Inhibition of Primary ADP-Induced

Platelet Aggregation in Normal Subjects after

Administration of Nitrofurantoin (Furadantin)

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A B S T R A C T The evidence indicating that platelets may play a role in the occurrence of certain thromboembolic phenomena has stimulated a search for inhibitors of platelet function. This report presents data to indicate that nitrofurantoin is a potent inhibitor of primary ADP-induced platelet aggregation. The addition of 10 μM nitrofurantoin to citrated platelet-rich plasma obtained from 12 normal subjects produced a $29\pm6\%$ (2 SD) inhibition of the velocity of platelet aggregation induced by 2 μM ADP. The inhibitory effect of nitrofurantoin demonstrated competitive kinetics in respect to ADP. The intravenous (180 mg) or oral (200 mg) administration of nitrofurantoin produced a serum nitrofurantoin concentration ranging from 2.7 to 23 μM in 28 normal subjects. Platelet-rich plasma obtained from these subjects demonstrated inhibition of the velocity of ADP-induced platelet aggregation that correlated with the log of the serum nitrofurantoin concentration ($P < 0.001$). Collagen-induced platelet aggregation was also inhibited in a dose-related manner, and the bleeding time was significantly prolonged in the two subjects with the highest serum nitrofurantoin concentration. These studies indicate that nitrofurantoin in vivo inhibits platelet function to a degree that is proportional to the serum nitrofurantoin concentration.

INTRODUCTION

When platelets adhere to collagen (1, 2) they release ADP (3), which, in turn, produces platelet aggrega-

tion (4). Although these platelet functions are important for hemostasis (5), the formation of platelet aggregates in diseased blood vessels may cause occlusion (6). For this reason inhibitors of platelet function have been sought in the hope that such drugs might decrease the morbidity and mortality associated with vascular disease.

Aspirin prolongs the bleeding time (7–12) and inhibits collagen-induced platelet aggregation (10, 11, 13). Sulfinpyrazone prolongs the survival time of ^{51}Cr-labelled platelets (14) and also inhibits platelet aggregation induced by collagen (15). Both drugs are inhibitors of the platelet release reaction (11, 15–17), and they are currently being tested for their effectiveness in the treatment of various forms of thrombotic disease (18, 19).

The search for a potentially useful inhibitor of primary ADP-induced platelet aggregation has not been as successful. Inhibitors such as prostaglandin E_1 (PGE$_1$)[1] (20), adenosine (21), and chloroadenosine (22) can not be used in patients because of various deleterious effects (23, 24), and 2-methylthio-AMP (25) must be given intravenously to be effective. Recently we reported that furosemide inhibited primary ADP-induced platelet aggregation in vitro (26). These studies led us to investigate the effects of another furfuryl drug, nitrofurantoin, upon platelets. Our results indicate that nitrofurantoin is a potent inhibitor of primary ADP-induced platelet aggregation in vitro and that its inhibitory effect can be demonstrated in platelet-rich plasma (PRP) obtained from normal subjects after oral administration of nitrofurantoin.

[1] *Abbreviations used in this paper: K_i, inhibitor constant; LT, light transmission; PGE$_1$, prostaglandin E_1; PPP, platelet-poor plasma; PRP, platelet-rich plasma; TTP, thrombotic thrombocytopenic purpura.*

METHODS

Blood was drawn from healthy human volunteers and placed in 3.8 g/100 ml trisodium citrate in a 9:1 ratio. In some experiments heparin (4 U/ml of blood) was used as the anticoagulant. PRP was prepared by centrifugation at 150g for 10 min at room temperature.

Materials. Nitrofurantoin sodium crystals (Eaton Laboratories, Inc., Div. of Norwich Pharmacal Co., Norwich, N. Y.) were dissolved in water. Dilutions of the nitrofurantoin stock solution (10 mg/ml) were prepared in a solution containing 100 mM Tris-HCl (pH 7.5), 95 mM NaCl, and 3 mM KCl. Solutions of ADP (Sigma Chemical Co., St. Louis, Mo.) were also prepared in the above Tris-HCl buffer. Collagen was prepared from human skin according to the method of Green, Dunne, Schmid, and Rossi (27). Solutions of PGE$_1$, theophylline, and epinephrine were prepared as described elsewhere (26).

In vitro studies. Platelet aggregation studies were performed according to the method of Born (28). A platelet aggregometer (Chrono-Log Corp., Broomall, Pa.) was used, and changes in light transmission were inscribed on an EU-20B servo recorder (Heath/Schlumberger Scientific Instruments Div., Heath Co., Benton Harbor, Mich.).

0.25 ml of PRP was placed into a siliconized 1.5 ml cuvette containing a stir bar and 0.02 ml of the drug to be tested (final concentrations: nitrofurantoin, 5–30 μm; PGE$_1$, 15 nM; or theophylline, 1 mM). The cuvette was placed in the aggregometer, which was adapted to accept 1.5-m cuvettes, and 0.02 ml of an ADP solution that gave a final concentration of 1, 1.5, 2, 3, or 6 μM was added to the cuvette. In other experiments, epinephrine (final concentration 2 μg/ml) or collagen was used to induce platelet aggregation. The aggregation observed in samples containing inhibitors was compared to that observed in appropriate control samples diluted with 0.02 ml of Tris buffer.

