Effect of a New Anti-Inflammatory Drug, Fenoprofen, on Platelet Aggregation and Thrombus Formation (36183)

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The inhibitory effect of aspirin on platelet aggregation is well known. Other nonsteroidal, anti-inflammatory drugs have also been reported to inhibit certain platelet functions including aggregation (1, 2). They have relatively little effect on ADP-induced aggregation *in vitro* but inhibit the platelet release reaction and collagen-induced aggregation (3). A new anti-inflammatory compound developed in our laboratories (4), fenoprofen sodium (*dl*-2-3-phenoxyphenyl propionic acid sodium salt) was therefore examined with respect to its effect on the platelet.

Materials and Methods. Blood sampling. Human blood samples were obtained as described earlier (5). Rabbit arterial blood was obtained under pentobarbital anesthesia from a polyethylene carotid artery cannula. The blood was allowed to flow into a siliconized centrifuge tube, containing 4.0 ml of 3.8% sodium citrate in saline, to the 40 ml mark. The blood and citrate solution were mixed by capping with Parafilm and gently inverting the tube 6 times. Guinea pigs (250–350 g body wt) were anesthetized with pentobarbital and the peritoneum was opened by a midline incision. A disposable 20 gauge needle and a 10 ml plastic disposable syringe containing 1.0 ml of citrate was used to puncture the heart via the diaphragm at the site where the heart could be seen beating between 2 lobes of the lung. After the heart was entered with a clean puncture, 0.20 ml of the citrate was expelled and 30 sec later blood was slowly drawn into the syringe to the 8.0 ml mark. This procedure was followed to minimize contamination of the blood with thromboplastic material.

Platelet-rich plasma (PRP) was prepared at room temperature by centrifugation at 100*g* for 20 min and platelet-poor plasma (PPP) by centrifugation at 7000*g* for 15 min. Both PRP and PPP were stored at room temperature until used.

In vitro *aggregation studies.* Preparation of the collagen suspension and measurement of collagen-induced platelet aggregation were performed as described previously (5). ADP-induced aggregation was determined from the height of the aggregation curve at 3 min after the addition of ADP. Saline dilutions of the stock collagen suspension were prepared as needed.

Drug solutions. Aspirin was brought into solution in saline by the addition of 1.5 times its weight of sodium acetate, instead of addition of NaOH to effect solution, to minimize pH change and thereby avoid possible hydrolysis. Fenoprofen sodium was dissolved in saline and the pH was adjusted to 7.4. Phenylbutazone was dissolved in a small volume of 1 *N* NaOH in saline, then diluted with saline and the pH was adjusted to 7.4.

Calculation of percentage inhibition, in vitro *studies.* The collagen-induced aggregation response was expressed as the tangent of the angle of a line drawn through the point of addition of the collagen suspension and tangent to the aggregation curve. Thus, this value incorporates both the time lag and height of the aggregation response. A control response was obtained before, and after, every 3 drug experiments and the average of the two control tangents was used to calculate the inhibition produced by the drugs. Each experiment was repeated at least 3 times to give an average percentage inhibition for

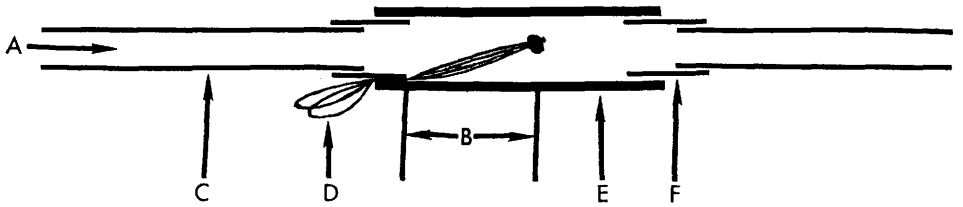

FIG. 1. Extracorporeal Shunt: (a) direction of blood flow; (b) 1 in. of thread exposed to blood; (c) polyethylene tubing (PE 200) for cannulation; (d) 12 in. length of pure silk black thread size A (Belding Corticelli) folded over twice to give 4 strands; a triple knot is tied at one end and the loose ends are cut off; (e) 2 in. length of Tygon tubing ⅛ in. i.d., 3/16 in. o.d.; (f) 1 cm length of Tygon tubing 1/16 in. i.d., ⅛ in. o.d. The shunt is assembled by pushing the pieces of tubing together, no adhesive is required.

each drug concentration. The lowest drug concentration giving an average of at least 20% inhibition was designated as the lowest inhibitory concentration.

