The Effects of Indomethacin on the Generalized Shwartzman Reaction

Edward Lee Howes, Jr., MD, Marion Tong Kwok, AB, and Donald G. McKay, MD

The antiinflammatory drug indomethacin, an inhibitor of prostaglandin synthesis, prevents the generalized Shwartzman reaction produced in rabbits by two intravenous injections of bacterial endotoxin. Indomethacin has this effect if given before the first but not the second injection of endotoxin. Measurements of circulating white blood cells, platelets, partial thromboplastin time, prothrombin time, fibrinogen, plasminogen, and soluble fibrin were made at several times after either the first or second injection of endotoxin in treated and nontreated rabbits. Four hours after the first injection of endotoxin, leukopenia and thrombocytopenia were somewhat greater in treated rabbits and the prolongation of the activated partial thromboplastin time was shortened. Twenty-one hours after injection of endotoxin, leukocytosis and elevation of plasma fibrinogen were not as great in treated animals. Four hours following the second injection of endotoxin a decrease in fibrinogen, prolongation of the prothrombin time, and the elaboration of soluble fibrin were consistently found in rabbits with the generalized Shwartzman reaction. In treated rabbits, none of these changes occurred. Indomethacin prevents the generalized Shwartzman reaction by preventing the development of the prepared state in this endotoxin model. (Am J Pathol 90:7–22, 1978)

SEVERAL OF THE EFFECTS of circulating bacterial endotoxin involve the elaboration of prostaglandins and related substances and can be inhibited by indomethacin, a potent inhibitor of prostaglandin synthetase. Pyrogen-induced fever in rats is associated with elevations of prostaglandins of the E series in the cerebrospinal fluid and can be inhibited by indomethacin.[1] An altered vascular permeability is produced by the direct injection of prostaglandins in the rabbit eye,[2] prostaglandins are elevated in ocular inflammation produced by endotoxin,[3] and the ocular response to both local and intravenous endotoxin can be inhibited by indomethacin.[3,4] Finally, prostaglandins are elaborated in endotoxin shock in dogs,[5] and this shock can be prevented by indomethacin pretreatment.[6]

The following study was undertaken to determine the effects of indomethacin on the generalized Shwartzman reaction produced in rabbits by two intravenous injections of endotoxin given 21 hours apart. It was found that indomethacin prevented the reaction if given before the first or preparatory endotoxin injection and that the prevention was the result of a failure of intravascular coagulation. No prevention was found if in-

From the Department of Pathology, University of California Service, San Francisco General Hospital, San Francisco, California.

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Address reprint requests to Dr. Donald G. McKay, Department of Pathology, University of California Service, San Francisco General Hospital, San Francisco, CA 94110.

domethacin was given before the second or provocative endotoxin injection.

Materials and Methods

Generalized Shwartzman Reaction

Two intravenous injections of bacterial endotoxin were made 21 hours apart and the generalized Shwartzman reaction was determined by the presence of glomerular fibrin thrombi on histopathologic study 4 hours after the second injection. The endotoxin employed was the lipopolysaccharide of *Escherichia coli* 055:B5 (Difco Laboratories, Detroit, Mich.) and each injection contained 200 μg dissolved in 2.0 ml of sterile isotonic saline.

Indomethacin

Indomethacin was given in varying quantities via oral and intraperitoneal routes before the first or before the second injection of endotoxin.

Oral Administration

A suction catheter (18 French, Bard Parker) was inserted into the stomach. Both 3 and 1 hours before endotoxin, indomethacin dissolved in 10 ml of water was administered. The quantities varied from 40 to 160 mg/kg at each time period, and initially the contents of commercially available capsules were used.

Intraperitoneal Administration

Indomethacin sodium was prepared from indomethacin powder (Merck, Sharpe and Dohme Research Laboratories, West Point, Pa). The aqueous solution was prepared fresh daily by neutralizing with Na_2CO_3 (anhydrous) by adding 0.012 to 0.023 g of Na_2CO_3 to 8.0 ml of water containing 20 to 60 mg/kg of indomethacin. Intraperitoneal injections were made 1 hour before the first or 1 hour before the second injection. Untreated animals were injected with the diluent only.

Animal Studies

New Zealand white male and female rabbits weighing 1.8 to 2.2 kg were fed rabbit chow and given water *ad libitum*. The experiments were divided into two parts.

Effects of Dosage and Route of Administration of Indomethacin

Rabbits were pretreated with various quantities of indomethacin. Oral pretreatment consisted of 40, 100, or 160 mg/kg of indomethacin given twice, ie, 3 and 1 hours before the first or second injection of endotoxin. Intraperitoneal pretreatment was with 20, 40, or 60 mg/kg of indomethacin given once, ie, 1 hour before the first or before the second injection of endotoxin. In all hematologic studies, intraperitoneal injections with 40 mg/kg of indomethacin was the form of treatment.

Hematologic Studies

Blood samples were taken at varying times either after the first or after the second endotoxin. To facilitate the obtaining of blood, a small skin incision was made over the middle portion of the central ear artery and a fine polyethylene tubing was inserted into the artery (Intramedic R; Clay Adams, Parsippany, NJ). This operative procedure required 5 minutes with a minimum of trauma and was performed with a local anesthetic (lidocaine [Xylocaine] hydrochloride 1%). The patency of this arterial line was maintained by a continuous pump infusion of sterile pyrogen-free saline at a rate of 2.5 ml/hr (Unita I, B.

