Glucocorticoids Inhibit the Generation of Leukocyte Procoagulant (Tissue Factor) Activity

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Human leukocytes may be induced to generate powerful procoagulant (tissue factor) activity (TFa). The TFa, generated by three different inducers—bacterial endotoxin, complement-activated plasma, or antigen–antibody interaction—was suppressed by glucocorticoids. In most instances, micromolar concentrations of methylprednisolone (MP) caused 50% or greater suppression of the procoagulant activity, offering a rationale for the use of steroids in diseases related to these inducers.

Demonstration of Fibrin Deposits, Leukocytes, and Complement in the Tissues of Patients with Acute Respiratory Distress Syndrome

Several aspects of the pathogenic process could be diminished or abolished if leukopenia is induced or coagulation inhibited.1-3 Leukocytes develop procoagulant activity in vitro in response to bacterial endotoxins,4,5 antigen–antibody complexes,6 some plasma lipoproteins,7 lytic products of the fifth component of complement,8 and phytohemagglutinins.9 Bacterial endotoxins are also effective inducers in vivo, and the leukocytes so stimulated may be thrombogenic when infused into normal rabbits.5 Corticosteroids, which are well known antiinflammatory agents, in high doses have been shown to have a beneficial effect on the outcome of such disease entities as adult respiratory distress syndrome (ARDS).10 This syndrome, associated with thromboembolic events as suggested by tissue necrosis, fibrin deposition, and fibrin degradation products, is accompanied by leukocyte infiltration.11 At very high doses, corticosteroids decrease granulocyte aggregation in vitro when induced by complement-activated serum, and it has been suggested that these findings could serve as rationale for the high doses used in the therapy of ARDS.12

Prydz and Allison13 and Maynard et al.14 have studied the effect of corticosteroids on TFa. We extend these observations and report that corticosteroids impede development of TFa in peripheral blood leukocytes when this activity has been caused by bacterial endotoxin, antigen–antibody stimulation, or complement-activated plasma.

Materials and Methods

Endotoxin from E. coli 026B6, rabbit brain thromboplastin (Difco Lab., Detroit, Mich.); Dextran 250 (Sigma, St. Louis, Mo.); trypsin blue (GIBCO, Grand Island Biological, Grand Island, N.Y.); Medium 199 (M.A. Bioproducts, Walkersville, Md.); renal dialysis membranes (Gambro, Inc., Newport News, Va.); Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, N.J.); and penicillin-streptomycin (Microbiological Associates, Walkersville, Md.) were purchased.

Methylprednisolone hemisuccinate lots 946 GH, 919K, and 369 HW, hydrocortisone hemisuccinate lot 397 GJ, and medroxyprogesterone lot 977 EE were gifts from Upjohn Co., Kalamazoo, Mich. Leukocytes were obtained from human citrated blood by Dextran sedimentation and Ficoll-Hypaque sedimentation by the method of Béyem.15 The leukocytes, 2 x 10⁷/ml, were incubated with endotoxin, 10 μg/ml (unless specified otherwise), MP, and Medium 199 containing 20 U penicillin and 20 μg of streptomycin/ml, then processed and assayed as described.4,16

The coagulant activity was tested by the one-stage test on human citrated plasma18 and the time expressed in seconds. The tissue factor activity was assayed by the two-stage assay16,17 and expressed in units. One milliliter of rabbit brain thromboplastin, prepared as per the manufacturer's instructions, contained 1000 U of tissue factor activity. Percent inhibition was derived from this assay. Each point is the average of at least 3 separate experiments. ABO antibody titer was assayed as described.18

Results

Effects of Corticosteroids on Endotoxin-Stimulated Leukocytes.

When doses of methylprednisolone (MP) of 1.6 x 10⁻² and 0.8 x 10⁻³ M were incubated with leukocytes and endotoxin, TFa generated by leukocytes was completely suppressed. At about 2 μM concentration, a 50% inhibition was observed, and suppression was noticeable even at a concentration of MP down to 10⁻⁴ M (Fig. 1).

