0031-3998/84/1811-1141$02.00/0
PEDIATRIC RESEARCH

Vol. 18, No. 11, 1984
Printed in U.S.A.

Effects of Methylprednisolone on the Response to Group B Streptococcal Toxin in Sheep

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ABSTRACT. The effects of pretreatment with methylprednisolone on the reaction to a toxin isolated from group B β-hemolytic streptococci, type III, were studied in seven sheep instrumented for chronic measurements of pulmonary lymph flow and pulmonary artery and left atrial pressure. Each sheep was infused with toxin alone on one day and with methylprednisolone plus toxin on a different day in random order. The toxin alone caused a two-phase reaction. After the infusion of toxin, alone, in the initial phase, pulmonary artery pressure increased from 16 ± 1 to 45 ± 5 mm Hg and the rectal temperature rose from 39.5 ± 0.14 to 40.8 ± 0.18° C. During the second phase, the peripheral blood granulocyte count decreased to 10% of baseline values and the lung lymph protein clearance increased from 5.1 ± 1.1 to 11.2 ± 1.8 ml/h, suggesting increased pulmonary vascular permeability. Methylprednisolone pretreatment did not alter the initial phase of pulmonary hypertension or the febrile response but completely abolished the granulocytopenia and the increased pulmonary vascular permeability. These effects are unlikely to be related to inhibition of prostaglandin synthesis. Prevention of the lung vascular injury by methylprednisolone may be related to inhibition of granulocyte accumulation in the lung. (*Pediatr Res* 18:1141–1144, 1984)

Abbreviations

TxB_2, thromboxane B_2
PG, prostaglandin

Early onset group B streptococcal disease in newborn infants is characterized by respiratory failure, leukopenia, and cardiovascular collapse. The mortality rate in some series is as high as 50% (10). Current treatment regimens which include antibiotics, volume expansion, and ventilatory support often do not prevent irreversible shock and death. A better knowledge of the pathophysiologic mechanisms involved in this disease may result in the design of new therapeutic approaches.

We have previously described that the infusion of a toxin isolated from type III group B *Streptococcus* into sheep causes a two-phase reaction characterized by an initial phase of pulmonary hypertension, followed by a second phase of granulocytopenia, and increased pulmonary vascular permeability to protein (8, 13, 14). The similarity of these changes to those seen in newborn infants provides an animal model which is well-suited to studies of the pathophysiology of this syndrome. We showed earlier that cyclooxygenase inhibition with indomethacin blocks the pulmonary hypertensive phase, without modifying the granulocytopenia or the increased pulmonary vascular permeability to protein (5).

There are several theoretical reasons to expect corticosteroids to moderate the pathophysiologic changes present in streptococcal toxemia, either by inhibiting arachidonic acid release from membrane phospholipids (9) or by altering granulocyte function (6, 19). Large doses of corticosteroids have been shown to have beneficial effects on other shock states (1, 18).

The purpose of this investigation was to determine the effects of pretreatment with methylprednisolone on the reaction to group B streptococcal toxin in adult unanesthetized sheep instrumented for chronic monitoring of vascular pressures and collection of pulmonary lymph.

MATERIALS AND METHODS

General. Seven yearling sheep (35–45 kg) were prepared for chronic lung lymph collection as has been previously described (13). Through bilateral thoracotomies, catheters were placed directly into the main pulmonary artery, left atrium, and the efferent duct of the caudal mediastinal lymph node. The tail of the node was resected to eliminate nonpulmonary lymph. Lymph collected from animals prepared in this way is primarily derived from the lung since lymph flow increases when pulmonary vascular pressure is increased but lymph flow does not increase when systemic venous pressure is increased. Catheters were also placed in the right atrium and thoracic aorta through neck vessels.

All experiments were done with the sheep unanesthetized, standing unrestrained in a cage. Vascular pressures were measured continuously with pressure transducers (Statham P23Gb, Gould-Statham Instruments, Inc., Hato Rey, Puerto Rico) and an electronic recorder (Hewlett-Packard Co., Palo Alto, CA). Lymph flow (QL) was measured at 15-min intervals by recording the volume drained into a graduated tube. Total protein concentration was measured in plasma separated from blood drawn each hour and in lymph pooled at 30-min intervals, using an automated system (Auto Analyzer, Technicon Instrument Corp., Tarrytown, NY) by a modified biuret method.

Lung lymph protein clearance (C_{prot}) was calculated with the formula:

$$C_{prot} = QL(L/P)$$

where L/P is the lymph/plasma total protein concentration ratio.

