to a theoretical 1500 mesh).

These findings were amplified by conducting appropriate studies in humans to obtain information on the effect of crystal size (surface area) on the urinary excretion rate, urinary concentration, and total excretion of nitrofurantoin following single oral doses to couple maintenance of efficacy with reduced nausea potential. Fifteen volunteer subjects were used in comparative studies, each serving as his own control. Subjects ate a standard breakfast immediately prior to administration of a selected crystal size in a single test dose of 100 mg. nitrofurantoin in a capsule. Marketed tablets[2] (100 mg.) were included in the study for comparison. Fluid intake was controlled and caffeine and alcohol were prohibited. Total urine collections were made at specified time intervals and nitrofurantoin concentrations determined on each collection from each subject by a recently developed method (22) or modification of an earlier one (23).

Table II presents a summary of the results. From the five columns it will be noted that: (*a*) the maximum per cent of nitrofurantoin excreted in *any* 2-hr. collection period decreased as the crystal size was increased, being definitely reduced in the case of the coarsest material, demonstrating decreased absorption rate with larger crystal sizes. (*b*) The average time at which maximum percentage excretion occurred increased with particle size. (*c*) The maximum urinary concentration attained was lower with the larger crystals, partially a result of the extended excretion period. (*d*) The average time at which maximum urinary concentration was reached was later for larger crystals, again reflecting slower absorption. (*e*) The total amount of nitrofurantoin excreted (*i.e.*, per cent of the dose given) in the urine decreases with increased particle size, but only in a degree to cause serious concern in crystal sizes larger than 50 mesh.

DISCUSSION

It appears that these human studies basically agree with those conducted in rats. From a potentially useful range of 50 to 400 mesh a more practical range of nitrofurantoin crystals of the 80–200 mesh size has been selected as providing ample urinary concentration with satisfactory total urinary excretion. Accordingly, such nitrofurantoin crystals, termed "macrocrystals," have now been introduced into clinical trial for critical testing of efficacy and emetic action in patients having urinary tract infections who, in addition, suffer nausea and emesis from commercial nitrofurantoin tablets. In the crossover studies planned, each patient will serve as his own control, making such tests more meaningful with reasonable numbers, rather than to test the macrocrystals on all patients using nitrofurantoin for urinary tract infections where nausea and emesis percentages are relatively low.

[2] Furadantin Tablets, Norwich Pharmacal Co.

TABLE II—SUMMARY OF CRITERIA MEASURED IN HUMAN VOLUNTEERS RECEIVING VARIOUS SIZES OF NITROFURANTOIN CRYSTALS[a]

Crystal Size[b] of Nitrofurantoin	(1) Max. % Excreted in Any 2-hr. Period	(2) Av. Time of Max. % Excretion, hr.	(3) Max. Urinary Concn. Attained, mg./L.	(4) Av. Time Max. Urinary Concn. Attained, hr.	(5) Total % of Initial Dose Excreted
50–60 mesh	8.3	4.9	83	5.5	19.6
80–120 mesh	12.9	4.6	124	5.0	29.8
140–200 mesh	16.6	3.8	159	4.1	32.3
200–400 mesh	17.8	3.6	156	3.4	35.4
Marketed nitrofurantoin tablets (Provide fine crystals in the 10 μ range)	20.0	3.6	151	3.0	36.1

[a] Each value based on 15 complete individual studies—no pooling of samples. Exceptions: 10 individuals only were available from same population for 200–400 mesh and 14 for tablets. [b] Size checked by microscopic measurement.

SUMMARY

A study of the effect of crystal size of orally administered nitrofurantoin on its urinary excretion in the rat and man demonstrates a slower rate of absorption from larger crystal size than from fine crystals with ample total urinary excretion from a suitably selected size or size range.

Studies with dogs reveal that the larger crystals are much less provocative of emesis than the smaller crystal size.

Based on quite favorable relationships between nitrofurantoin crystal size, total urinary excretion, and reduction of emesis established by these studies, nitrofurantoin crystals ranging from 80 to 200 mesh and of about 150 mesh average size, or its equivalent in surface area as determined by solubility rate, have been selected for clinical study.

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