ADP release by platelets in the presence of collagen and nitrofurantoin was measured in samples of PRP obtained from five normal subjects. Before an ADP release experiment was performed, a dose-response curve with various dilutions of collagen was performed upon each sample of PRP in order to determine the lowest concentration of collagen required to produce at least 50% aggregation within 5 min in that particular sample of PRP. 2 ml of PRP were placed in each of two siliconized 10-ml cuvettes. One cuvette contained 0.2 ml nitrofurantoin solution (final concentration, 40 μM) while the other contained 0.2 ml Tris buffer. The cuvettes were placed in sequence in an aggregometer set at 37°C, and 0.2 ml of the appropriate predetermined dilution of collagen was added. The amount of aggregation present after 5 min of constant stirring was recorded, and the cuvette was removed from the aggregometer. Nitrofurantoin or Tris buffer was then added to the test or control sample so that the nitrofurantoin concentration and volume of all samples would be constant through the deproteinization and assay procedures. An additional control assay consisting of platelet poor plasma (PPP), collagen, and nitrofurantoin was performed with each set of tests. A calibration curve for ADP was prepared by assaying mixtures of PPP, nitrofurantoin, and known amounts of ADP. The removal of platelet aggregates, the preparation of deproteinized plasma supernates, and the enzymatic assay of ADP were performed as described by Weiss, Aledort, and Kochwa (11).

The effect of nitrofurantoin upon the retention of platelets by glass bead columns was studied in samples of whole blood to which nitrofurantoin was added. The method employed is described in detail elsewhere (29). In summary, 11.5-ml blood samples were drawn sequentially into 20-ml plastic syringes containing 1 ml of a nitrofurantoin solution. A 38 mM nitrofurantoin solution prepared in 0.425 g/100 ml NaCl was the highest concentration used in these experiments. This solution had an osmolarity of 240 mosmol and a pH of 9. Since the addition of Tris-HCl buffer to this solution resulted in the precipitation of nitrofurantoin, the solution was not buffered. For this reason, control "adhesiveness" studies were performed using a 240 mosM NaCl solution adjusted to pH 9 with NaOH. The syringes were placed on a syringe pump, and blood was forced through a glass bead column constructed by packing 2.6 grams of type 070 Super-Brite Beads (Reflective Products Div., 3M Corporation, St. Paul, Minn.) into lengths of polyvinyl tubing. (ID 0.118 inches, Abbott Laboratories, North Chicago, Ill.). The flow rate employed was 6 ml/min. Four aliquots (2 ml each) of effluent blood were collected in plastic tubes each containing 5.4 mg of sodium EDTA. The difference between the platelet counts before and after passage through the column divided by the platelet count before passage represented the percent of platelets retained by the column.

In vivo studies. 27 healthy volunteers each ingested 200 mg nitrofurantoin in a single dose. Blood samples were drawn for serum nitrofurantoin levels and platelet aggregation studies before and at 2 and 4 h after nitrofurantoin ingestion. A different venipuncture site was used for each blood sample obtained from the same individual. In one volunteer, 180 mg of sodium nitrofurantoin in 500 c³ 5% glucose in H$_2$O was given intravenously over a 2½-h period. Blood samples were drawn before infusion, 1½ h after its initiation and 15 min after its conclusion. Serum nitrofurantoin levels were determined according to a modification of the method of Buzard, Vrablic, and Paul (30).

The volume of citrate-PRP obtained from pre- and postnitrofurantoin blood samples did not show significant variation in any individual. Platelet counts were obtained on all samples of PRP. Variation in the platelet counts of PRPs obtained after nitrofurantoin ingestion did not exceed a 16% variation. The platelet count in the postnitrofurantoin PRP was not equilibrated to the count obtained in the prenitrofurantoin PRP. In this manner, the time interval between harvest and testing of PRPs was both minimal and constant. Pre- and postnitrofurantoin PRPs were tested with fresh ADP solutions that gave final concentrations of 1, 1.5, 2, 3, and 6 μM. Aggregation studies of each PRP were begun within 2 min of harvest and were completed within 10 min time.

In order to study the primary wave of ADP-induced platelet aggregation, the speed of the paper in the recorder was set at 4 inches/min. The velocity of aggregation was measured by drawing by eye a line tangential to the initial rapid phase of platelet aggregation (31). The difference in light transmission (LT) along this tangent at 15 s from the initiation of aggregation was recorded. The value obtained with a postnitrofurantoin PRP was mathematically corrected for differences in platelet count from the prenitrofurantoin PRP by multiplying the ΔLT (postnitrofurantoin PRP) by the ratio platelet count prenitrofurantoin PRP/platelet count postnitrofurantoin PRP. The percent inhibition of the velocity of platelet aggregation after nitrofurantoin administration was calculated for each ADP concentration according to the following formula:

$$[\Delta LT \text{ control PRP} -\Delta LT \text{ nitrofurantoin PRP (corrected for } \Delta \text{ in platelet count]}/\Delta LT \text{ control PRP}.$$

The retention of platelets by glass bead columns (platelet adhesiveness) was measured before and after nitrofurantoin administration in five normal subjects according to the method of Bowie, Owen, Thompson, and Didisheim (32) with the exception that heparin was not used (29). Bleeding times were measured according to the method of Mielke, Kaneshiro, Maher, Weiner, and Rapaport (12). Platelet counts were done by phase microscopy (33).

RESULTS

In vitro studies. Nitrofurantoin in vitro inhibited primary ADP-induced platelet aggregation in all samples of citrated-PRP obtained from 12 normal subjects. The effect of nitrofurantoin was immediate and proportional to its concentration. In a 10 μM concentration, nitrofurantoin produced a $29\pm6\%$ (2 SD) inhibition of the velocity of aggregation induced by 2 μM ADP. The effect of nitrofurantoin was demonstrated in heparinized as well as in citrated PRP, and it was not corrected in citrated PRP by calcium in concentrations up to 20 mM. The inhibitory effect of 10 μM nitrofurantoin in combination with either 15 nM PGE$_1$ (an adenyl cyclase stimulator [34]) or 1 mM theophylline (a phosphodiesterase inhibitor [35]) was also tested. In both combinations an additive increase of inhibition was observed. Potentiated inhibition, as obtained when PGE$_1$ and theophylline are combined (36), was not observed (Table I).