Blood for white cell studies was drawn from the aortic catheter. Samples were obtained twice during baseline periods and then

Received November 11, 1983; accepted March 19, 1984.
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This work was supported by SCOR Grant HL 14214 from the National Institutes of Health. This work was done during the tenure of K. L. B. as an Established Investigator of the American Heart Association.

at 30, 60, 180, and 300 min after the infusion of toxin. Total leukocyte counts were performed electronically in a model ZBI Coulter Counter (Coulter Electronics, Hialeah, FL), specifically adjusted for counting sheep leukocytes. Blood smears were stained with Wright's stain and subjected to a 200-cell differential count. Total granulocyte count was calculated by multiplying the total leukocyte count with the percentage of granulocytes.

The isolation of the toxic polysaccharide has been described elsewhere (8). The dose used in all infusions was 60 μg/kg suspended in 80 ml of 0.9% NaCl solution. Each sheep received toxin on one day and the same dose of toxin after pretreatment with methylprednisolone on another day. In three sheep, a third experiment was done using methylprednisolone alone. The sequence of studies was varied to avoid bias and a minimum of 7 days was allowed between infusions.

All statistical calculations were done using a two-tailed paired *t* test. A *p* value of less than 0.05 was considered significant.

Specific Protocols. Toxin alone. After a stable baseline period of at least 90 min, 60 μg/kg of toxin were infused intravenously over 15 min and hemodynamic and lymph flow data were recorded for at least 5 h after the infusion.

Methylprednisolone and toxin. After a stable baseline period, a loading dose of 1.25 g of methylprednisolone (Upjohn) was infused over 30 min. This was followed by a maintenance dose of 0.5 g/h during 4 h. Methylprednisolone was mixed immediately before each experiment with sterile water. The total volumes for the loading dose and maintenance dose were 30 and 120 ml, respectively. After infusion of the methylprednisolone loading dose, 60 μg/kg of toxin were infused intravenously and the animal was monitored for at least 5 h.

The loading dose of methylprednisolone corresponds to 30 mg/kg which is similar to the dose recommended for the treatment of endotoxin shock (18).

Methylprednisolone alone. After a stable baseline period, methylprednisolone was infused in doses identical to those in the experiment described above. The animals were then monitored for 5 h.

Analysis of Thromboxane A_2 and Prostacyclin Metabolites. TxB_2 and 6-keto-$PGF_{1\alpha}$, stable hydrolysis products of thromboxane A_2 and prostacyclin, respectively, were measured in lung lymph. Specimens were collected during the baseline period, 30 min after the peak in pulmonary artery pressure and 1 h into the steady state second phase. Analyses were performed by radioimmunoassay, employing rabbit anti-TxB_2 and anti-6-keto-$PGF_{1\alpha}$ antibodies obtained from Dr. J. Bryan Smith (Cardeza Foundation, Philadelphia, PA). The anti-TxB_2 antibody cross-reacts <1% with PGE_2, $PGF_{2\alpha}$, and 6-keto-$PGF_{1\alpha}$ and <3% with PGD_2. The 6-keto-$PGF_{1\alpha}$ antibody cross-reacts <3% with $PGF_{2\alpha}$ and <1% with PGE_2, PGD_2, and TxB_2. Authentic prostaglandins and TxB_2 were generously supplied by Dr. John Pike (Upjohn Company, Kalamazoo, MI). Radiolabeled (5,6,8,9,11,12,14,15-^{3}H)TxB_2 and (5,6,8,9,11,12,14,15-^{3}H)6-keto-$PGF_{1\alpha}$ were purchased from New England Nuclear (Boston, MA).

The radiolabeled ligand (≃2000 cpm/tube) was first mixed with bovine γ-globulins (10 mg/ml in Trizma, pH 7.4). To 100-μl aliquots of this mixture were added 100-μl aliquots of sample or unlabeled standard dilutions. The binding reaction was initiated by addition of 100 μl of antibody diluted to produce 60% binding of radioactivity in the absence of unlabeled ligand. The binding reaction continued for 60 min at 37° C and was terminated by precipitation of the immune complexes with ammonium sulfate at a final concentration of 50% of saturation. After centrifugation at 2500 × *g* at 4° C for 10 min, 300 μl of supernatant was counted in Aquasol (New England Nuclear, Boston, MA). Each sample was assayed in duplicate. Duplicate determinations differed by <10%. The detection limit of both assays was <20 pg.

