Inhibition of Human Platelet Aggregation by Dimethylsulfoxide, Dimethylacetamide, and Sodium Glycerophosphate[1] (36751)

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Dimethylsulfoxide (DMSO), dimethylacetamide (DMAC), and sodium glycerophosphate reduce platelet damage associated with freezing (1–3). DMSO and DMAC, with dextrose, improved the ability of frozen platelets to support clot retraction, and improved morphologic preservation of platelets observed by phase-contrast microscopy (1–2). Recovery of frozen rat platelets after transfusion into thrombocytopenic rats also was enhanced by exposure of platelets to DMSO or DMAC and dextrose at the time of freezing (1–2). Sodium glycerophosphate alone provided partial protection of human platelets against loss of platelet aminopeptidase activity after freezing (3). During studies of the ultrastructural characteristics of freeze dried blood platelets in this laboratory, platelet aggregation was noted to be diminished by cryoprotective agents. Aggregation of platelets has a fundamental role in hemostasis and therefore the reduction of aggregation by cryoprotective agents has been examined in detail.

Materials and Methods. Human venous blood was collected with 0.1 vol acid citrate anticoagulant (containing 3 vol of 0.1 *M* Na citrate and 2 vol 0.1 *M* citric acid) and platelet-rich plasma (PRP) prepared by centrifugation at 21° and 450*g*. Aggregation was evaluated at 37° by a turbidimetric method (4), with a Chrono-log aggregometer, 1 hr after collection of PRP. PRP (0.4 ml) was incubated for 2 min at 37° with 0.05 *M* Tris buffered saline (TBS) (0.05 ml), or with a solution of a cryoprotective agent in TBS prior to addition of the aggregating agent. Final concentrations of aggregating agents were: adenosine diphosphate (ADP), 2×10^{-6} *M*; epinephrine, 5×10^{-5} *M*; thrombin, (0.3 U/ml). In each series of experiments, aggregation with TBS was compared to that with different concentrations of cryoprotective agents. Only those samples which showed a biphasic response with TBS were observed for the effects of cryoprotective agents on epinephrine induced aggregation. The extent of aggregation of PRP was determined from the ratio of change of transmittance of PRP to the transmittance of similarly diluted platelet poor plasma from the same blood samples. Adenosine-5′-diphosphate and epinephrine bitartrate were obtained from Calbiochem (Los Angeles, CA) and bovine thrombin from Parke Davis and Company (Detroit, MI).

Results. Platelet aggregation induced by ADP, epinephrine, or thrombin was decreased by each cryoprotective agent studied. The effects are summarized in Table I. DMSO reduced aggregation by ADP, epinephrine, and thrombin but only at concentrations greater than 1%. Epinephrine caused minimal aggregation in the presence of 2.5% DMSO whereas a biphasic response occurred with 1% DMSO (Fig. 1). DMAC blocked aggregation at lower concentrations than DMSO (Table I and Fig. 2). In the presence of 5% DMAC, ADP, epinephrine and thrombin failed to cause aggregation in 20 of 22 experiments, whereas with 5% DMSO aggregation was absent in 10 of 21 experiments. Significant inhibition of the extent of aggregation occurred with 0.5% DMAC. A double wave of aggregation occurred after addition of epinephrine in 3 of 5 experiments with 0.5% DMAC. Aggregation by ADP but not

[1] Supported in part by a grant in aid from the American Heart Association.

TABLE I. Mean Percentage Change of Transmittance During Aggregation of Human Platelet-Rich Plasma with Added DMSO, DMAC, and Sodium Glycerophosphate.[a]

Conen of cryoprotective agent	Aggregating agent: ADP (2×10^{-6} *M*)	Epinephrine (5×10^{-5} *M*)	Thrombin (0.3 U/ml)
DMSO (%)			
1.0	41 ± 4[b]	68 ± 12[b]	38 ± 13[b]
2.5	31 ± 3	9 ± 3	23 ± 8
5.0	31 ± 3	0	9 ± 8
DMAC (%)			
0.05	37 ± 8[b]	75 ± 6[b]	61 ± 9[b]
0.1	33 ± 5	58 ± 12[b]	57 ± 6[b]
0.5	21 ± 4	43 ± 10	28 ± 8
1.0	9 ± 3	12 ± 4	11 ± 3
5.0	1 ± 1	0	1 ± 1
Sodium glycerophosphate (*M*)			
0.05	25 ± 4	46 ± 13	50 ± 13[b]
0.15	21 ± 5	3 ± 3	4 ± 4
0.25	14 ± 5	0	0
Control (0.05 *M* TBS)	52 ± 4	78 ± 4	57 ± 7

[a] Mean value ± SEM.

[b] Value not significantly different from aggregation of TBS control sample. All other values were significantly different from control ($p \leq .01$).