Braun, Melsunger, West Germany). All blood samples were taken from this arterial tubing. All tubing and containers were siliconized. Each sample of blood was anticoagulated with 3.8% trisodium citrate (7.2 ml blood: 0.8 ml citrate). Samples were routinely obtained before the first or second injection of endotoxin and 0.5 and 4 hours after the injection. In some rabbits additional samples were obtained before indomethacin pretreatment and 2 and 21 hours after the first or second endotoxin injection.

To prepare plasma, the blood was centrifuged at 2300 rpm for 20 minutes, the plasma was pipetted, and portions were quick frozen in acetone cooled by dry ice. These specimens were stored at −70 C until used. Determinations of prothrombin time, activated partial thromboplastin time, and the protamine sulfate serial dilutions were performed within 30 minutes of collection. Fibrinogen and plasminogen determinations were done on the frozen plasma samples.

Hematocrit. Microhematocrits were performed on all blood samples with heparinized capillary tubes (Capilets; Dade Laboratories, Miami, Fla.).

White Blood Cell and Platelet Counts. The Unopette system (Becton-Dickinson, Rutherford, NJ) was used; 20 μl of blood was added to 2 ml of 1% ammonium oxalate. Total white blood cell counts were made in a standard fashion, and the number of rabbit heterophils was determined by counting 200 cells on stained blood smears. Platelet counts were made by phase microscopy.[7]

Prothrombin Time. A one-stage prothrombin test [8] was performed using a fibrometer (B.B.L., Becton-Dickinson, Rutherford, NJ).

Activated Partial Thromboplastin Time. A modification of the method of Nye and Brinkhous was used.[9] Four milliliters of Thrombofax reagent (Ortho Diagnostics, Raritan, NJ) was prepared with 4.0 ml of kaolin in Owren's buffer. Plasma (0.10 ml) was mixed with reagent (0.10 ml) and incubated for 3 minutes with mixing. $CaCl_2$ (0.025 M) was added and the time of clotting was determined by fibrometer.

Fibrinogen. A modification [10] of the method of Blomback and Blomback [11] was used.

Plasminogen. The determination of plasminogen was by a caseinolytic method.[12] Human urokinase (Hoffman-La Roche, Basel, Switzerland) was employed as activator (400 CTA units) and α-casein (Worthington Biochemicals, Freehold, NJ) was used as substrate. A pooled sample of plasma from 10 normal rabbits was used for control purposes. Values are expressed as a percent of starting values for each rabbit..

Circulating Soluble Fibrin. The serial-dilution protamine sulfate test (SDPS) was employed.[13] Dilutions of protamine sulfate of 1/5, 1/10, 1/20, 1/40, and 1/80 were made. A positive test was determined by the presence of fibrin strands or gel in dilutions of 1/20 or higher after standing 30 minutes at room temperature.[14]

Experimental Design for Hematologic Study

Blood samples were taken just before and at selected times either after the first or after the second endotoxin.

Rabbits were divided into the following groups for hematologic study:

After the first endotoxin injection (No. of rabbits in each group)

Group A Endotoxin alone (11)
Group B Indomethacin 40 mg/kg 1 hour before endotoxin (9)
Group C Normal rabbits (11)
Group D Indomethacin 40 mg/kg in normal rabbits (9)

After the second endotoxin injection (No. of rabbits in each group)

Group E Endotoxin alone (13)
Group F Indomethacin 40 mg/kg 1 hour before first endotoxin injection (9)
Group G Indomethacin 40 mg/kg 1 hour before second endotoxin injection (10)
Group H Indomethacin 40 mg/kg 22 hours before sampling (normal rabbits) (7)

Histopathologic Studies

Kidneys were removed 4 hours following the second injection of endotoxin, fixed in Zenker's solution, washed, and processed for light microscopy. Sections were stained by hemotoxylin and eosin and phosphotungstic acid hematoxylin. Fibrin strands and clumps in capillaries of renal glomeruli indicated the generalized Shwartzman reaction.

Statistical Evaluation

The Student t test was used to evaluate the significance of differences between mean values and mean percent changes in values. A P value of <0.01 was considered significant.