RESULTS

Toxin alone. The response was biphasic as previously described (13). There was an initial phase of pulmonary hypertension and high lymph flow followed by a second phase when pulmonary artery pressure was slightly above baseline and lung lymph flow remained elevated. The data are summarized in Table 1. The values for phase I, because it is a transient phase, were obtained at the peak of the pulmonary artery pressure. All phase II values are the average of at least 3 h of steady state.

During the second phase, lung lymph flow was 2 times higher than baseline, and the lymph to plasma protein concentration ratio returned to near baseline, resulting in a significant increase in lymph protein clearance.

Rectal temperature increased from 39.5 ± 0.14° C during the baseline period to 40.8 ± 0.18° C after the infusion of toxin ($p < 0.05$).

The baseline white blood cell values for all sheep were within the normal range for sheep as described by Schalm (17). The total white blood cell count dropped within the 1st h after the infusion of the polysaccharide to 30% of baseline values. This leukopenia was due primarily to a decrease in granulocytes. The absolute granulocyte count fell to 10% of baseline values by 60 min followed by a slow recovery over the next 4 h (Fig. 1).

Methylprednisolone and toxin. The methylprednisolone-loading dose caused no changes in white blood cell count, granulocyte

Table 1. *Summary of pressure and flow data in biphasic response**

	Mean pressure (mm Hg)				
	Pulmonary artery	Left atrium	Lymph flow (ml/h)	Lymph/plasma ratio	Protein clearance (ml)
Toxin alone ($n = 7$)					
Baseline	15.6 ± 0.4	4.0 ± 1.3	8.4 ± 1.7	0.67 ± 0.01	5.1 ± 1.1
Phase I	44.8 ± 4.6†	1.6 ± 0.9	21.4 ± 4.4†	0.50 ± 0.04†	
Phase II	21.1 ± 1.8†	4.2 ± 1.5	17.1 ± 2.6	0.69 ± 0.02	11.2 ± 1.8†
Methylprednisolone and toxin ($n = 7$)					
Baseline	17.2 ± 0.6	4.0 ± 0.6	9.6 ± 1.6	0.67 ± 0.01	6.6 ± 1.0
Phase I	36.7 ± 3.6†	0.6 ± 1.9	17.9 ± 2.5†	0.54 ± 0.02†	
Phase II	18.2 ± 0.8	3.6 ± 1.1	9.9 ± 1.4	0.67 ± 0.01	6.1 ± 0.8‡
Methylprednisolone alone ($n = 3$)					
Baseline	15.5 ± 1.7	4.5 ± 0.8	6.8 ± 1.1	0.69 ± 0.01	4.8 ± 0.9
Phase I	15.7 ± 1.5‡§	4.9 ± 0.9	7.1 ± 0.9‡§	0.69 ± 0.01‡§	
Phase II	15.8 ± 1.6	4.8 ± 0.9	6.3 ± 0.2‡	0.67 ± 0.02	4.2 ± 0.3‡

* All values are mean ± SEM.

† $p < 0.05$ compared to baseline.

‡ $p < 0.05$ compared to toxin.

§ $p < 0.05$ compared to methylprednisolone and toxin.

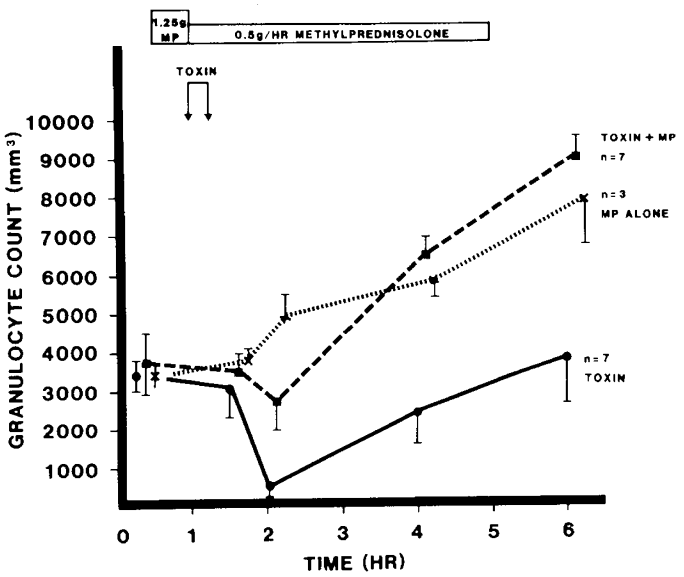

Fig. 1. Absolute granulocyte counts during the reaction to group B streptococcal toxin (——), after pretreatment with methylprednisolone (*MP*) (– – –), and for methylprednisolone alone (· · ·). At 2, 4, and 6 h, the absolute granulocyte count was significantly lower ($p < 0.05$) for the toxin when compared with pretreatment with methylprednisolone or methylprednisolone alone. There were no significant differences between both methylprednisolone groups. *Bars* represent mean ± SEM.