The inhibitory effect of in vitro nitrofurantoin upon the velocity of primary ADP-induced platelet aggregation, (i.e. the velocity of the increase in LT), is shown

TABLE I
The Effect upon Platelet Aggregation of Nitrofurantoin in Combination with Prostaglandin E$_1$ or Theophylline

Platelet-rich plasma + 2 μM ADP	% Aggregation
plus Tris buffer	100%*
plus 10 μM nitrofurantoin	72%
plus 15 nM PGE$_1$	64%
plus 1 mM theophylline	89%
plus 10 μM nitrofurantoin; 15 nM PGE$_1$	32%
plus 10 μM nitrofurantoin; 1 mM theophylline	65%
plus 15 nM PGE$_1$; 1 mM theophylline	0%

* All studies were performed on samples from the same specimen of citrated PRP (platelet count—338,000/mm³). The amounts of all substances added are expressed as final concentrations. The amount of platelet aggregation observed 1 min after the addition of 2 μM ADP to a sample of PRP containing Tris buffer was defined as 100% (control value). Aggregation values observed in the presence of inhibitors are expressed as percentage of the control value.
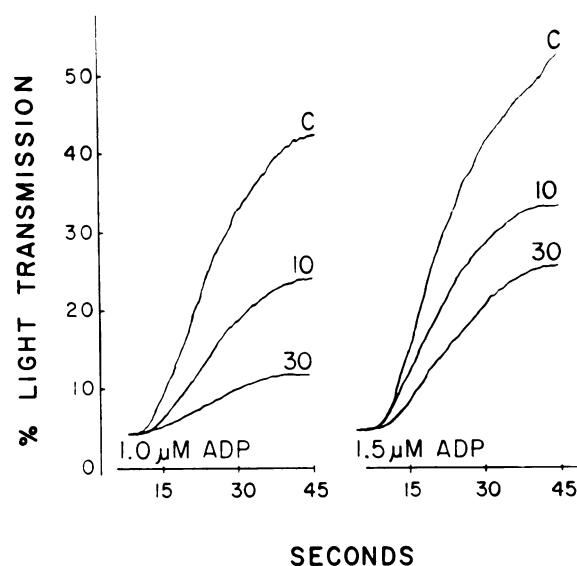

FIGURE 1 Inhibition of the first wave of ADP-induced platelet aggregation by nitrofurantoin. C = control. The number after each tracing indicates the final concentration (μM) of nitrofurantoin. The speed of the paper in the recorder was increased to 4 inches/min and samples from the same PRP were used for all tracings.

in Fig. 1. When the reciprocal of the velocity of aggregation was plotted against the reciprocal of the ADP concentration, inhibition by nitrofurantoin was revealed to be competitive in type, and the inhibitor constant ($K_{i\,nitrofurantoin}$) was 9–12 μM (Fig. 2).

Nitrofurantoin in vitro also inhibited platelet aggregation and ADP release induced by collagen (Table II), and the second wave of aggregation induced by epinephrine. In order to evaluate more fully the dual effect of nitrofurantoin upon primary ADP-induced platelet aggregation and collagen-induced platelet aggregation, a study comparing the in vitro effect of nitrofurantoin and acetylsalicyclic acid upon ADP- and collagen-induced platelet aggregation was performed upon the PRP from four normal subjects (Table III). The results showed that nitrofurantoin had an inhibitory effect upon the velocity of ADP-induced aggregation while acetylsalicylic acid had the greater inhibitory effect upon collagen-induced aggregation. The effect of a combination of nitrofurantoin and acetylsalicylic acid upon collagen-induced aggregation also was tested. In this experiment the addition of 10 μM nitrofurantoin to a sample of PRP produced 27% inhibition while 22 μg/ml acetylsalicylic acid produced 39% inhibition. When the drugs were combined in these concentrations and added to a duplicate sample taken from the same PRP, 67% inhibition was observed.
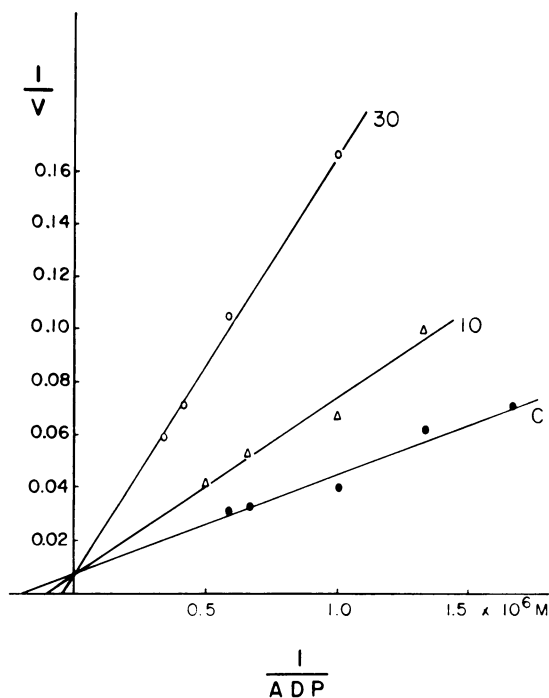
Inhibition of Platelet Aggregation by Nitrofurantoin 2459

FIGURE 2 The kinetics of the inhibitory effect of nitrofurantoin upon ADP-induced platelet aggregation. V, the velocity of the increase in LT (i.e., platelet aggregation), is determined by drawing by eye a line tangential to the initial rapid phase of platelet aggregation (31). When the reciprocal of the velocity is plotted against the reciprocal of the ADP concentration, the inhibitory effect of nitrofurantoin is revealed as competitive in type. The point on the abscissa intercepted by the line derived from the double reciprocal plot of a competitively inhibited reaction is given by the expression of $-1/K_m \cdot K_i/K_i + (I)$, where I equals the inhibitor concentration and $-1/K_m$ equals the intercept when $I = 0$. When I is known the equation can be solved for K_i. In this experiment the $K_{i\text{nitrofurantoin}} = 12 \mu M$ (when $I = 10 \mu M$) and $9 \mu M$ (when $I = 30 \mu M$).