Results

Effect of Dosage and Route of Administration of Indomethacin

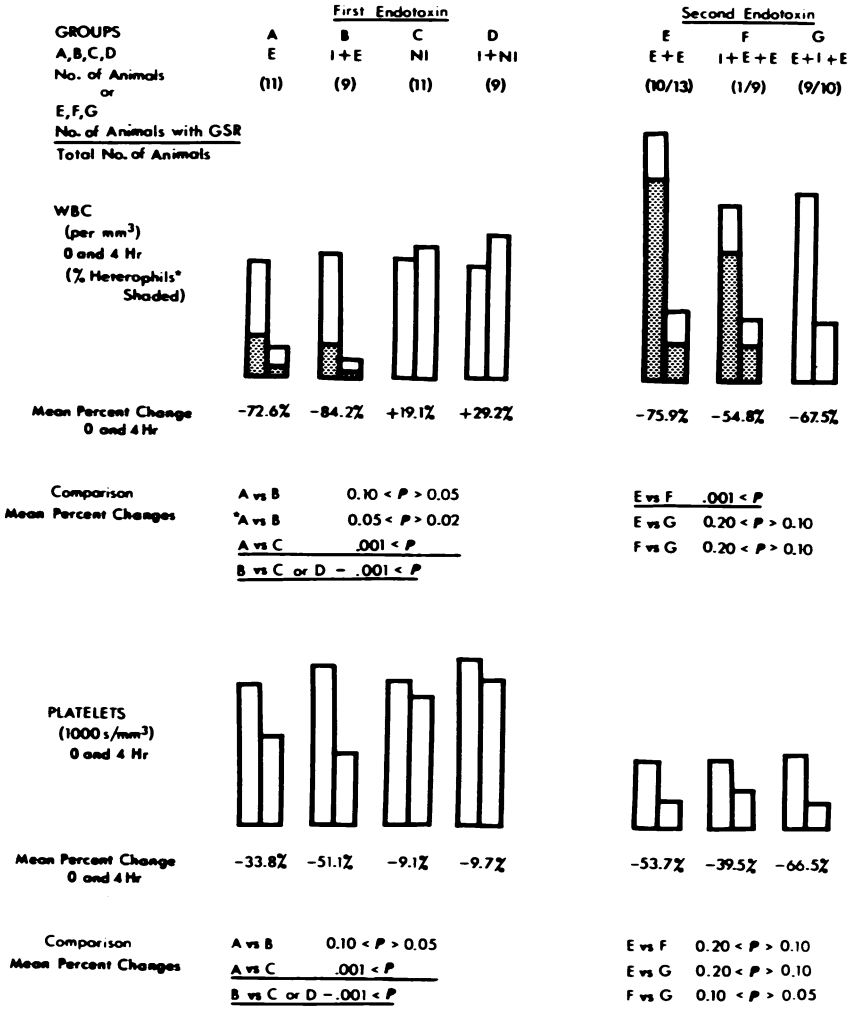
The effects of varying quantity, route of administration, and timing of indomethacin treatment are recorded in Table 1. Indomethacin given orally in quantities of 100 mg/kg, 3 and 1 hours prior to the first endotoxin injection, prevented the generalized Shwartzman reaction in all but 1 of 8 rabbits. A single intraperitoneal injection of indomethacin of 40 mg/kg 1 hour before the first injection of endotoxin was equally effective in prevention; 20 mg/kg given by this route was somewhat less effective, and 3 of 8 rabbits developed the generalized Shwartzman reaction. Treatment with indomethacin prior to the second injection was not successful in preventing the reaction, regardless of quantity of indomethacin used or route of administration employed (Table 1). In all subsequent hematologic studies, indomethacin in quantities of 40 mg/kg was given intraperitoneally 1 hour prior to either the first or second injection of endotoxin.

Table 1—Effect of Indomethacin on the Generalized Shwartzman Reaction

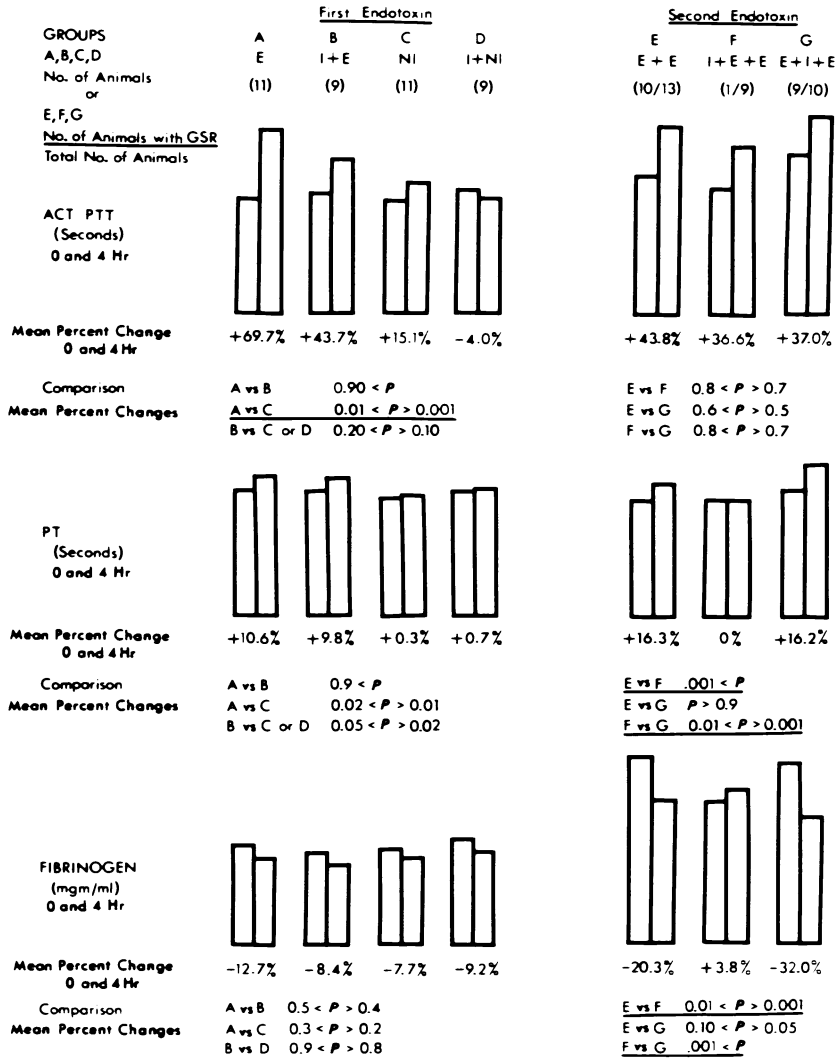
Dosage and route of administration	Incidence of generalized Shwartzman reaction	
	Treated	Endotoxin Alone
Oral indomethacin		
Before first endotoxin injection		
40 mg/kg 3 and 1 hr before	4/4	4/5
100 mg/kg 3 and 1 hr before	1/8	8/8
Before second endotoxin injection		
40 to 100 mg/kg 3 and 1 hr before	6/6	5/5
160 mg/kg 3 and 1 hr before	6/7	6/8
Intraperitoneal indomethacin		
Before first endotoxin injection		
40 mg/kg 1 hr before	1/9	7/8
20 mg/kg 1 hr before	3/8	7/8
Before second endotoxin injection		
40 to 60 mg/kg 1 hr before	5/6	6/6