count, or pulmonary artery pressure. After the infusion of toxin, the pulmonary artery pressure rose to levels similar to those with toxin alone. High flow of protein-poor lymph occurred during this phase. During the second phase, the lymph flow returned to baseline. There was no change in protein clearance.

Rectal temperature increased from 39.8 ± 0.23° C during the baseline period to 40.4 ± 0.42° C, similar to the infusion of toxin alone ($p < 0.05$).

The absolute granulocyte count decreased slightly by 60 min and was followed by a significant increase to 1.7 times higher than baseline at 180 min and 2.4 times higher than baseline at 300 min (Fig. 1).

Methylprednisolone alone. Methylprednisolone had no effects on pulmonary artery or left arterial pressure. Lymph flow, lymph to plasma protein ratio, and protein clearance also remained unchanged throughout the experiment.

Rectal temperature did not change and the absolute granulocyte count slowly increased throughout the experiment to values 2.3 times higher than baseline by 5 h.

Thromboxane A_2 and prostacyclin metabolites. Table 2 shows the results of the measurements of thromboxane B_2 and 6-keto-$PGF_{1\alpha}$ concentration in lung lymph for seven experiments with toxin and four of the experiments where there was pretreatment with methylprednisolone. As described previously (15), TxB_2 concentration in lung lymph increased during the initial pulmonary hypertension and remained somewhat elevated during the second phase. There was also an increase on 6-keto-$PGF_{1\alpha}$ during the initial phase which returned to baseline during phase II. Methylprednisolone did not modify the increase in TxB_2 during phase I, although it abolished it during phase II. The increase in 6-keto-$PGF_{1\alpha}$ was abolished by methylprednisolone.

Table 2. *TxB_2 and 6-keto-$PGF_{1\alpha}$, stable metabolites of thromboxane A_2 and prostacyclin, in lung lymph for experiments done with toxin alone and pretreatment with indomethacin**

	TxB_2 (ng/ml)	6-Keto-$PGF_{1\alpha}$ (ng/ml)
Toxin alone ($n = 7$)		
Baseline	0.151 ± 0.05	0.148 ± 0.08
Phase I	1.969 ± 0.69†	0.823 ± 0.33†‡
Phase II	0.721 ± 0.49‡	0.224 ± 0.05‡
Methylprednisolone and toxin ($n = 4$)		
Baseline	0.138 ± 0.04	0.089 ± 0.07
Phase I	2.151 ± 1.82†	0.068 ± 0.05
Phase II	0.134 ± 0.02	0.068 ± 0.04

* All values are mean ± SEM.
† $p < 0.05$ compared to baseline.
‡ $p < 0.05$ compared to methylprednisolone.

DISCUSSION

Pulmonary hypertension and granulocytopenia are an integral part of early onset group B streptoccal disease in newborn infants (10). The infusion of a toxin isolated from the same organism into unanesthetized sheep reproduces these findings, providing a model in which to study the pathophysiologic mechanism of the disease. In this study, pretreatment with high doses of methylprednisolone (in the range suggested for use in humans with septic shock) (18), prevented the fall in circulating granulocytes as well as the increase in pulmonary vascular permeability, but did not change the transient pulmonary hypertensive phase or the febrile response. These results are consistent with those seen with the infusion of Gram-negative endotoxin after pretreatment with corticosteroids in the same animal model (1).

We have previously shown that cyclooxygenase inhibition with indomethacin blocks the pulmonary hemodynamic changes and the febrile response that follow the infusion of group B streptococcal toxin into sheep (15). The conversion of arachidonate to prostaglandin endoperoxides seems necessary for these changes. The granulocytopenia and increased pulmonary vascular permeability appear to be independent of prostaglandin synthesis. Under some circumstances, corticosteroids inhibit endogenous production of arachidonate products, apparently by preventing the release of arachidonate from membrane phospholipids (9). This does not appear to be the mechanism by which corticosteroids modify the response to group B streptococcal toxin since the pulmonary hypertension and the febrile response are preserved, as well as the increase in thromboxane A_2 metabolites.

