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Shwartzman Reaction
endotoxin
cortisone

coagulation
kidney
cortical necrosis
rabbit

Factor V
fibrinogen
Factor VIII

Quantitative Aspects of Blood Coagulation in the Generalized Shwartzman Reaction

II. Effect of Cortisone

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Extract

In the previous paper quantitative changes in the coagulation mechanism accompanying each of the two injections of endotoxin required for the production of the generalized Shwartzman reaction (GSR) were described. It was demonstrated that the development of cortical necrosis of the kidneys was dependent both on the induction of intravascular clotting and on the state of preparation of the rabbits in response to the first dose of endotoxin. Conversely, the adequacy of preparation could be assessed by the response to a standard dose of endotoxin measured by the degree of intravascular clotting induced and the incidence of cortical necrosis of the kidneys.

In 1952 THOMAS and GOOD reported that the GSR could be produced by a single injection of *Serratia marcesens* or meningococcus endotoxin if rabbits were pre-treated with ACTH or cortisone. It was the purpose of the present investigations to compare the degree of preparation induced by cortisone and by Thorotrast with that induced by *E. coli* endotoxin employing a provocative dose of endotoxin which had been shown capable of inducing intravascular clotting and cortical necrosis of the kidneys in adequately prepared rabbits.

Rabbits were pre-treated with cortisone 25 mg IM for four days and given a single dose of endotoxin on the third day according to the regimen of THOMAS and GOOD. With provocation using the standard dose of endotoxin (0.100 mg/kg), renal cortical necrosis was not observed in any of 20 animals. In contrast this same provocative dose induced renal cortical necrosis in 74 and 77 % of animals prepared with 0.01 and 0.1 mg/kg of endotoxin. With the same regimen of cortisone preparation provocative doses of endotoxin of 0.2 and 0.5 mg/kg likewise failed to induce cortical necrosis. With a provocative dose of 1.0 mg/kg two of fifteen animals developed cortical necrosis of the kidneys. Five animals receiving 2.0 mg/kg of endotoxin exhibited no GSR. With cortisone preparation of 100 and 250 mg/day for four days provocation with 1.0 mg/kg of endotoxin resulted in cortical necrosis in only 1 of 6 animals in each group (table I). Coagulation studies were carried out in animals prepared with cortisone, 25 mg/day for four days, and provoked with the standard dose of endotoxin of 0.1 mg/kg. Significant decreases were noted in white cells, platelets, Factors II and VIII, but no significant changes occurred in Factor V or fibrinogen concentrations (table II). These changes are almost identical to those found with saline preparation.

Thorotrast was given to eleven rabbits in a dose of 3.0 ml/kg intravenously and eighteen hours later 0.1 ml/kg of endotoxin was given. Five of the animals developed cortical necrosis of the kidneys. With Thorotrast preparation the coagulation mechanism was activated in its entirety, with highly significant falls in all of the measured coagulation factors observed (table III).

Conclusions: Cortisone preparation, even in doses ten times those employed by THOMAS and GOOD did not lead to development of intravascular clotting or cortical necrosis of the kidneys following a single injection of *E. coli* endotoxin. This held true even when the provocative dose of endotoxin was raised to ten times the standard dose or forty times the minimum effective dose with endotoxin preparation. Thorotrast preparation, on the other hand, was followed by intravascular clotting and cortical necrosis of the kidneys as response to a single injection of endotoxin in the standard amount.

Speculation

The ability to prepare animals for intravascular clotting and the development of the generalized Shwartzman phenomenon does not seem to be an inherent property of cortisone but appears to be dependent upon the type of endotoxin used in provocation. The implications that others have drawn from the data of THOMAS and GOOD that the use of cortisone may be hazardous in treatment of Shwartzman-like clinical conditions in the human appear to be premature in the present state of knowledge.

Introduction

In the previous paper the quantitative changes in the coagulation mechanism accompanying each of the two injections of endotoxin required for the production of the generalized Shwartzman reaction (GSR) were described [1]. It was demonstrated that the development of cortical necrosis of the kidney was dependent both on the induction of intravascular clotting and on the state of preparation of the rabbits in response to the first dose of endotoxin. Conversely the adequacy of preparation could be assessed by the response to a standard dose of endotoxin, this response being measured by the degree of intravascular clotting induced and by the incidence of cortical necrosis of the kidneys observed.