Hematologic Studies

Results are presented in graphic form in Text-figures 1 and 2. Average values and standard deviations before and 4 hours after either the first or second injection of endotoxin are given in Tables 2 and 3. Values recorded just before the second injection of endotoxin are actually 21 hours follow-



TEXT-FIGURE 1—Effects of indomethacin on the generalized Shwartzman reaction. White blood cells (WBC) and platelets. Group A = endotoxin only; Group B = indomethacin before endotoxin; Group C = normal rabbits; Group D = indomethacin in normal rabbits; Group E = endotoxins 21 hours apart; Group F = indomethacin before first of two endotoxins; Group G = indomethacin before second of two endotoxins. Bars represent average values just before and 4 hours after first or second endotoxin. Times after second endotoxin are 21 and 25 hours after first endotoxin. Significant differences between mean percent changes are underlined.



TEXT-FIGURE 2—Effects of indomethacin on the generalized Shwartzman reaction. Activated partial thromboplastin times (Act PTT), prothrombin times (PT), plasma fibrinogens. Group A = endotoxin only; Group B = indomethacin before endotoxin; Group C = normal rabbits; Group D = indomethacin in normal rabbits; Group E = endotoxins 21 hours apart; Group F = indomethacin before first of two endotoxins; Group G = indomethacin before second of two endotoxins. Bars represent average values just before and 4 hours after first or second endotoxin. Times after second endotoxin are 21 and 25 hours after first endotoxin. Significant differences between mean percent changes are underlined.

Table 2—Changes Following the First Injection of Endotoxin

Experimental groups (No. of animals)	WBC (cells/cu mm) Percent heterophils			Platelets (1000s/cu mm)			Activated partial thromboplastin time (sec)			Prothrombin time (sec)			Fibrinogen (mg/ml)			Plasminogen (%Δ 0-4 hr)	Protamine sulfate (No. positive at 4 hr)
	0	4	%Δ	0	4	%Δ	0	4	%Δ	0	4	%Δ	0	4	%Δ		
Endotoxin alone (11)	5690 ± 2420 35.5 ± 10.2	1380 ± 820 31.7 ± 18.4	−72.6 ± 19.8 −73.6* ± 26.3	342 ± 112	217 ± 83	−33.8 ± 22.0	56.2 ± 12.4	90.0 ± 15.7	+69.7 ± 54.0	6.1 ± 0.5	6.8 ± 0.9	+10.6 ± 9.7	2.45 ± 0.43	2.13 ± 0.39	−12.7 ± 9.5	−14.5 ± 15.0	8/11
Endotoxin after indomethacin (9)	6060 ± 2490 26.5 ± 10.6	850 ± 440 16.5 ± 14.0	−84.2 ± 8.3 −90.2* ± 7.8	385 ± 98	178 ± 56	−51.1 ± 15.5	58.6 ± 17.3	75.0 ± 16.8	+43.7 ± 63.4	6.1 ± 0.6	6.9 ± 0.8	+9.8 ± 8.8	2.24 ± 0.40	1.99 ± 0.26	−8.4 ± 13.7	−18.2 ± 13.0	6/9
Normal rabbits (11)	5720 ± 2540	6420 ± 1970	+19.1 ± +38.7	352 ± 83	317 ± 57	−9.1 ± 18.8	55.3 ± 8.4	63.9 ± 10.3	+15.1 ± 18.0	5.7 ± 0.3	5.8 ± 0.2	+0.3 ± 5.0	2.33 ± 0.40	2.13 ± 0.37	−7.7 ± 6.4	−2.9 ± 17.0	2/11
Normal rabbits (9) and indomethacin	5520 ± 1300	6900 ± 1850	+29.2 ± 36.0	407 ± 143	358 ± 142	−9.7 ± 29.0	60.6 ± 20.1	56.3 ± 12.4	−4.0 ± 51.0	6.0 ± 0.5	6.1 ± 0.5	+0.7 ± 5.0	2.55 ± 0.30	2.27 ± 0.28	−9.2 ± 7.0	−0.5 ± 32.0	2/9

Mean values at 0 and 4 hours ±SD and mean changes ±SD.
* Percent change in total heterophils/cu mm.