The retention of platelets by glass bead columns was not affected by nitrofurantoin unless inordinately large concentrations of the inhibitor were used. When blood was mixed with nitrofurantoin in final concentrations ranging between 10 μM and 1 mM, there was no effect upon the retention of platelets by glass bead columns (seven experiments). However, with concentrations above 1 mM an effect could be demonstrated. Fig. 3 (Part A) shows the results of three adhesiveness studies performed on the same individual, in which whole blood was mixed with either nitrofurantoin (final concentration, 1.5 or 3 mM) or a saline solution appropriately controlled for osmolarity and pH. Note that the initial retention of platelets from the first and second aliquots of effluent blood was unaffected by nitrofurantoin while retention from the third and fourth aliquots was decreased in a dose-dependent manner. In a second

subject (Fig. 3, Part B) 5.7 mM nitrofurantoin decreased platelet retention in all four aliquots. Larger concentrations of nitrofurantoin could not be tested because of difficulties with dissolution.

In vivo studies. Since the amount of nitrofurantoin required to inhibit ADP-induced aggregation in vitro was within the range achievable in vivo (37), we attempted to demonstrate inhibition of ADP-induced platelet aggregation in the PRP of normal subjects after nitrofurantoin administration. Platelet aggregation studies were performed before and after infusion of 180 mg nitrofurantoin over a 2½-h period in a normal healthy subject. The serum nitrofurantoin concentration after completion of the infusion was 3.6 $\mu g/ml$ (13.8 μM), and primary ADP-induced platelet aggregation
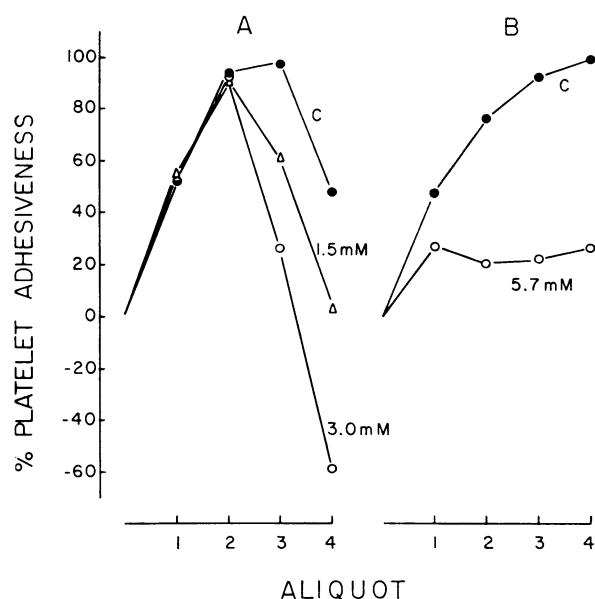

FIGURE 3 The effect of nitrofurantoin upon the retention of platelets by glass bead columns. A. 11.5 ml of blood was drawn into a plastic syringe containing 1 ml of a 38 mM nitrofurantoin solution (final concentration, 3 mM) and a platelet adhesiveness study was performed. A second test was performed with 19 mM nitrofurantoin (final concentration, 1.5 mM). The control adhesiveness test (C) was performed with 1 ml of a 240 mosM NaCl solution adjusted to pH 9 with NaOH (see Methods). At the conclusion of this experiment the pH of plasma samples from all three tests ranged from 7.67 to 7.75. The negative adhesiveness (29) seen in the fourth aliquot of the 3 mM nitrofurantoin test indicates that this aliquot of effluent blood contained more platelets than originally present before passage through the column (actual platelet counts were: before passage, 222,000/mm³; fourth aliquot, 353,000/mm³). B. 11.5 ml of blood was drawn into a syringe containing 2 ml of 38 mM nitrofurantoin (final concentration, 5.7 mM). The control adhesiveness was similarly altered. The plasma pHs on completion of this experiment were, test 7.78, control 7.88.

TABLE II

The Effect of in Vitro Nitrofurantoin upon Collagen-Induced Aggregation and ADP Release

| Subject | Aggregation* | | ADP Release | |
	Collagen	Collagen + 40 μM nitrofurantoin	Collagen	Collagen + 40 μM nitrofurantoin
	%		*nmol/10⁹ platelets*	
1	88	35	11	6.5
2	90	13	16	10
3	94	35	24	2
4	60	21	9	1
5	84	16	18	7
Mean	83±13	24±10	16±6	5±4
P value	<0.001		<0.05	

* In these experiments, the LT values for PRP and PPP were defined as 0% and 100% aggregation, respectively. The results are expressed in relationship to this scale, which was determined in each individual experiment.

was inhibited (Fig. 4). A reciprocal plot of these results confirmed the competitive nature of the inhibition, and the $K_{i nitrofurantoin}$ was virtually identical to that observed with the in vitro studies (Fig. 5).

27 normal subjects ingested 200 mg of nitrofurantoin in a single dose. 2 and 4 h after drug ingestion blood samples for platelet aggregation studies and serum samples for nitrofurantain levels were obtained. In 24 subjects the peak nitrofurantoin concentrations observed in the 2- or 4-h sample was between 0.7–6.0 μg/ml (2.7–23 μM). In the other three subjects the peak concentration was only 0.2 μg/ml (0.8 μM) in one, while no serum nitrofurantoin could be detected in samples obtained from the other two subjects. Some

individuals complained of mild nausea after drug ingestion. This symptom seemed to occur more frequently in subjects in whom blood levels greater than 2 μg/ml (8 μM) were achieved.