The most striking finding in this study is the prevention of the granulocytopenia and increased pulmonary vascular permeability by pretreatment with corticosteroids. Granulocytes, oxygen radicals, and lysosomal enzymes may injure vascular endothelium (3, 16). Granulocyte depletion prevents some of the pulmonary vascular changes after endotoxemia (7) or microembolization (5). We have recently shown that large numbers of granulocytes accumulate in the lungs following the infusion of group B streptococcal toxin into sheep (14). Pulmonary leukostasis and leukopenia are also known to occur during the adult respiratory distress syndrome (11), hemodialysis (4), and cardiopulmonary bypass (2). Complement activation may be the cause since similar changes develop in several animal species shortly after the infusion of plasma which has been exposed to complement-activating agents (4). Corticosteroids may prevent the granulocytopenia and increased vascular permeability by inhibiting complement-induced granulocyte aggregation (6) and the subsequent granulocyte trapping in the lungs.

Pulmonary endothelial cells can synthesize prostacyclin (12). Corticosteroids blocked the increase in 6-keto-$PGF_{1\alpha}$ that follows the infusion of group B streptococcal toxin, although they did not seem to modify production of TxB_2. Corticosteroids may have abolished the release of prostacyclin by preventing vascular injury rather than directly blocking its synthesis.

In summary, pretreatment with corticosteroids in doses similar to those suggested for the treatment for septic shock (18) abolishes the late phase increase in lung vascular permeability that follows the infusion of group B streptococcal toxin in sheep without modifying the early phase of pulmonary hypertension. These effects are unlikely to be due to inhibition of prostaglandin synthesis. Prevention of lung vascular injury by methylprednis-

olone may be related to inhibition of granulocyte accumulation in the lung. In addition, this study provides further evidence that the two phases of the reaction are pathogenically separable.

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0031-3998/84/1811-1144$02.00/0
PEDIATRIC RESEARCH

Vol. 18, No. 11, 1984
Printed in U.S.A.

Inherited Lactic Acidosis: Correction of the Defect in Cultured Fibroblasts

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ABSTRACT. We report a case of familial lactic acidosis, lethal in the newborn period. Studies in intact fibroblasts identified a defect in the oxidative pathway of pyruvate metabolism. Although assay of pyruvate dehydrogenase on cell sonicates was not appreciably reduced, flux through the enzyme and other mitochondrial multienzyme dehydrogenases was severely impaired in intact cells. Deficient lactate conversion to carbon dioxide could be repaired by the addition to the incubation medium of electron acceptors such as methylene blue (25 μg/ml) or dichlorophenolindophenol (25 μg/ml). (*Pediatr Res* 18: 1144–1148, 1984)

Abbreviation

PDH, pyruvate dehydrogenase

Received January 12, 1983; accepted April 19, 1984.
This work was supported by the MRC Genetics Group, the Réseau de Médecine Génétique du Québec, and the McGill University-Montreal Children's Hospital Research Institute. P. R. G. is a recipient of the McGill University-Montreal Children's Hospital Junior Scientist Award. This is Publication No. 84033 of the McGill University-Montreal Children's Hospital Research Institute.

Inherited forms of lactic acidosis have been recognized since the early 1960s (7, 12, 17). Recently, these disorders have been classified into errors of the gluconeogenic or oxidative pathways leading from pyruvate.

In the gluconeogenic group, lactic acidosis is accompanied by hypoglycemia and is exacerbated by fasting. The most frequent cause is deficiency of pyruvate carboxylase (EC 6.4.1.1), an intramitochondrial enzyme which can be measured in fibroblasts (1, 20) and amniocytes (9, 11) making antenatal diagnosis possible. In isolated pyruvate carboxylase deficiency, therapy has generally been unsatisfactory (13, 27) and prognosis is poor. When other carboxylases are affected, the disorder involves attachment of biotin to the apocarboxylase or biotin availability and may be successfully treated by pharmacologic doses of this cofactor (22, 25). Phosphoenolpyruvate carboxykinase (EC 4.1.1.32) may be measured in fibroblasts, but deficiencies of this enzyme associated with lactic acidosis are rare (14, 21). Fructose-1,6-diphosphatase (EC 3.1.3.11) and glucose-6-phosphatase (EC 3.1.3.9) deficiencies are diagnosed in gluconeogenic tissues and may be managed by frequent carbohydrate feeds (2, 15, 16).

Errors of the oxidative pathway of pyruvate metabolism are less amenable to therapy. Patients with abnormal enzymes of the