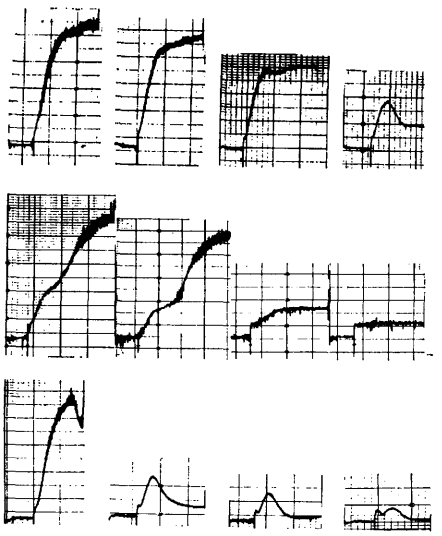

FIG. 1. Effect of DMSO on the aggregation of human platelets by 2×10^{-6} *M* ADP (top), 5×10^{-5} *M* epinephrine (middle), and 0.3 U/ml thrombin (bottom). Left to right in each row: TBS control, 1% DMSO, 2.5% DMSO, 5% DMSO. (ordinate) Light transmission units; (abscissa) time (1/4 in. = 1 min).

epinephrine or thrombin was reduced by 0.1% DMAC, whereas 0.05% DMAC caused no significant reduction with any aggregating agent (Table I). Addition of 5% dextrose to DMAC or DMSO prior to aggregation of PRP did not appear to alter the inhibition of aggregation caused by the cryoprotective agents (8 experiments).

Sodium glycerophosphate effectively inhibited aggregation at concentrations of 0.15 and 0.25 *M*. Aggregation by epinephrine and thrombin was absent after addition of 0.25 *M* sodium glycerophosphate (Table I and Fig. 3).

Each of the experiments was reviewed to determine whether cryoprotective agents reduced primary aggregation or only the second wave of aggregation. DMSO, DMAC and sodium glycerophosphate reduced primary aggregation by ADP and caused an accelerated reversal of aggregation. In each case in which the release reaction (second wave) appeared to be diminished the extent of primary aggregation was also decreased. All cryo-

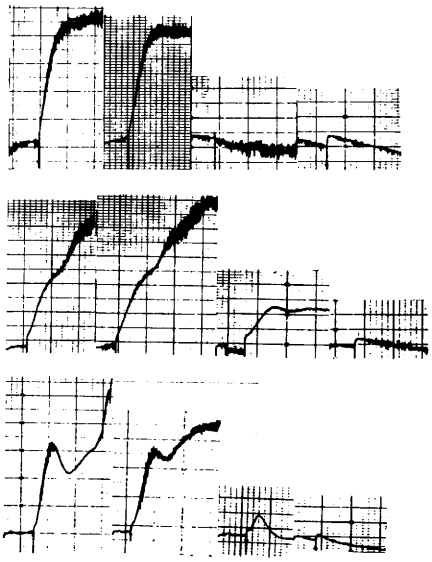

FIG. 2. Effect of DMAC on the aggregation of human platelets by 2×10^{-6} *M* ADP (top), 5×10^{-5} *M* epinephrine (middle), and 0.3 U/ml thrombin (bottom). Left to right in each row: TBS control, 0.05% DMAC, 1% DMAC, 5% DMAC. (ordinate) light transmission units; (abscissa) time (1/4 in. = 1 min).

protective agents also decreased the extent of primary aggregation by epinephrine and thrombin. Initial aggregation by epinephrine was diminished by DMSO and DMAC even though the second wave of aggregation remained intact. In two experiments in which aggregation was caused by thrombin a second wave appeared in the presence of DMAC and sodium glycerophosphate, although absent in control samples. Thus, the dominant effect of cryoprotective agents appeared to be an inhibition of primary aggregation, and not of the release reaction.

In a separate series of six experiments TBS (control), 5% DMSO, or 5% DMAC in TBS were added to platelet-rich plasma, which was centrifuged (10 min; 450*g*; 21°) to form a platelet button. The platelets were resuspended in platelet-poor plasma (PPP) from the same blood sample to which DMSO and DMAC had not been added. Aggregation by ADP, epinephrine and thrombin was reduced, or blocked completely, except for platelets exposed to DMSO and aggregated by ADP (Fig. 4).

Discussion. The clinical need to preserve platelets for subsequent transfusion has lead to the freezing of platelets with cryoprotective agents, including DMSO and DMAC. These agents have been clearly demonstrated to enhance posttransfusion increments of platelet counts (2).