After the ingestion of nitrofurantoin, the velocity of ADP-induced platelet aggregation was inhibited in all of the PRPs in which this was studied (20 subjects). The inhibition was competitive in type, and in each subject the sample containing the highest concentration of nitrofurantoin showed the greatest inhibition of ADP-induced platelet aggregation. The percent inhibition of primary ADP-induced platelet aggregation was proportional to the log of the serum nitrofurantoin concentration. Positive correlations were observed with

TABLE III

Comparative Study of the Effects of in Vitro Nitrofurantoin and Aspirin upon ADP- and Collagen-Induced Platelet Aggregation

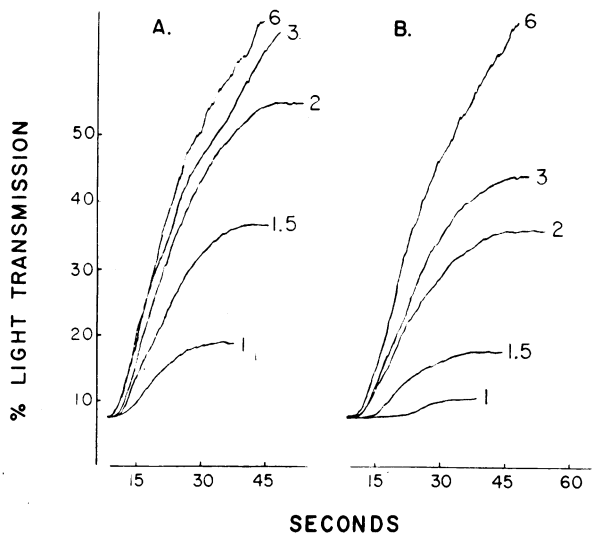| Subjects	10 μM Nitrofurantoin	30 μM Nitrofurantoin	110 μg/ml Aspirin (0.6 mM)	220 μg/ml Aspirin (1.2 mM)
Velocity of 2 μM ADP-induced platelet aggregation				
	% of control sample lacking drug			
1	76	34	100	100
2	85	57	103	95
3	84	52	94	94
4	54	36	100	100
Mean	75±14%	45±11%	99±4%	97±3%
P values	<0.05	<0.005	NS	NS
Collagen-induced platelet aggregation				
1	92	0	0	0
2	96	56	4	0
3	92	52	5	2
4	98	31	10	0
Mean	95±3%	35±26%	5±4%	0.5±1%
P values	<0.05	<0.02	<0.001	<0.001

FIGURE 4 Inhibition of ADP-induced platelet aggregation after intravenous infusion of nitrofurantoin in a normal subject. A. ADP-induced platelet aggregation observed in citrated PRP before the administration of nitrofurantoin. The PRP contained 404,000 platelets/mm^3. The number after each tracing represents the final ADP concentration (μM) used to produce aggregation. B. ADP-induced platelet aggregation observed in a specimen of citrated PRP obtained 15 min after the completion of an intravenous infusion of nitrofurantoin. The nitrofurantoin concentration in the plasma was 3.6 μg/ml (13.8 μM) and the PRP contained 394,000 platelets/mm^3.

1, 1.5, and 2 μM ADP, and they were all significant at the 0.1% level (Fig. 6).

The effect of nitrofurantoin ingestion upon collagen-induced platelet aggregation was also investigated. In all of 12 subjects studied, collagen-induced platelet aggregation was inhibited after nitrofurantoin ingestion (Fig. 7). Although the responsiveness of platelets to collagen varied greatly from one person to another, in
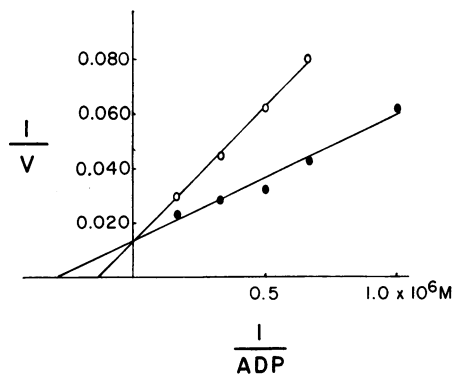

FIGURE 5 Double reciprocal plots of ADP-induced platelet aggregation curves (shown in Fig. 4) obtained before (●——●) and after (○——○) intravenous infusion of nitrofurantoin. I = 13.8 μM ; $K_{i\,\text{nitrofurantoin}}$ = 10 μM.

each individual the sample containing the highest concentration of nitrofurantoin also showed the greatest inhibition of collagen-induced platelet aggregation. The inhibitory effect of nitrofurantoin upon collagen-induced platelet aggregation could be overcome in part by increasing the collagen concentration.

The effect of nitrofurantoin upon the bleeding time was studied in 17 normal subjects. The baseline bleeding time in these individuals was 5.6 min (range 2–9 min), which agrees closely with the results obtained by Mielke et al (12). In these individuals, bleeding
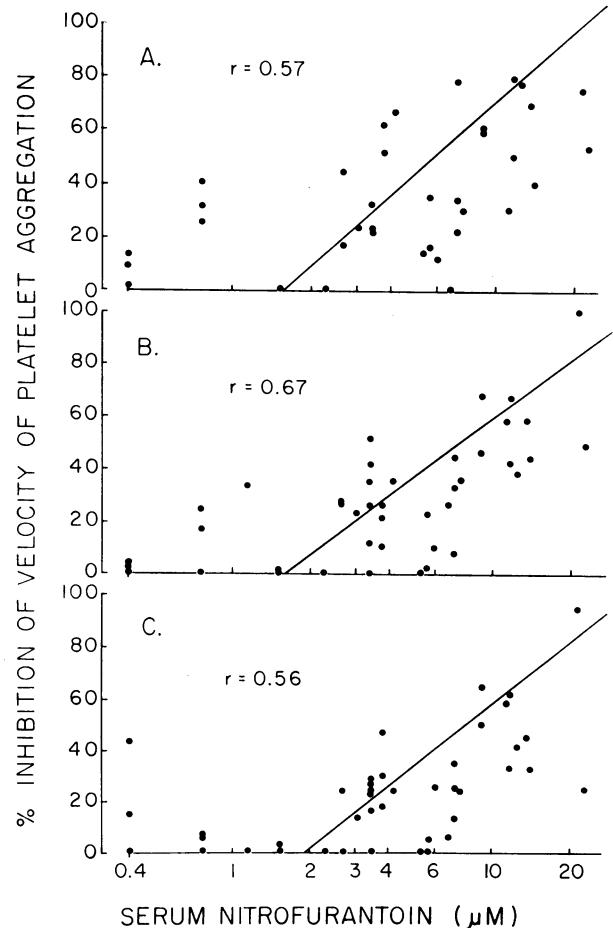