In 1952 THOMAS and GOOD reported that the GSR could be produced by a single injection of endotoxin if rabbits were pre-treated with ACTH or cortisone [2]. These investigators also found that cortical necrosis of the kidneys could be produced by a single injection of endotoxin if rabbits were pre-treated with Thorotrast or trypan blue [3].

It was the purpose of the present investigations to compare the degree of 'preparation' induced by cortisone and by Thorotrast with that induced by endotoxin, employing the experimental model described in the previous paper. For this study a dose of endotoxin was used which had been shown to be capable of inducing intravascular clotting and cortical necrosis of the kidneys in adequately prepared rabbits. The results to be described revealed that cortisone preparation, even in doses ten times those employed by THOMAS and GOOD did not lead to the development of intravascular clotting or cortical necrosis of the kidneys following a single injection of endotoxin. This held true even when the provocative dose of endotoxin was raised to ten times the standard endotoxin dose or 40 times the minimum effective dose with endotoxin preparation. Thorotrast preparation, on the other hand, was followed by intravascular clotting and cortical necrosis of the kidneys in response to a single injection of endotoxin in the standard amount.

Materials and Methods

1. White New Zealand rabbits of either sex, weighing 1.0 kg were obtained through the same breeder, housed in a temperature controlled animal room, and fed Purina pellets and water, *ad lib.* All animals used in these experiments were free of obvious illness.

2. The endotoxin of *Escherichia coli* 0127:B8, Boivin type (Difco Laboratories, Detroit), was used. The endotoxin was prepared daily by using 0.9 % sterile pyrogen free saline as the diluent. The appropriate amount of endotoxin was injected via the marginal ear vein in a constant volume of 1.5 ml. The 'standard' dose of endotoxin employed was 0.100 mg/kg rabbit body weight, a dose previously shown to provoke intravascular clotting in rabbits prepared with 0.001 mg/kg of endotoxin and *both* intravascular clotting and renal cortical necrosis in animals prepared with 0.01 and 0.1 mg/kg of endotoxin [1].

3. Cortisone acetate, 25 mg/ml (Merck, Sharp and Dohme, West Point, Pa.), was administered to rabbits according to the schedule reported by THOMAS and GOOD i.e., 25 mg intramuscularly once a day for four days. Endotoxin was then given on the third steroid day [2].

4. Thorotrast, a sterile, stabilized, colloidal suspension of thorium dioxide (24 to 26 % by volume; Fellows-Testagar Co., Inc., Detroit, Mich.) was given intravenously in a dose of 3.0 ml/kg body weight.

5. Sampling techniques, blood counts and assays for coagulation Factors II, V, VIII, and fibrinogen were performed as described in an accompanying paper [1]. The white blood cells and platelets are expressed as cells/mm^3; factors II, V, VIII and the prothrombin complex level (corresponding to the prothrombin time) as percent activity as compared to a normal human standard, and fibrinogen as mg/100 ml plasma.

6. All animals that died during the experiment were autopsied. Survivors were sacrificed by cervical subluxation 24 hours following the provocative endotoxin injection in the endotoxin, Thorotrast, and saline prepared animals, and 30 hours in the cortisone group. The rabbits were considered to have the GSR when the kidneys showed gross evidence of cortical necrosis with or without massive hemorrhages.

Results

1. Effect of Cortisone Pre-treatment and a Single Injection of Endotoxin

Rabbits were pre-treated with cortisone, 25 mg IM for four days and given a single dose of endotoxin on the third day, according to the regimen of THOMAS and GOOD [2]. Table I demonstrates that with provocation using the standard dose of endotoxin (0.100 mg/kg) renal cortical necrosis was not observed in any of 20 animals. In contrast, this same provocative dose induced renal cortical necrosis in 74 and 77 % of animals prepared with 0.01 and 0.1 mg/kg of endotoxin respectively [1]. With the same dosage regimen of cortisone preparation, provocative doses of endotoxin of 0.2 and 0.5 mg/kg likewise failed to induce renal cortical necrosis in any of the animals tested. When the provocative dose was raised to 1.0 mg/kg two of five animals developed cortical necrosis of the kidneys. Repeat of this experiment with an additional 10 animals resulted in no instance of cortical necrosis. Likewise, five animals receiving 2.0 mg/kg of endotoxin exhibited no GSR. An increase in the preparative dose of cortisone to 50 mg/day for four days did not induce cortical necrosis in response to the standard provocative dose. With cortisone preparation of 100 and 250 mg/day for four days provocation with 1.0 mg/kg of endotoxin resulted in cortical necrosis of the kidneys in only 1 of 6 animals in each group.