In vivo *dosing*-in vitro *testing*. The guinea pigs (fasted for 24 hr) were dosed orally; and 1 hr after dosing blood samples were taken for *in vitro* testing as described above.

Rabbit blood samples were obtained, without anesthesia, from the central ear artery with a cannula consisting of a 20 gauge disposable needle (with hub removed), to which was attached a 5 inch length of polyethylene tubing, PE160. The needle was inserted into the artery with a clean puncture and after discarding the first ml, the blood was allowed to flow into a siliconized graduated 10 ml centrifuge tube, containing 0.8 ml of citrate, to the 8.0 ml mark. A control blood sample was taken prior to drug administration and at 1 and 3 hr after dosing. Thus, each rabbit served as its own control. The validity of this procedure was verified by oral dosing with saline instead of drug. It was found that after saline administration the aggregation response was not statistically different from that before dosing.

Extracorporeal shunt thrombus formation. Albino rabbits were used for this study. The shunt is described in Fig. 1. With the rabbit under pentobarital anesthesia, the shunt was introduced between the left carotid artery and the left jugular vein. Since the shunt is easily disassembled from the polyethylene tubing, several shunts can be introduced successively in the same animal. The thrombus forms on the silk thread which is immediately removed and gently rinsed with saline for weight determination after drying in an oven at 100° for 1 hr. Statistical evaluation of thrombus formation was calculated from the $\log_{10}$ of the dry weights (6).

Mesentery micropuncture bleeding time. The procedure used for this study was described previously for the mouse (7). The same procedure was used for the guinea pig by employing a larger animal holder.

Results. Table I presents the comparative inhibitory effects of fenoprofen sodium, aspirin, and phenylbutazone on collagen induced-

TABLE I. *In Vitro* Inhibition of Platelet Aggregation.

Drug	Lowest inhibitory conc (*M*)		
	Human PRP 1:16 collagen dilution	Rabbit PRP 1:8 collagen dilution	Guinea pig PRP undiluted collagen
Fenoprofen sodium	4.4×10^{-5}[a]	4.4×10^{-5}	4.4×10^{-5}
Aspirin	2.3×10^{-4}	1.1×10^{-4}	2.3×10^{-4}
Phenylbutazone	5×10^{-4}	1.4×10^{-4}	1.4×10^{-4}

[a] Based on free acid.

TABLE II. Effect on *in Vitro* Platelet Aggregation after *in Vivo* Dosing and on Hemostatic Plug Formation.

	Lowest active oral dose (mg/kg)			
	In vivo dosing-*in vitro* testing; 1:2 collagen dilution		Mesentery micropuncture bleeding time	
Drug	Rabbit	Guinea pig	Mouse	Guinea pig
Fenoprofen sodium	25	12.5	10	250
Aspirin	25	100	20	500[a]
Phenylbutazone	50	5	5	400[a]

[a] These values represent highest inactive dose tested.

platelet aggregation *in vitro*. The platelet counts (platelets/mm^3) of the PRP preparations ranged from 4 to 7 $\times$ 10^5, 5 to 7 $\times$ 10^5, and 4 to 6 $\times$ 10^5 for human, rabbit, and guinea pig, respectively. The intensity (dilution) of the collagen challenge used to induce aggregation was chosen so that a relative activity relationship could be established. The data indicate that fenoprofen sodium is a more potent inhibitor of collagen-induced platelet aggregation *in vitro* than either aspirin or phenylbutazone. Furthermore, it was found that fenoprofen sodium, like aspirin and phenylbutazone, had little, if any, effect on ADP-induced platelet aggregation.

The inhibitory effectiveness of these drugs, in the rabbit, on platelet aggregation *in vitro* after *in vivo* dosing was also examined. None of these drugs had any observable effect on the *in vivo* platelet counts. The results obtained 1 hr after oral dosing are presented in Table II. It is apparent that fenoprofen sodium is an active platelet aggregation inhibitor after oral dosing and is possibly superior to either aspirin or phenylbutazone. Three hours after dosing, the inhibitory activity of both aspirin and phenylbutazone was equal to that observed at 1 hr whereas the activity of fenoprofen sodium had decreased considerably.

The mesentery micropuncture bleeding time assay involves the time required for hemostasis to occur by the formation of a platelet plug closing the standardized micropuncture wound. An increase in bleeding time could indicate a decreased ability of the platelets to adhere to injured tissue and to cohere to each other and form the hemostatic plug. The bleeding time for the normal guinea pig was found to be about 10 sec longer than that of the mouse, but the increase required for significance was similar to that of the mouse (7). The results are presented in Table II and show that fenoprofen sodium is an active inhibitor of platelet function *in vivo*. In the guinea pig, it is considerably more active than either aspirin or phenylbutazone, while in the mouse, it has intermediate activity.