FIGURE 6 Correlation of the percent inhibition of the velocity of ADP-induced platelet aggregation with the log of the serum nitrofurantoin concentration. The results shown were obtained with the following ADP concentrations: A, 1.0 μM; B, 1.5 μM; and C, 2.0 μM. All data obtained on 20 normal subjects who had ingested 200 mg nitrofurantoin orally and on the one subject who received intravenous nitrofurantoin are included (two points per subject). 6 of the 42 points obtained with 1 μM ADP (A) were omitted because the control PRPs in those instances were not sufficiently responsive to 1 μM ADP to permit an accurate determination of aggregation velocity. The P values for the correlation coefficients were all < 0.001.
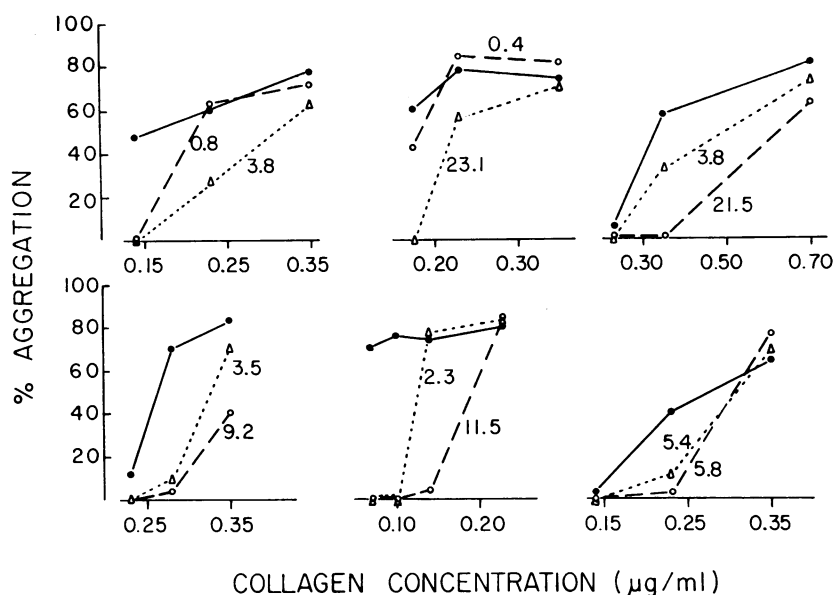
COLLAGEN CONCENTRATION (μg/ml)

FIGURE 7 Inhibition of collagen-induced platelet aggregation in six normal subjects after the ingestion of 200 mg nitrofurantoin. A sample of PPP was prepared from each sample of PRP obtained from each individual. The amount of aggregation observed 5 min after the addition of collagen was expressed in percent on a scale in which the LT values obtained with PRP and PPP were defined as 0% and 100%, respectively. Several determinations of collagen-induced aggregation using various concentrations of collagen (assay performed by Dr. Arthur Veis), were performed on each sample of PRP. The collagen concentration given in the abscissa refers to final concentration in PRP. ●——●, before nitrofurantoin ingestion; O— —O, 2 h after ingestion; △......△, 4 h after ingestion. The numbers accompanying the results obtained at 2 and 4 h refer to the serum nitrofurantoin concentration (μM), which was measured in a blood sample drawn at the same time. Note that in each individual the greatest inhibition of aggregation was observed in the PRP that had the highest nitrofurantoin concentration.

times 2 and 4 h after nitrofurantoin ingestion were repeated immediately before obtaining blood samples for nitrofurantoin levels. The two nitrofurantoin serum levels obtained on each subject were classified as "high" or "low" values. The high nitrofurantoin concentration obtained in these subjects (9.3±6.1 μM) was associated with a prolongation of the bleeding time (0.8±0.4 min; $P < 0.05$). Conversely, the mean bleeding time associated with the low nitrofurantoin concentration (2.3 ±1.8 μM) was similar to the baseline bleeding time (Table IV). Subjects 3 and 5, who achieved the highest nitrofurantoin concentrations (23.1 μM [4 h] and 21.5 μM [2 h], respectively), also showed the greatest prolongation in bleeding time (3.5 min). In both subjects, the prolongation in bleeding time was observed at the time when the high nitrofurantoin concentration was achieved (Fig. 8). When the prolongation of the bleeding time in all 17 subjects was plotted against the nitrofurantoin concentration, a positive correlation was noted. ($r = 0.53$, $t = 2.415$, $P < 0.05$).

Since the prolongation of the bleeding time and the percent inhibition of ADP-induced platelet aggregation

both correlated in a positive fashion with the serum nitrofurantoin concentration, one might expect in this study a positive correlation between the aforementioned parameters of platelet function as well. This correlation was sought. Measurements of ADP-induced platelet aggregation were performed in 12 of the 17 subjects who received nitrofurantoin and in whom changes in the bleeding time were studied. When the prolongation of the bleeding time was plotted against the inhibition of ADP-induced platelet aggregation, positive correlations were obtained (Fig. 9).

As expected, the retention of platelets by glass bead columns was not affected by nitrofurantoin administration in the five subjects tested. The highest concentration of nitrofurantoin achieved in any of these subjects was 13.8 μM.

DISCUSSION

Nitrofurantoin in vitro inhibits the velocity of primary ADP-induced platelet aggregation. The inhibition is competitive in type, and the $K_{i\,nitrofurantoin}$ is approximately 10 μM. It also inhibits collagen-induced platelet

aggregation and release and the second wave of epinephrine-induced aggregation. When nitrofurantoin is mixed with whole blood in a final concentration of 1–5 mM, the retention of platelets by glass bead columns is also inhibited.