Table 3—Changes Following the Second Injection of Endotoxin

Experimental groups (No. animals of total with generalized Shwartzman reaction)	WBC (Cells/cu mm) Percent heterophils			Platelets (1000s/cu mm)			Activated partial thromboplastin time (sec)			Prothrombin time (sec)			Fibrinogen (mg/ml)			Plasminogen	Protamine sulfate	
	0	4	% Δ	0	4	% Δ	0	4	% Δ	0	4	% Δ	0	4	% Δ	% Δ 0–4 hr	No positive 0 hr	4 hr
Endotoxin twice, 21 hr apart (10/13)	12,110 ± 6970	3410 ± 2490	−75.9 ± 7.3	162 ± 80	65 ± 40	−53.7 ± 19.0	67.6 ± 16.3	91.3 ± 12.5	+43.8 ± 47.0	5.8 ± 0.4	6.4 ± 0.4	+16.3 ± 7.0	4.54 ± 1.26	3.52 ± 0.70	−20.3 ± 16.0	+2.3 ± 12.0	4/13	12/13
	80.9 ± 5.5	55.0 ± 13.7	−83.3* ± 8.0															
Endotoxin twice, 21 hr apart (1/9) (indomethacin before first injection)	8604 ± 3047	3067 ± 1280	−54.8 ± 14.3	168 ± 90	92 ± 44	−39.5 ± 22.0	61.9 ± 12.0	81.2 ± 17.6	+36.6 ± 41.0	5.6 ± 0.3	5.6 ± 0.4	0.0 ± 0.4	3.49 ± 0.47	3.73 ± 0.56	+3.8 ± 14.0	−1.5 ± 12.0	2/9	4/9
	72.9 ± 16.3	58.4 ± 8.5	−62.4* ± 17.7															
Endotoxin twice, 21 hr apart (9/10) (indomethacin before second injection)	9162 ± 4950	2920 ± 2330	−67.5 ± 21.4	181 ± 54	59 ± 35	−66.5 ± 54.0	78.3 ± 19.7	97.4 ± 31.0	+31.0 ± 45.0	6.3 ± 0.5	7.3 ± 0.5	+16.2 ± 11.0	4.40 ± 0.90	3.04 ± 1.22	−32.0 ± 15.0	+8.0 ± 10.8	3/10	9/10
Normal rabbits, (0/7) (indomethacin 22 hr before)	5190 ± 1070	4690 ± 1320	−8.8 ± 21.6	480 ± 137	415 ± 107	−12.2 ± 10.6	52.3 ± 10.2	49.4 ± 10.0	−3.9 ± 18.0	5.7 ± 0.3	5.8 ± 0.4	+5.2 ± 3.9	3.24 ± 0.54	3.12 ± 0.60	−2.0 ± 13.1	+9.0 ± 10.8	1/7	1/7

Mean values at 0 and 4 hours ±SD and mean percent changes ±SD (0 hours is 21 hours after first injection of endotoxin).
* Percent change in total heterophils/cu mm.

ing the first endotoxin (Table 3). Samples were taken additionally at 0.5, 2, and 21 hours after the first endotoxin injection and in some cases prior to indomethacin. Average values of these determinations did not further contribute to interpretation and are not included in the tables.

Hematocrits at 0 and 4 hours decreased by no more than 15% and averaged a 6% decrease. No attempt was made to correct for this blood loss in the presentation of the results of hematologic studies in normal control and treated normal control rabbits (Groups C and D, Text-figure 1) (Table 2) which were available for direct comparison. Neither the method of blood sampling nor the loss of blood had other than minimal effects on results in normal rabbits.

Leukocytes and Platelets

A significant leukopenia and thrombocytopenia occurred 4 hours following endotoxin in both nontreated (Group A) and treated groups (Group B, Text-figure 1) (Table 2). Four hours following the first injection of endotoxin, a somewhat greater decrease in total leukocytes, heterophils, and platelets was noted in treated rabbits ($-84.2 \pm 8.3\%$, $-90.2 \pm 7.8\%$, $-51.1 \pm 15.5\%$) compared with nontreated rabbits ($-72.6 \pm 19.8\%$, $-73.6 \pm 26.3\%$, $-33.8 \pm 22.0\%$), but in no case was the difference statistically significant (Text-figure 1).

Twenty-one hours following the first and just before the second endotoxin injection (Text-figure 1 and Table 3), the average WBC count was 12,110 ± 6970/cu mm, with 80.9 ± 5.5% heterophils in rabbits given endotoxin alone and 8064 ± 3044/cu mm with 72.9 ± 16.3% heterophils in rabbits pretreated with endotoxin. Four hours following a second injection of endotoxin, mean percent decreases in WBC counts of 75.9 ± 7.3% and in heterophils of 83.3 ± 8.0% were noted in nontreated rabbits (Group E), and mean percent decreases in WBC counts of 54.8 ± 14.3% and in heterophils of 62.4 ± 17.7% were noted in rabbits pretreated with indomethacin prior to the first endotoxin (Group F). The mean percent changes in both WBCs and heterophils were significantly different in the two groups, and this difference was primarily due to the extent of the leukocytosis prior to the second endotoxin injection in untreated rabbits. In rabbits pretreated with indomethacin prior to the second injection of endotoxin (Group F) the mean percent decrease in leukocytes was not significantly different from the other groups (Text-figure 1 and Table 3).

Twenty-one hours following the first injection of endotoxin, the numbers of platelets were decreased in a similar fashion in all groups. Following a second injection of endotoxin, the mean percent decreases were not significantly different in any group (Text-figure 1 and Tables 2 and 3).

Activated Partial Thromboplastin Time

Four hours following the first injection of endotoxin, the activated partial thromboplastin time was increased by a mean percent change of 69.7 ± 54.0% in untreated rabbits and 43.7 ± 63.4% in treated rabbits. Because of the marked variability from rabbit to rabbit these changes were not significant (Text-figure 2 and Table 2). Four hours following a second injection of endotoxin, all groups showed a prolongation of the activated partial thromboplastin time of approximately the same magnitude (Text-figure 2 and Table 3).

Prothrombin Time

Four hours following the first injection of endotoxin, a prolongation of the average prothrombin time was noted in both treated and untreated rabbits. The mean percent change was essentially the same in the two groups; although the prothrombin times were increased, the change was not significantly different from normal rabbits and normal rabbits given indomethacin (Text-figure 2 and Table 2).

