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Proquazone (Sandoz 43-715), An Unusually Potent Inhibitor of the Platelet Release Reaction and Malondialdehyde Formation[1] (39908)

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Blood platelets have proved useful in analyzing the mode of action of acetylsalicylic acid (aspirin), the most widely used nonsteroidal anti-inflammatory drug (NSAID), and indomethacin, one of the most potent (1, 2). Secretion of serotonin and nonmetabolic adenine nucleotides by the electron-dense granules of platelets in human citrated platelet-rich plasma (PRP) is inhibited by these drugs when it is triggered by ADP- or epinephrine-induced aggregation or contact with either a moderate concentration of collagen or a low concentration of thrombin.

Release and its inhibition by NSAID can readily be monitored by measuring secretion of ^{14}C-labeled serotonin or aggregation produced either with a moderate concentration of collagen or as a second wave following the primary response to exogenous ADP or epinephrine (3–10). Collagen-induced release is inhibited 50% after 15-min incubation of PRP with about 20 μM aspirin (4). Salicylic acid has virtually no effect (4, 5), and indomethacin is 5 to 10 times more active than aspirin (4, 6).

The platelet release reaction (i.e., secretion) is associated with the conversion of endogenous or exogenous arachidonic acid to prostaglandins, endoperoxides [see (11) for references], and thromboxanes (12, 13). Some of these derivatives induce platelet shape change and aggregation directly as well as indirectly by stimulating secretion of ADP and serotonin (12, 14). Aspirin and indomethacin prevent the synthesis of active compounds (13, 15) as well as a by-product, malondialdehyde (MDA) (11). Thus the concentration of MDA following an appropriate stimulus can be used to monitor the release reaction, and inhibition of its formation suggests that an inhibitor acts at the same site as aspirin and indomethacin (16).

Most NSAIDs are acidic compounds (17), and others besides aspirin and indomethacin inhibit the platelet release reaction (4, 6, 7, 18). There are also at least 10 groups of *nonacidic* compounds whose anti-inflammatory activity has already been demonstrated by *in vivo* biologic tests. In many cases their mechanism of action is unknown (19). Proquazone (1-isopropyl-7-methyl-4-phenyl-2-[^1H]quinazolinone; Fig. 1) is a nonacidic NSAID belonging to the quinazolin group. It is 35 times more active than indomethacin in preventing bradykinin-induced bronchoconstriction in guinea pigs after intravenous injection, and about equally active in other tests when given orally to rats (20, 21). This report demonstrates that proquazone is at least 50 times more active than indomethacin in its ability to inhibit platelet secretion and that it also inhibits MDA formation.

Materials and methods. Proquazone [SaH 43-715; compound 27 in Ref. (20)] is stable at 4° in dimethylsulfoxide (DMSO), and was diluted in isotonic saline before use so that 1 ml of test PRP contained at most 2 μl of DMSO. Aspirin (Amend Co., New York, N.Y.) was kept frozen as a 1 mM solution in isotonic saline. Epinephrine was obtained as a 1:1000 solution (Parke, Davis & Co., Detroit, Mich.). ADP (sodium salt, Sigma Chemical Co., St. Louis, Mo.) was kept frozen at 0.01 M in isotonic saline. Acid-soluble collagen was either prepared as described by Holmsen and others (22) and diluted with 16.7 mM acetic acid, or obtained from Hormon-Chemie (Munich, West Germany) and diluted with the recommended buffer. Thrombin was obtained from the Upjohn Co., Kalamazoo, Michi-

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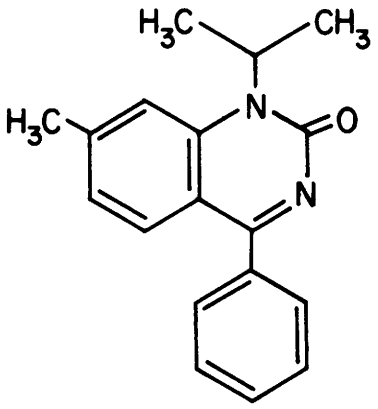

FIG. 1. Structural formula of proquazone.

gan, and *N*-ethylmaleimide (NEM) from Aldrich Chemical Co., Metuchen, New Jersey. The dilutions of aggregating agents were kept cold and discarded at the end of the day. Arachidonic acid (99% pure, Sigma) was dissolved in benzene to 0.1 *M* and stored at −70° under nitrogen. Before use, the benzene was evaporated by a stream of nitrogen and replaced with 0.1 *M* Na_2CO_3.

Unless otherwise noted, blood donors had not ingested an aspirin-containing drug for 1 week or any other drug for 24 hr. Citrated PRP was prepared and platelets were labeled with [^{14}C]serotonin as described elsewhere (5). The PRP was kept in a capped plastic tube at 20° to preserve the platelets' ability to undergo the release reaction. Four-tenths milliliter of PRP and 0.05 ml of proquazone or its vehicle were incubated in a glass aggregometer cuvette for 5 min at 37°, unless otherwise noted; samples with added aspirin (100 μM) were incubated for 15 min. The cuvette was placed in a Payton aggregation module (Payton Associates, Buffalo, N.Y.) or a Chrono-Log aggregometer (Chrono-Log Corp., Havertown, Pa.), and aggregation was induced in the stirred PRP by adding 0.05 ml of aggregating agent, for final concentrations of 30 μg or less of collagen/ml, 27.5 μM epinephrine, or 2 or 5 μM ADP. Aggregation was recorded as the maximal change in light transmission units. At 5 min, the samples were chilled and centrifuged, and the supernatants were used to measure the release of platelet-bound radioactivity (5).