The mechanism of action of nitrofurantoin is not known. Chelation of calcium is eliminated as a possible mechanism by the failure of calcium to correct the inhibitory effect. Moreover, the additive rather than potentiated inhibition observed when nitrofurantoin is combined with PGE₁ or theophylline suggests that nitrofurantoin does not stimulate adenyl cyclase or inhibit phosphodiesterase. However, a clear definition of the mechanism of action of nitrofurantoin must await further studies.

The fact that nitrofurantoin inhibits both primary ADP-induced platelet aggregation and collagen-induced release is not unexpected. Other inhibitors of primary ADP-induced platelet aggregation such as adenosine, PGE₁, theophylline, and dipyridamole have also been shown to inhibit the platelet release reaction (17, 38, 39). However, it is difficult to determined whether these drugs do in fact inhibit collagen-induced release or merely seem to do so because of their effects upon the ADP-platelet interaction. Platelets adherent to collagen

may still release ADP in the presence of nitrofurantoin. However, inhibition of the effect of this released ADP may be sufficient to prevent subsequent ADP-induced ADP release from platelets not adherent to collagen. The solution to this problem requires methods that can distinguish the actual ADP release induced by collagen from the ADP release induced by ADP.

The ambiguity of nitrofurantoin's effect upon platelet release is in contrast with the more distinct effect of aspirin. Since aspirin does not inhibit ADP-induced aggregation, its effect upon collagen-induced platelet aggregation must be due to interference with the collagen-induced platelet release reaction. The studies comparing the effects of nitrofurantoin and aspirin help to place the multiple effects of nitrofurantoin in perspective. Concentrations of nitrofurantoin that inhibited ADP-induced aggregation did not inhibit collagen-induced aggregation nearly as effectively as aspirin. This observation not only draws distinctions between these two inhibitors, it also supports the possibility that nitrofurantoin's effects upon collagen-induced aggregation and release may be phenomena that are merely secondary to nitrofurantoin's primary effect upon the ADP-platelet interaction.

TABLE IV

The Effect of Nitrofurantoin upon the Bleeding Time

Subject	Time after drug ingestion when the high serum level was found	The high serum nitrofurantoin level	Bleeding time—"d"* from baseline value (high nitrofurantoin)	Time after drug ingestion when the low serum level was found	The low serum nitrofurantoin level	Bleeding time—"d" from baseline value (low nitrofurantoin)
	h	µM		h	µM	
1	2	7.3	+1.5	4	0.4	−0.5
2	4	3.8	+0.5	2	0.8	0
3	4	23.1	+3.5	2	0.4	−1.0
4	2	7.3	+2.0	4	0.4	+1.0
5	2	21.5	+3.5	4	3.8	+1.5
6	2	13.8	−1.0	4	0.0	0
7	2	0.8	0	4	0.0	0
8	2	7.0	0	4	2.7	−0.5
9	4	5.0	+2.5	2	3.5	+2.0
10	2	5.8	0	4	5.0	0
11	2	7.0	0	4	3.5	0
12	2	3.8	−2.0	4	3.5	−1.0
13	2	9.2	0	4	3.5	0
14	2	12.0	+1.0	4	2.7	−1.5
15	2	11.5	0	4	2.3	−2.0
16	2	14.2	+0.5	4	0.8	+1.0
17	2	5.8	+1.5	4	5.4	+1.0
			+13.5			0.0
Mean		9.3±6.1	0.8±0.4		2.3±1.8	0.0±0.3
P values			<0.05			NS

* "d" refers to the difference between the bleeding times before and after nitrofurantoin ingestion, given in minutes. Positive numbers denote prolongation and negative numbers denote diminution of the bleeding time.
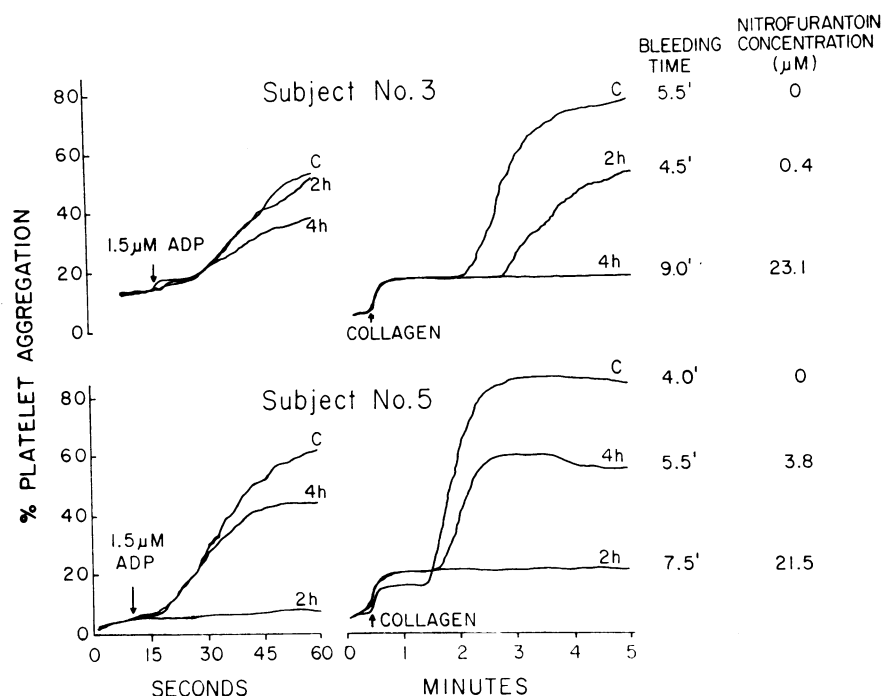
2464   E. C. Rossi and N. W. Levin

FIGURE 8 The changes in ADP-induced platelet aggregation, collagen-induced aggregation, and bleeding time that were observed in the two subjects with the highest serum nitrofurantoin concentration (Subjects 3 & 5, Table IV). The platelet counts of the PRPs used in these studies were as follows: Subject 3, $C = 473,000/mm^3$, 2 h $= 402,000/mm^3$, 4 h $= 504,000/mm^3$; Subject 5, $C = 484,000/mm^3$, 2 h $= 411,000/mm^3$, 4 h $= 407,000/mm^3$. The variation in platelet count did not obscure the fact that the greatest inhibition of ADP- and collagen-induced aggregation, and the greatest prolongations in bleeding time were associated with the highest serum nitrofurantoin concentration.