Four hours following the second endotoxin injection, no change in average prothrombin time was observed in rabbits pretreated with endotoxin prior to the first endotoxin injection (Group F). But in rabbits receiving endotoxin only (Group E) and rabbits treated prior to the second endotoxin injection (Group G), the mean percent prolongation of the prothrombin times was 16.3 ± 7.0% and 16.2 ± 11.0%, respectively; both changes are significantly different from those of Group F (Text-figure 2 and Table 3).

Fibrinogen

Four hours following the first injection of endotoxin, a decrease in plasma fibrinogen of a similar magnitude was noted in all groups (Text-figure 2 and Table 3). Twenty-one hours after the first endotoxin injection the average plasma fibrinogen in untreated rabbits was 4.54 ± 1.26 mg/ml and 3.49 ± 0.47 mg/ml in rabbits pretreated with indomethacin ($0.05 > P > 0.02$). The average plasma fibrinogen was 2.68 ± 0.89 mg/ml 21 hours after cutdown in normal rabbits and significantly different from rabbits receiving endotoxin alone ($0.01 < P < 0.001$). The average plasma fibrinogen level 21 hours after indomethacin was 3.24 ± 0.54 mg/ml (Group H), and this value was not significantly different from the normal ($0.3 < P > 0.2$) (Table 3).

Four hours following a second endotoxin injection, a slight increase in plasma fibrinogen was noted in rabbits pretreated with indomethacin prior to the first injection of endotoxin (Group F). By contrast, 4 hours

after a second endotoxin injection in untreated rabbits (Group E) and rabbits treated only just prior to the second endotoxin injection (Group G), there was a mean percent decrease of 20.3 ± 16.0% and 32.0 ± 15.0%, respectively (Text-figure 2 and Table 3); both values are significantly different from those of Group F ($0.01 < P > 0.001$ and $0.001 < P$, respectively).

Plasminogen

Four hours following the first endotoxin injection a mean percent decrease was noted in treated and nontreated rabbits of 18.2 ± 13.0% and 14.5 ± 15%, respectively, but these changes were not significantly different from normal controls due to a wide scatter of results (Table 2). Four hours following a second injection of endotoxin, no definite changes could be detected in any group.

Circulating Soluble Fibrin

Results are seen in Tables 2 and 3. Four hours after the first endotoxin injection approximately two thirds of both treated and untreated rabbits had positive tests (Table 2). Four hours following a second injection of endotoxin, virtually all rabbits developed evidence of circulating soluble fibrin with the exception of Group F rabbits pretreated with indomethacin prior to the first injection of endotoxin (four ninths positive).

Discussion

The generalized Shwartzman reaction can be prevented by indomethacin, an inhibitor of prostaglandin synthetase. This effect was observed if indomethacin was given before the first but not the second injection of endotoxin. Prevention was associated with an absence of fibrinogen consumption, no prolongation in prothrombin time, and formation of soluble fibrin in some animals only. By contrast, rabbits not treated in this manner showed a decrease in circulating fibrinogen, a prolongation of the prothrombin time, and a consistent production of soluble fibrin. The effect of indomethacin thus constitutes a prevention of the state of preparation for the generalized Shwartzman reaction and provides an opportunity to study the events that are important in the prepared state.

Of the hematologic parameters observed following the first injection of endotoxin, several differences were noted in treated animals. Four hours following the first endotoxin injection, there was a tendency toward a somewhat greater decrease in leukocytes and platelets compared with values in untreated rabbits, and the prolongation of the activated partial

thromboplastin time was not as great in treated rabbits, but none of these differences by themselves was significant. Twenty-one hours after endotoxin administration, elevations in plasma fibrinogen and leukocytosis were somewhat less in treated rabbits, but again the average values by themselves were not significantly different from values in untreated rabbits. The mean percent decrease in leukocytes was significantly greater 4 hours following a second endotoxin injection in untreated rabbits, primarily because of the leukocytosis before endotoxin administration in this group.

From the present study, it would appear that prostaglandins and related products are important in preparation and that indomethacin prevents their formation. It should be pointed out that large quantities of indomethacin were needed to produce these effects, and conceivably a toxic effect has been produced. Different species, however, vary greatly in the levels of indomethacin required to have a therapeutic effect,[15] and it has been demonstrated that indomethacin in approximately the quantities used in the present study does not have a pronounced effect in rabbits if given daily over a 9-day period.[16] It is also possible that indomethacin may have functions other than the inhibition of prostaglandin synthesis. Northover[17] has suggested that indomethacin may reduce influx of calcium into endothelial cells and thus prevent their damage by histamine.

A variety of therapeutic agents inhibit the generalized Shwartzman reaction, but prevention by an action(s) on the preparatory phase is somewhat unique. Most agents are effective just prior to provocation, a timing definitely not effective in the present study. Anticoagulants such as heparin prevent the reaction if given just prior to provocation,[18] and dicumarol is effective if the animals are anticoagulated throughout the experiment.[19] Corticosteroids can prevent the generalized Shwartzman reaction if given just before the second endotoxin injection, presumably through their effects on blood clotting factors.[20] It is also well recognized that treatment with corticosteroids over a 3-day period can induce a state of preparation so that a single injection of endotoxin is required to produce a generalized Shwartzman reaction, an effect attributed in part to sensitization to α-adrenergic stimulation.[21] We have been unable to induce a state of preparation with indomethacin.[22] A variety of therapeutic agents known to produce vasodilation diminish the incidence of the generalized Shwartzman reaction if given before the second injection.[23] Dipyridamole, a potent inhibitor of platelet aggregation and a vasodilatory drug, inhibits intravascular fibrin accumulation induced by endotoxin.[24] Finally, α-adrenergic blocking agents can prevent the generalized Shwartzman reaction in pregnant rats.[25]