Platelets play a major role in arterial thrombus formation, and they may also be involved in venous thrombi (8, 9). The effect of these drugs on thrombus formation in an extracorporeal shunt was therefore determined. The rabbit was used for this study, and several types of shunt were investigated. The shunt described in Fig. 1 was chosen because it appeared to afford better repoducibility. Visual inspection of the fresh thrombus adhering to the thread under a microscope (100$\times$ magnification) indicated that these thrombi consisted of a major platelet component.

Two shunts (60 min flow time each) were introduced in each rabbit to give a mean dry thrombus weight. Shunt 1 was introduced 1 hr after oral dosing and a 8.0 ml blood sample was obtained 2 hr after dosing for assay of *in vitro* platelet aggregation. The second shunt was introduced immediately after blood sampling. The results are presented in Table III and show that inhibition of plate-

TABLE III. Inhibition by Fenoprofen Sodium and Aspirin of Thrombus Formation in an Extracorporeal Shunt and of Platelet Aggregation in the Rabbit.

No. of rabbits	Drugs	Oral dose (mg/kg)	Thrombus formation: Thrombus dry wt (mg)	Thrombus formation: Inhibition (%)	Inhibition (%) of *in vitro* platelet aggregation; aggregation inducer: Collagen undiluted suspension	Collagen 1:4 dilution	ADP $2.1 \times 10^{-5} M$
6	Saline control		9.09				
6	Fenoprofen sodium	200	4.80[a]	47	59[a]	93[a]	0
6		100	5.51[a]	39	43[a]	80[a]	4
7		50	9.30	0	31	63[a]	1
6	Aspirin	200	6.91	24	43[a]	81[a]	0
7		100	7.69	15	63[a]	96[a]	0
	Analysis of variance						
	Source	*DF*					
	Total	41					
	Treatment	5	0.4865[b]		0.4199[b]	0.4409[b]	7.083
	Fenoprofen Sodium vs. aspirin (100 and 200 doses)	1	0.6997[b]		0.0905	0.0050	4.127
	Error	36	0.0628		0.0559	0.0231	7.516

[a] Treatments significantly different from control, $p<.05$, according to Dunnett's method (10) of multiple comparison.

[b] Significant at $p<.01$.

let aggregability can indeed reduce thrombus formation under the experimental conditions employed. Fenoprofen sodium at 50 mg/kg inhibited thrombus formation by 47%, whereas a dose of 200 mg/kg of aspirin was required to reduce thrombus formation 21%.

Discussion. The results just presented show that fenoprofen sodium possesses inhibitory activity on collagen-induced platelet aggregation both *in vitro* and *in vivo*. Its action was found to be similar to that of aspirin and phenylbutazone in that it also had little effect on ADP-induced aggregation. Collagen is a normal physiological substance and represents an example of the induction of platelet aggregation via the adherence of platelets to a foreign surface with subsequent release of platelet constituents, leading to coherence and aggregation. Inhibition of the initiating foreign surface interaction may very well be sufficient to prevent or at least retard, the formation of *in vivo* thrombi. Indeed, in the extracorporeal shunt experiments, it was found that thrombus formation was inhibited by both fenoprofen sodium and aspirin. Furthermore, the results indicate a relationship between inhibition of thrombus formation and inhibition of collagen-induced aggregation *in vitro* as shown in Table III. A fairly strong inhibition of platelet aggregation, as measured by the *in vitro* assay, appears to be required before an appreciable inhibition of thrombus formation is evident. Quite possibly the silk thread in the shunt presents a stronger challenge to the platelets than the collagen in the *in vitro* assay. This shunt probably presents stronger challenge than might be expected *in vivo* under physiological conditions. If this is true, smaller doses of fenoprofen sodium would likely be effective.

Summary. Fenoprofen sodium (a new anti-inflammatory drug), aspirin, and phenylbutazone were compared with respect to their inhibitory activity on platelet function. These compounds inhibit collagen-induced platelet aggregation both *in vitro* (human, rabbit, and guinea pig) and *in vivo* (rabbit and guinea pig).

In an extracorporeal shunt experiment in the rabbit, both fenoprofen sodium and aspirin inhibited thrombus formation.

Of the three compounds tested, fenoprofen sodium appears to be the most active inhibitor of platelet function.

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