The concentration of nitrofurantoin required to produce inhibition of platelet aggregation in vitro is obtainable in vivo. In the rat, an oral dose of nitrofurantoin is absorbed primarily in the small bowel and appears rapidly in the urine (40). Early clinical studies demonstrated that oral administration of nitrofurantoin produced high drug concentration (as measured by antibacterial activity) in urine but not in serum (41). However, the colorimetric determination for nitrofurantoin does demonstrate measurable blood levels. Felts, Hayes, Gergen, and Toole (37) gave 100 mg oral nitrofurantoin to 11 normal subjects and obtained peak blood levels of 0.9–4.8 μg/ml (3.4–18 μM) 2–6 h after ingestion. In our study 200 mg oral nitrofurantoin produced peak blood levels of 0.7–6.0 μg/ml (2.7–23 μM) in 24 of 27 subjects at 2 or 4 h after ingestion. At these drug concentrations inhibition of platelet function was demonstrable.

PRP obtained from normal subjects after the ingestion of nitrofurantoin showed inhibition of ADP- and collagen-induced platelet aggregation. Moreover, the percent inhibition of the velocity of primary ADP-induced platelet aggregation was proportional to the

serum nitrofurantoin concentration. Prolongation of the bleeding time was also observed. Although the degree of prolongation was slight at best, it nonetheless served to confirm the inhibitory effect of nitrofurantoin upon platelets by means of an independently measured parameter of platelet function. This conclusion was strengthened when the degree of prolongation of the bleeding time was found to correlate with both the serum nitrofurantoin concentration and the percent inhibition of the velocity of ADP-induced platelet aggregation. Platelet retention by glass bead columns was not affected by the oral ingestion of nitrofurantoin. This was predicted by the in vitro studies that indicated that 1–5 mM nitrofurantoin was required before this test was affected.

Inhibitors of platelet function may be useful in the treatment of some forms of thrombotic disease (42). Sulfinpyrazone has been shown to be beneficial in patients with attacks of transient cerebral ischemia (18). Aspirin in one study (43) decreased the incidence of venous thrombosis after hip arthroplasty but failed in another (19) to affect the incidence of postoperative venous thrombosis. It is tempting to speculate that treat-
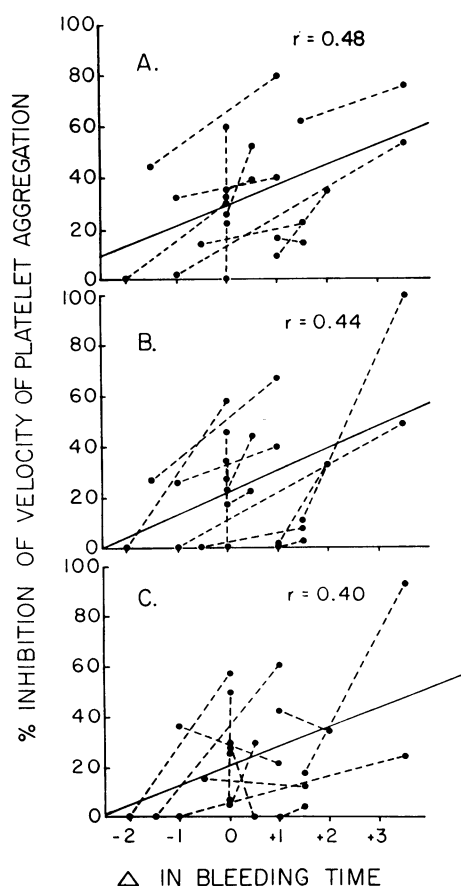
FIGURE 9 Correlations between the percent inhibition of the velocity of platelet aggregation and the prolongation in bleeding time. The Δ in bleeding time represents the difference between the bleeding times obtained before and after nitrofurantoin administration. Values are given in minutes with positive numbers representing prolongation and negative numbers representing diminution of the bleeding time. Points connected by dash lines represent values obtained on the same subject (12 subjects, 24 points). The results shown were obtained with the following ADP concentrations: A, 1.0 μM; B, 1.5 μM; and C, 2.0 μM. The P values for the correlation coefficients were A, < 0.02; B, < 0.05; and C, < 0.10.

ment with aspirin or sulfinpyrazone in combination with an inhibitor of ADP-induced platelet aggregation would be more effective than treatment with a platelet release inhibitor alone. The combination of aspirin and dipyridamole has been reported to be useful in the treatment of thrombotic thrombocytopenic purpura (TTP) (44, 45). However, dipyridamole is only a weak inhibitor of ADP-induced platelet aggregation (46), and it is uncertain whether its beneficial effects in patients with TTP (44, 45) and patients with prosthetic heart valves (47, 48) can be attributed to inhibition of this particular platelet function.

Since nitrofurantoin inhibits ADP-induced platelet aggregation at concentrations that can be achieved in vivo, one might consider using this drug for that purpose. However, nitrofurantoin has certain disadvantages. Its inhibitory effect upon platelet function is rapidly reversed as its concentration in the blood declines, and the prolonged administration of nitrofurantoin may be associated with signs of neurotoxicity (37). A less toxic inhibitor would be preferable. Conceivably, an investigation of nitrofurantoin analogues might lead to the discovery of such an inhibitor.

ACKNOWLEDGMENT

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