Both leukocytes and platelets have been defined as important elements in the generalized Shwartzman reaction. Changes in both total white blood cells and heterophils occurred in the present study and the expected changes were to some extent altered by indomethacin. Exudate white cells primed with endotoxin can produce a disseminated intravascular coagulation and the generalized Shwartzman reaction.[26] It is well known that a leukopenia induced by nitrogen mustard prevents the reaction [27] and that exudate leukocytes can restore the reaction in leukopenic animals.[28] It has been suggested that a substance(s) is elaborated in white blood cells that has a capacity to interact with soluble fibrin and thus cause fibrin deposition and the generalized Shwartzman reaction,[14] a theory originally proposed by Thomas et al.[29]

Little is known of the effects of indomethacin on leukocytes. A reduced phagocytic capacity of treated leukocytes has been demonstrated *in vitro*,[30] and it has been established that prostaglandins are released from human neutrophils on contact with zymosan, a release that is inhibited by indomethacin.[31] Although it would be tempting to incriminate the leukocyte or a leukocyte product as important, evidence is insufficient from the present study.

Indomethacin is also a potent inhibitor of platelet aggregation and release of prostaglandins,[32] but in the present study there was little evidence of a selective effect in treated rabbits. A lack of correlation between decreases in platelet numbers and the development of the generalized Shwartzman reaction has been noted by others.[33] It is possible that a platelet or white cell function could be altered without being reflected in numbers of circulating formed elements. It has been found that to produce an inhibition of the reaction by an immune platelet depletion, it is necessary to remove virtually all platelets.[34]

The combination of a leukocytosis and an elevated fibrinogen prior to the second endotoxin greatly enhances the chances that intravascular fibrin deposition will occur.[35] Indomethacin decreased both of these parameters in the present study, but exactly how these changes are related to the state of preparation is not known.

It has been proposed that the state of preparation involves at least three components: α-adrenergic sensitization, reticuloendothelial blockade, and altered fibrinolysis.[36] Any one or all of these could be affected by indomethacin. A blockade seems unlikely, for indomethacin apparently decreases phagocytic function *in vitro*.[30] The fibrinolytic system may be altered, but this change could not be demonstrated in the present study. It seems most likely that a sensitization to α-adrenergic stimulation is inhibited by indomethacin. Prostaglandins are known to modulate the response

to adrenergic stimulation, and PGE_1 and PGE_2 can inhibit the effects of norepinephrine on perfused segments of isolated rabbit artery, an inhibition reversed by indomethacin.[37] Prostaglandins may also play a role in the stimulation of corticotropin secretion in response to stress,[38] raising the possibility of another possible mechanism whereby α-adrenergic sensitization could be prevented by indomethacin by preventing the release of corticosteroids during the Shwartzman reaction.[21]

An interesting aspect of the present study was the failure of consumption of fibrinogen in previously treated rabbits. Indomethacin may directly interfere with fibrinogen synthesis or utilization or alternately cause an elaboration of a substance(s) that has these effects. It is equally possible that indomethacin may prevent the elaboration of a tissue thromboplastin or "potential" thromboplastin activity and that this prevention is due to an inhibition of synthesis of prostaglandins and related products throughout the body. The ocular response to circulating endotoxin is primarily due to an alteration in vascular permeability,[39] but endothelial injury also occurs, in part as fibrin forms locally.[40] We have assumed that ocular blood vessels and vessels of the choroid plexus are uniquely sensitive to the effects of endotoxin, but conceivably blood vessels in different regions and in particular anatomic sites may be equally sensitive.[41] Multiple foci in blood vessels throughout the body might be the sites of development of thromboplastic or potential thromboplastic activity that triggers a massive clotting episode when a second injection of endotoxin is given. Conceivably, indomethacin could prevent the development of this activity.

References

1. Feldberg W, Gupta KP, Milton AS, Wendlandt S: Effect of pyrogen and antipyretics on prostaglandin activity in cisternal C.S.F. of unanaesthetized cats. J Physiol (Lond) 234:279–303, 1973
2. Beitch BR, Eakins KE: The effects of prostaglandins on the intraocular pressure of the rabbit. Br J Pharmacol 37:158–167, 1969
3. Bhattacherjee P: Ocular responses and the release of prostaglandin-like substances by Shigella endotoxin. J Physiol (Lond) 246:38p, 1975
4. Howes EL Jr, McKay DG: The effects of aspirin and indomethacin on the ocular response to circulating bacterial endotoxin in the rabbit. Invest Ophthalmol 15:648–651, 1976
5. Kessler E, Hughes RC, Bennett EN, Nadela SM: Evidence for the presence of prostaglandin-like material in the plasma of dogs with endotoxin shock. J Lab Clin Med 81:85–94, 1973
6. Erdös EG, Hinshaw LB, Gill CC: Effect of indomethacin in endotoxin shock in the dog. Proc Soc Exp Biol Med 125:915–919, 1967
7. Brecher G, Cronkite EP: Morphology and enumeration of human blood platelets. J Appl Physiol 3:365–377, 1950
8. Quick AJ: Hemorrhagic Diseases and Thrombosis. Philadelphia, Lea & Febiger, 1957