As 10 μg/ml of endotoxin exceeds the highest amount of endotoxin necessary for optimal stimulation in these experiments, investigation of the effect of MP on lower amounts of endotoxin appeared of interest. As shown in Fig. 2, where actual TFa is depicted, lesser amounts of TFa are generated with lower amounts of endotoxin. The TFa generated with 0.01 μg endotoxin/ml is quite weak but completely abolished by 1.6 x 10⁻³ M MP. One-tenth of a microgram of endotoxin

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costeroids included studies of the uptake of Trypan blue dye by leukocytes. Less than 3% of leukocytes took up the dye with concentration of MP of $1.6 \times 10^{-4} M$ or lower, suggesting that cell death was not responsible for this effect. When disrupted leukocytes containing tissue factor activity were incubated with MP for up to 5 hr at $37^\circ$C, no inhibition of activity was observed, suggesting that MP did not neutralize TFa directly. The possibility that MP exerted its effect through granulocytes was tested and excluded. Indeed, preparations containing only mononuclear cells gave the same results with or without added granulocytes.

**Effect of MP on Antigen–Antibody-Generated TFa**

Tissue factor activity was generated by incubation of leukocytes with ABO-incompatible plasma. Substantial coagulant activities were generated as judged by tissue factor assay (Fig. 1.) and the one-stage test (results not shown). With group A cells, inhibition of activity was observed with several concentrations of MP and 50% obtained with about $1.6 \times 10^{-5} M$. However, less effective inhibition was observed when group B cells were used (Fig. 1). Differences were significant ($p < 0.05$) at concentration of MP from $1.6 \times 10^{-3}$ to $1.6 \times 10^{-5} M$. Antibody titration of the group O plasma detected 7S antibody to B cells at $1/512$.
dilution, but only 1/64 dilution to A cells. Plausibly, as with endotoxin, the effects of somewhat lesser stimuli are easier to inhibit.

**Effect of MP on Leukocyte TFa Induced by Complement-Activated Plasma**

Complement activation of plasma by renal dialysis membranes induces leukocyte TFa. Methylprednisolone suppressed this TFa. Ninety percent or higher inhibition was observed with concentrations of about 1.6 × 10⁻⁴ M (Fig. 1). Results of the one-stage test (not shown) concorded with the two-stage assay. Hydrocortisone also suppressed leukocyte TFa generation in response to endotoxin. However, medroxyprogesterone, a nonglucocorticoid, had no suppressive effect on leukocyte TFa induced by either endotoxin or antigen–antibody stimulation.

**DISCUSSION**

The results demonstrate that leukocyte tissue factor activity, when induced by three different stimuli, can be inhibited by corticosteroids. Methylprednisolone at a concentration of 1.6 × 10⁻³ M induced nearly complete inhibition. Fifty percent inhibition of all three stimuli is obtained, in most instances, with approximately 2 μM concentration. As illustrated in experiments with lower amounts of endotoxin and group A and B leukocytes, the degree of inhibition may also depend on the degree of stimulation. The dose-dependent inhibition curves with various inducers are different. The endotoxin curve appears the most complex. Methylprednisolone causes approximately 20% inhibition at concentrations from 10⁻¹⁴ to 10⁻⁸ M. This was not observed with the other inducers, suggesting different mechanisms of inhibition. Furthermore, at concentrations from 1.6 × 10⁻³ M to 1.6 × 10⁻¹ M, the endotoxin curve tends to be upwardly concave, whereas the other curves at these concentrations tend to reach a plateau (Fig. 1).

Prydz et al. studied the effect of dexamethasone on leukocyte TFa and concluded that dexamethasone had no stimulatory effect on the activity. Maynard et al. demonstrated inhibition by corticosteroids of TFa generated by WISH amnion cells. To generate TFa, WISH amnion cells require subculture and trypsin treatment. These inducers are ineffective on leukocyte TFa generation. Conversely, endotoxin may not induce WISH cells to generate TFa, suggesting different mechanisms for TFa induction between the two cell lines.

It has been proposed that leukocyte aggregates may be responsible for shock-induced ARDS. Steroids, which inhibit leukocyte adherence to nylon fibers, may also, at very high doses (approximately 1 × 10⁻⁶ M), cause 50% inhibition of leukocyte aggregation induced by complement-activated plasma. This finding has been proposed as a rationale for the use of high doses of steroids in the therapy of shock-induced ARDS. We note that the amounts that are necessary to achieve inhibition of leukocyte aggregation are more than 100-fold higher than those used in our experiments and possibly not achievable in clinical medicine. Moreover, complement-induced leukocyte aggregation is a reversible phenomenon, while fibrin deposition may be a more durable event. Thus, deleterious effects attributed to complement-activated leukocytes may be in part related to their coagulant properties, which could be impeded by corticosteroids.

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