These agents, however, clearly diminish the extent of platelet aggregation by ADP, epinephrine, and thrombin and appropriate concentrations may effectively prevent aggregation. The primary action of cryoprotective agents appeared to be a reduction in initial aggregation and not inhibition of the release reaction. Inhibition of aggregation was observed at lower concentrations of DMAC than DMSO. Similar inhibition of platelet aggregation by DMSO was noted by Streiff and co-workers (5), using much larger concentrations of DMSO (11%), and by White (6), who noted that "1% DMSO affects platelet aggregation." Once platelets were exposed to 5% DMSO or DMAC the presence of the cryoprotective agent was not required. The inhibitory effect on platelet aggregation re-

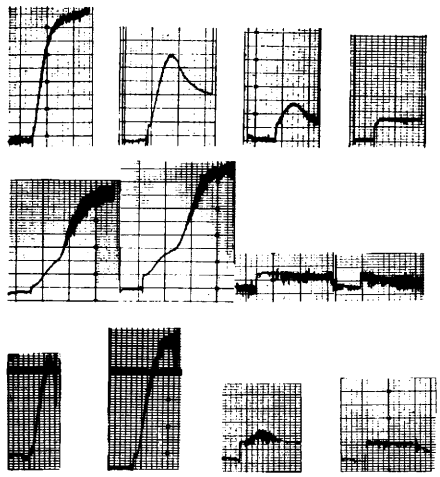

FIG. 3. Effect of sodium glycerophosphate on the aggregation of human platelets by 2×10^{-6} *M* ADP (top), 5×10^{-5} *M* epinephrine (middle), and 0.3 U/ml thrombin (bottom). Left to right in each row: TBS control, 0.05 *M* sodium glycerophosphate 0.15 *M* sodium glycerophosphate, 0.25 *M* sodium glycerophosphate. (ordinate) Light transmission units. (abscissa) time (1/4 in = 1 min).

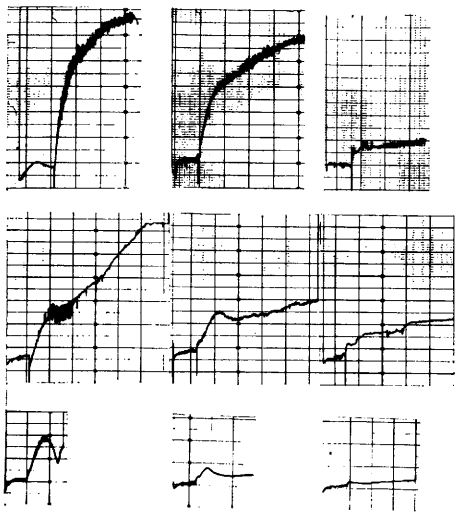

FIG. 4. Aggregation of human platelets incubated with DMSO or DMAC and resuspended in PPP without DMSO or DMAC. Aggregating agents: 2×10^{-6} *M* ADP (top), 5×10^{-5} *M* epinephrine (middle), and 0.3 U/ml thrombin (bottom). Left to right: incubation with TBS, 5% DMSO, 5% DMAC. (ordinate) Light transmission units; (abscissa) time (1/4 in. = 1 min).

mained when platelets were resuspended in platelet-poor plasma which did not contain DMSO or DMAC.

DMAC is a more effective cryoprotective agent when dextrose is added (2), and DMAC and DMSO are most often used with 5% dextrose. The addition of dextrose to PRP with DMSO and DMAC did not restore the ability of platelets to aggregate in the presence of ADP, epinephrine, or thrombin, however.

DMSO not only blocked platelet aggregation, but has been reported to damage platelets with release of platelet amino acids, adenine nucleotides, acid phosphatase, and β-glucuronidase from washed platelets (7). It has been suggested that DMSO induced changes mainly in the platelet plasma membrane, possibly as a result of lipid peroxidation. The possible effect of dextrose in reducing release of platelet enzymes and amino acids, by DMSO, apparently has not been evaluated.

DMSO is believed to protect cells against freezing injury by exerting a colligative action (8). DMSO also has been postulated to bind sulfhydryl groups (9). Since sulfhydryl groups are important in the normal aggregation of platelets by ADP (10, 11), the reduction of aggregation by DMSO may be effected, in part, by sulfhydryl binding.

Prostaglandin E_1 (PGE_1), which is a potent inhibitor of platelet aggregation, recently has been used in the preparation and storage of platelet concentrates (12). PGE_1 in concentrations which did not inhibit aggregation by ADP, collagen, and epinephrine, reduced platelet clumping in whole blood, aided in resuspension of platelets, and did not reduce the platelet life span (12). Cryoprotective agents and PGE_1 may inhibit aggregation by different mechanisms, but the recovery and life spans of frozen platelets were not improved by PGE_1 added to DMSO (13). Because cryoprotective agents inhibit aggregation, their role in the hemostatic effectiveness of frozen platelets, with or without other inhibitors of platelet aggregation, deserves further study.

Summary. DMSO, DMAC, and sodium glycerophosphate reduced aggregation of human platelets by ADP, epinephrine, and thrombin. Initial aggregation by ADP and the secondary wave of aggregation associated with the release of platelet ADP were inhibited. DMSO and DMAC with 5% dextrose blocked aggregation as effectively as DMSO or DMAC alone. Platelets exposed to DMSO, DMAC or sodium glycerophosphate and resuspended in PPP free of cryoprotective agents did not aggregate as did platelets exposed to TBS, suggesting that platelets had been irreversibly altered *in vitro*.

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Received April 7, 1972. P.S.E.B.M., 1972, Vol. 141.