Table I. Incidence of GSR with varying doses of cortisone preparation and a single injection of varying amounts of endotoxin provocation

Cortisone (mg/day × 4)	Endotoxin (mg/kg)	Renal cortical necrosis (Incidence/total No. animals)
25	0.1	0/20
25	0.2	0/5
25	0.5	0/5
25	1.0	2/5
25	1.0	0/10
25	2.0	0/5
50	0.1	0/5
100	1.0	1/6
250	1.0	1/6
Saline	1.0	0/5

Table II. Studies in cortisone treated rabbits receiving a single dose of endotoxin

	0 hr.	4 hr.	% fall	p
WBC				
mean	7150/mm^3	1335	81	0.001
S.E.	±573	192		
Platelets				
mean	722,000/mm^3	526,000	27	0.001
S.E.	±16,000	28,000		
Factor VIII				
mean	244 %	129	47	0.001
S.E.	±25	13		
Pro. time				
mean	380 %	257	32	0.001
S.E.	±15	10		
Pro. assay				
mean	212 %	138	35	0.05
S.E.	±32	4		
Factor V				
mean	527 %	486	7	0.10
S.E.	±47	52		
Fibrinogen				
mean	204 mg %	183	9	0.10
S.E.	±9	12		
No. animals	9	19		

Cortisone acetate 25 mg/day for four days, I.M.
E. coli 0127:B8 Endotoxin 0.10 mg/kg
S.E. = Standard error

Coagulation studies were carried out in animals prepared with cortisone, 25 mg/day for four days, and provoked with the standard dose of endotoxin of 0.1 mg/kg (table II). Significant decreases were noted in the white blood cells, platelets, Factor VIII, Factor II, and the prothrombin complex, but no significant changes occurred in Factor V and most important, fibrinogen concentration. These changes are almost identical to those found with saline preparation (table III).

Table III. Various modes of preparation

'Preparation' provocation	Mean percent change four hours after provocation			
	Saline (1.0 ml/day × 4, IM) Endotoxin (0.1 mg/kg)	Cortisone (25 mg/day × 4, IM) Endotoxin (0.1 mg/kg)	Endotoxin (0.01 mg/kg) Endotoxin (0.1 mg/kg)	Thorotrast (3.0 ml/kg) Endotoxin (0.1 mg/kg)
No. animals	19	28	15	12
WBC	—74	—81	—87	—19
Platelets	—41	—27	—66	—40
Factor VIII	—30	—47	—74	—18
Factor V	—14	—7	—48	—56
Factor II	—8	—35	—54	—43
Prothrombin time	—25	—32	N.D.[1]	N.D.[1]
Fibrinogen[2]	—2 (—4)	—9 (—21)	—36 (—144)	—42 (—135)

[1] Not determined.

[2] Fibrinogen values are expressed as percent change and in parentheses, mg/100 ml.

In order to determine whether cortisone treatment might actively protect against the development of the GSR animals were given cortisone, 25 mg IM for four days and then 0.1 mg/kg endotoxin on the third steroid day and 24 hours later a provoking or second dose of 0.1 mg/kg endotoxin. Seven of eleven rabbits or 64 % exhibited bilateral renal cortical necrosis. Cortisone pretreatment, therefore, did not protect the animals from the development of the GSR when two injections of endotoxin were employed.