9. Nye SW, Graham JB, Brinkhous KM: The partial thromboplastin time as a screening test for the detection of latent bleeders. Am J Med Sci 243:279–287, 1962
10. Lerner RG, Rapaport SI, Siemsen JK, Spitzer JM: Disappearance of fibrinogen ^{131}I after endotoxin: Effects of a first and second injection. Am J Physiol 214:532–537, 1968
11. Blömback BJ, Blömback M: Purification of human and bovine fibrinogen. Arkiv Kemi 10:415–443, 1957
12. Bruhn HD, Müller L, Duckert F: Quantitative determination of plasminogen: A caseinolytic method. Thromb Diath Haemorrh 23:191–201, 1970
13. Gurewich V, Hutchinson E: Detection of intravascular coagulation by a serial-dilution protamine sulfate test. Ann Intern Med 75:895–902, 1971
14. Lipinski B, Nowak A, Gurewich V: The organ distribution of ^{125}I-fibrin in the generalized Shwartzman reaction and its relation to leucocytes. Br J Haematol 28:221–231, 1974
15. Ferreira SH, Vane JR: New aspects of the mode of action of nonsteroid anti-inflammatory drugs. Annu Rev Pharmacol 14:57–73, 1974
16. Hanna C, Keatts HC: Indomethacin in ocular inflammation in rabbits. Arch Ophthalmol 77:554–558, 1967
17. Northover AM: Action of histamine on endothelial cells of guinea pig isolated hepatic portal vein and its modification by indomethacin or removal of calcium. Br J Exp Pathol 56:62–61, 1975
18. Good RA, Thomas L: Studies on the generalized Shwartzman reaction. IV. Prevention of the local and generalized Shwartzman reactions with heparin. J Exp Med 97:871–888, 1953
19. Shapior SS, McKay DG: The prevention of the generalized Shwartzman reaction with sodium warfarin. J Exp Med 107:377–381, 1958
20. Latour JG, McKay DG, Nasu K: Prevention of the generalized Shwartzman reaction by glucocorticoids. Am J Obstet Gynecol 113:863–867, 1972
21. Latour JG, Prejean JB, Margaretten W: Corticosteroids and the generalized Shwartzman reaction: Mechanism of sensitization in the rabbit. Am J Pathol 65:189–202, 1971
22. Howes EL Jr, McKay DG: Unpublished observations
23. Nasu K, Latour JG, McKay DG: Modification of the generalized Shwartzman reaction by therapeutic agents. Am J Obstet Gynecol 109:991–996, 1971
24. Gurewich V, Lipinski B, Wetmore R: Inhibition of intravascular fibrin deposition by dipyridamole in experimental animals. Blood 45:569–575, 1975
25. Müller-Berghaus G, McKay DG: Prevention of the generalized Shwartzman reaction in pregnant rats by α-adrenergic blocking agents. Lab Invest 17:276–280, 1967
26. Niemetz J, Fani K: Thrombogenic activity of leukocytes. Blood 42:47–59, 1973
27. Thomas L, Good RA: Studies on the generalized Shwartzman reaction. I. General observations concerning the phenomenon. J Exp Med 96:605–624, 1952
28. Bohn E, Müller-Berghaus G: The effect of leukocyte and platelet transfusion on the activation of intravascular coagulation by endotoxin in granulocytopenic and thrombocytopenic rabbit. Am J Pathol 84:239–258, 1976
29. Thomas L, Smith RT, von Korff R: Studies on the generalized Shwartzman reaction. VII. The role of fibrinogen in the deposition of fibrinoid after combined injections of endotoxin and synthetic acidic polymer. J Exp Med 102:263–278, 1955
30. Chang Y-H: Studies on phagocytosis. II. The effect of non-steroidal anti-inflammatory drugs on phagocytosis and on urate crystal-induced canine joint inflammation. J Pharmacol Exp Ther 183:235–244, 1972
31. Zurier RB, Sayadoff DM: Release of prostaglandins from human polymorphonuclear leukocytes. Inflammation 1:93–101, 1975
32. Vane JR: Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature [New Biol] 231:232–235, 1971

33. Müller-Berghaus G, Bohn E, Höbel W: Activation of intravascular coagulation by endotoxin: The significance of granulocytes and platelets. Br J Haematol 33: 213–220, 1976
34. Margaretten W, McKay DG: The role of the platelet in the generalized Shwartzman reaction. J Exp Med 129:585–590, 1969
35. Gurewich V, Lipinski B, Hyde E: The effect of the fibrinogen concentration and the leukocyte count on intravascular fibrin deposition from soluble fibrin monomer complexes. Thromb Haemostas 36:605–614, 1976
36. McKay DG: Acute inflammation of the circulating blood. Pathobiology Annual, 1973. Edited by H Ioachim. New York, Appleton-Century-Crofts, 1973, pp 71–106
37. Malik KU, Ryan P, McGiff JC: Modification by prostaglandins E1 and E2, indomethacin, and arachidonic acid of the vasoconstrictor responses of the isolated perfused rabbit and rat mesenteric arteries to adrenergic stimuli. Circ Res 39:163–168, 1976
38. Hanukoglu I: Prostaglandins as first mediators of stress. N Engl J Med 296:1414, 1977
39. Howes EL Jr, McKay DG, Aronson SB: An ultrastructural study of the ciliary process in the rabbit following systemic administration of bacterial endotoxin. Lab Invest 24:217–228, 1971
40. Howes EL Jr, McKay DG, Margaretten W: The participation of the platelet in the vascular response to endotoxemia in the rabbit eye. Am J Pathol 70:25–44, 1973
41. Gaynor E: Increased mitotic activity in rabbit endothelium after endotoxin: An autoradiographic study. Lab Invest 24:318–320, 1971

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