To determine whether the effect of proquazone is reversible, release was also studied in platelets separated from the drug. PRP containing platelets labeled with [^{14}C]serotonin was incubated for 10 min with 1 μM proquazone or 100 μM aspirin, brought to pH 6.5 with one-fifth volume of a mixture of 65% 0.11 *M* sodium citrate and 35% 0.11 *M* citric acid, and centrifuged. The plasma was discarded, the tubes were carefully wiped, and the platelets were resuspended in a small volume of saline and brought to the original volume with citrated plasma. The resuspended platelets were treated with epinephrine and collagen in the aggregometer.

MDA production in PRP was measured after 3 min of incubation with thrombin (5 units/ml of PRP) (11) or 1 hr of incubation with 1 m*M* NEM (23). The method of Smith *et al.* (11) was used, except that samples were incubated with thiobarbituric acid for 30 min at 60°, rather than 15 min at 100°, to avoid the formation of a precipitate (C. Ingerman, personal communication).

Results and discussion. At a concentration of 220 n*M*, proquazone almost completely inhibited release induced by ADP or epinephrine. Concentrations as low as 11 n*M* were active in some experiments. Aggregation induced by 5 μM ADP does not depend on the release reaction and was not inhibited by as much as 22,000 n*M* proquazone. Collagen-induced release was inhibited 50% by about 100 n*M* proquazone

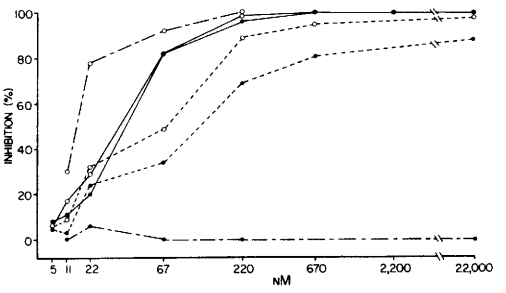

FIG. 2. Effect of different concentrations of proquazone on aggregation (●) or [^{14}C]serotonin release (○) in citrated platelet-rich plasma stirred with 27.5 μM epinephrine (——), 10 μg/ml of collagen (----), or 5 μM ADP (- - - -). Average of five experiments. Log scale on abscissa. With epinephrine, only the second wave of aggregation was measured.

TABLE I. EFFECT OF PROQUAZONE AND ASPIRIN SEPARATELY OR COMBINED ON PERCENTAGE OF PLATELET-BOUND [^{14}C]SEROTONIN RELEASED BY COLLAGEN.

Experiment No.	PRP, preaspirin ingestion + *in vitro* inhibitor[a]				PRP, postaspirin ingestion[b] + *in vitro* inhibitor[a]	
	None	Aspirin	Proquazone	Aspirin and proquazone	None	Proquazone
1	51	6	19	7	11	11
2	37	5	3	3	3	4
3	55	10	13	15	4	5
4	50	26	38	26	13	13

[a] Platelet-rich plasma incubated for 10 min with or without 100 μM aspirin, then for 5 min with or without 0.67 μM proquazone.

[b] Blood drawn 2 hr after ingestion of 0.65 g of aspirin.

(Fig. 1). The degree of inhibition at the low proquazone concentrations varied considerably with PRP samples. Arachidonic acid-induced aggregation and serotonin release were abolished by 1 μM proquazone; lower concentrations were not tested.

Proquazone was incubated with PRP for varying periods in two experiments. In one, epinephrine-induced release was completely inhibited when 165 nM proquazone was added at the same time as epinephrine, i.e., with no incubation. In the other, 35 nM proquazone caused maximal inhibition after 5 min of incubation, but not 2 min.

Even high concentrations of aspirin cannot prevent release and aggregation induced by a concentrated suspension of collagen; there is apparently a second mechanism for release which is not affected by NSAID (4). In the experiments shown in Table I, enough collagen was used so that aspirin did not entirely inhibit release. Since proquazone caused no inhibition beyond that induced *in vivo* or *in vitro* by aspirin alone, it presumably also inhibits cyclo-oxygenase. However, proquazone, unlike aspirin, causes reversible inhibition. Platelets which had been incubated with the drug and then separated showed marked aggregation and release on stirring with epinephrine or collagen, whereas platelets incubated with aspirin remained unresponsive even after separation from the drug.

Thrombin treatment of PRP led to the production of 1.5 and 1.0 nmoles of MDA/3×10^8 platelets, compared with 1.0 and 0.7 nmoles produced by NEM (two experiments). MDA production was prevented by prior 5-min incubation of the PRP with 100 μM aspirin or 0.5 μM proquazone.

Summary. Although proquazone is not an acid, its action on platelets is similar to that of a typical acidic NSAID: It inhibits the release reaction and associated production of MDA without affecting primary aggregation caused by ADP or epinephrine, and it fails to inhibit the collagen-induced release which remains after maximal inhibition by aspirin. It is unusually active; it may have an effect *in vitro* at 11 nM, and is at least 50 times more active than indomethacin (4) in preventing collagen-induced release from human platelets.

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