2. Effect of Thorotrast and a Single Injection of Endotoxin

Thorotrast was given to eleven rabbits in a dose of 3.0 ml/kg intravenously and eighteen hours later 0.1 mg/kg of endotoxin was given. Five of the animals developed cortical necrosis of the kidneys. Coagulation changes in this experiment are summarized on table III as contrasted with changes following saline, cortisone, and 0.01 mg/kg endotoxin preparation. Of importance is the fact that with Thorotrast preparation the coagulation mechanism was activated in its entirety with highly significant falls in all of the measured coagulation factors being observed. Most important the fibrinogen concentration was reduced by 135 mg/100 ml, an amount equivalent to that following endotoxin preparation with 0.01 mg/kg.

Discussion

The results demonstrate that in these experiments cortisone preparation of rabbits, carried out exactly in accordance with the regimen described by THOMAS and GOOD was not followed by the development of the GSR in response to a single dose of endotoxin of demonstrated potency. The failure of 'preparation' by cortisone was evidenced not only by the absence of renal cortical necrosis but also by the pattern of coagulation changes which were no different from those observed in animals 'prepared' with saline. In contrast animals prepared with small doses of endotoxin or with Thorotrast and provoked with the same standard dose of endotoxin developed the classical GSR and the coagulation changes which indicated intravascular clotting and which resulted in depletion of significant amounts of fibrinogen. Moreover, an increase in the cortisone dose to ten times that employed by THOMAS and GOOD and increase in the provocative dose of endotoxin to ten times the standard dose likewise failed to induce any significant incidence of the GSR.

Explanations for the differences in the results reported here from those described by THOMAS and GOOD include the type of endotoxin employed, the strain of rabbits or, possibly, variation in the cortisone itself. In the studies of THOMAS and GOOD a single dose of endotoxin derived from *Serratia marcescens* or meningococcus was capable of inducing renal cortical necrosis in cortisone treated animals. In dose-response studies with the *Serratia* endotoxin it was found that the amount of endotoxin needed was more than four times greater than the concentration which would consistently cause the GSR in non-cortisone treated animals given two injections 24 hours apart. While in the present studies *E. coli* endotoxin was employed it was shown in the previous paper that this endotoxin was quite capable of producing the GSR regularly over a wide range of both preparative and provocative doses. Furthermore the coagulation studies provided a additional means for determining and, to a degree, quantitating the potency and effectiveness of the endotoxin and of various modes of preparation. As indicated before, no evidence of preparation by cortisone could be detected. That the type of endotoxin utilized may be important is suggested also by the observations that the optimal times for a single effective injection of endotoxin in Thorotrast rabbits has been reported as between 3 to 12 hours for *Serratia* [3] and 10 to 18 hours for *E. coli* [4].

Variability of response to endotoxin in different strains of rabbits has been demonstrated [5]. However, the rabbits employed in our experiments regularly developed the GSR with two injections of endotoxin and with a single injection preceded by Thorotrast. That this strain was peculiarly resistant to cortisone seems unlikely.

With regard to the type of cortisone used there does not appear to be any reported difference, to our knowledge, between the cortisone acetate presently available and that used 15 years ago.

Based on the data of THOMAS and GOOD others have implied that the use of cortisone may be hazardous in the treatment of Shwartzman-like clinical conditions in the human [6, 7, 8, 9, 10]. It is evident from the data in the present study however that the influence of cortisone on the response to endotoxin in the production of the GSR may depend on the animal strain, the type of endotoxin or other variables. Therefore, it would appear that until further studies are forthcoming extrapolation of the experimental results to clinical situations is premature.

Summary

1. Renal cortical necrosis did not develop in a significant number of rabbits receiving a wide range of both cortisone pre-treatment and a single injection of *E. coli* endotoxin as provocation. The failure of 'preparation' by cortisone was evident also by the pattern of coagulation changes following a standard known provoking dose of endotoxin, these changes being no different from those observed in animals 'prepared' with saline.

2. Rabbits prepared with either small doses of endotoxin or with Thorotrast and provoked with the same standard dose of endotoxin developed both renal cortical necrosis and significant changes in the measured coagulation factors indicative of diffuse intravascular clotting.

3. These data, in addition to those of THOMAS and GOOD, suggest that the influence of cortisone in the production of the GSR may depend on the type of endotoxin, the strain of animals, or other variables and also suggest that until additional studies are performed generalizations concerning the use of cortisone in treatment of the human equivalents of the GSR would seem to be premature.

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