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The Reduction of Endotoxin-induced Loss of Protein into the Extra-cellular Space: Evidence for its Benefit in Shock?

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Intravenous injection of endotoxin (E. coli lipopolysaccharide, 0127:B5) 3-30 mg/kg brought about a dose-dependent decrease in protein levels in rat plasma. The decrease was however not accompanied by corresponding decrease in haematocrit. The packed cell volume was found to rise with the dose of endotoxin lipopolysaccharide given. Dexamethasone alone (20 mg/kg) did not affect the protein level, but when it was administered 20 minutes before the injection of 10 mg/kg lipopolysaccharide, it significantly prevented the reduction of protein levels by endotoxin ($p<0.05$). Interestingly, dexamethasone also prevented the rise of haematocrit induced by lipopolysaccharide. The dexamethasone prevention of the increase brought about by lipopolysaccharide indicates that dexamethasone relies on its effect in preventing leakage of protein material from the vasculature to the extracellular space. This appears to be the basis of its beneficial effect in shock, edema and inflammatory conditions.

Key Words: endotoxin, protein, haematocrit, dexamethasone

INTRODUCTION

It has long been recognized that when the quantity of plasma protein falls, it leads to lowered colloid osmotic pressure with the resultant fluid escape from the vascular spaces, especially the capillaries, into tissue spaces which then causes edema. Resembling this phenomena is a situation seen when endotoxin is injected into an animal resulting in shock. Previous observations of this phenomena have implicated the circulating bacterial endotoxin in the development of shock [1, 2, 3, 4]. Olson [5] demonstrated that as haematocrit increases in response to bacterial introduction, the plasma content also increases. Related findings show haematologic changes include leucopenia and hemoconcentration [6]. The changes observed in the blood coagulation values include a significant prolongation of the activated partial thromboplastin time and thrombin time and an insignificant prolongation of the prothrombin time. These and other hemodynamic effects of endotoxin shock are of clinical significance.

In animal studies, glucocorticoids have been reported to relieve the lethal effects of bacterial endotoxin [7, 8]. Although clinically, glucocorticoids are used in combination with other drugs in a number of conditions, including the treatment of asthma, allergies, certain forms of shock and cancer, neither their biochemical nor the pharmacological basis for those effects is completely known. In some cases, the actions of other hormones are observed only when the tissue has first been exposed to glucocorticoids. For instance, biological mediators like tumor necrosis factor (TNF) have been shown to be effected in presence of dexamethasone [9, 10]. Beutler and others [11] have proposed that steroids inhibit release of cachectin (tumor necrosis factor). Although a number of studies have shown the inhibitory ability of steroids on the LPS-dependent factors [12, 13, 14, 15] and thereby prevention of inflammation, Green et al [16] observed no such effect on the rabbit eye. The present study is an attempt to elucidate the basis of the interaction between glucocorticoid and bacterial endotoxins.

MATERIALS AND METHODS

Normal healthy Sprague-Dawley male rats, specific pathogen free, 8-10 weeks old, weighing between 250-300 grams, were used. They ate *ad libitum* and had free access to water. For the production of anesthesia, the rats were injected with intraperitoneal sodium pentobarbitone, 60 mg/kg body weight. Within about 10 minutes of the injection, the rats were checked for anaesthetic effect by assessing if there was an eye lid reflex. Anesthesia was maintained by occasional intraperitoneal administration of sodium pentobarbitone, 10-25 mg/kg body weight. Artificial respiration was instituted with a small animal pump (Nihon Kohden 21).

CANNULATION PROCEDURE

After full anesthesia was effected, the right femoral vein was cannulated with a polyethylene cannula (Clay Adams NJ USA, P.E. 50). The injection of endotoxin and infusion of dexamethasone were given through this cannula. The left femoral and carotid arteries were cannulated with a polyethylene cannula (Clay Adams NJ USA, P.E. 60). These were for the recording of systemic blood pressure and collection of blood samples respectively.

EXPERIMENTAL PROTOCOL

Each animal served as its own control. After the surgical procedure, the blood pressure and other parameters were allowed to stabilize for 30 minutes. Blood sample was taken for 3, 10 and 30 minutes.

Experimental Design For Endotoxin Studies

There were four (4) groups of animals, each group consisting of 11 male Sprague-Dawley rats weighing 250-300 gm.

Group 1: Consisted of animals in which only saline (0.5ml) was injected into the femoral vein.

Group 2: Consisted of animals in which endotoxin (E. coli), dissolved in saline at various concentrations, was injected into the femoral vein.

Group 3: Consisted of animals in which i.v. Dexamethasone (DEX) alone 20 mg/kg was injected.

Group 4: Consisted of animals in which intravenous dexamethasone 20 mg/kg was injected followed by endotoxin, 20 - 30 minutes later.

Doses of Endotoxin

Endotoxin (E. coli endotoxin lipopolysaccharide 0127:B5) was prepared in saline 3-30 mg/ml saline/kg) and doses were injected as a bolus into the right femoral vein. For control studies, only saline (1ml/kg) was injected. In another set of experiment in one set of experiments, DEX alone 20 mg/kg was injected and the fourth group of animals DEX (20mg/kg) was injected 20 minutes before the intravenous injection of 10 mg/kg endotoxin.

Measurement of Systemic Blood Pressure

The mean systemic blood pressure was measured by a pressure transducer (MPU-0.5, Nihon Kohden, Tokyo, Japan) attached to a cannula inserted into the right femoral artery and this parameter was recorded on a polygraph (RM-85 Nihon Kohden, Tokyo, Japan). The heart rates were recorded by a forced-displacement transducer.

Measurement of Protein Content in Blood

The levels of plasma protein were determined by the method of Lowry *et al* [17]. The carbonyl groups in the protein molecule react with $CuSO_4$ in alkaline medium to give purple color. Thus, the protein measurement was performed with Folin phenol reagent, taking albumin as a standard comparison on the basis of spectrophotometric reading at 500nm.

To 500µl of samples, 150µl of sodium desoxycholate was added and allowed to stand for 10 minutes at room temperature. Then 300µl reagent (D), was added and allowed to stand for a further 10 minutes. Finally 300µl Folin reagent, was added and the tubes were left to stand for 40-50 minutes at room temperature before reading in a spectrophotometer. This reading was compared with that of the standard.

Statistical Analysis

When variances were not heterogeneous, studentís t-test was used to evaluate the significance of differences. Other wise statistical analysis was performed by Wilcoxinís rank sum test.

RESULTS

Changes in Systemic Blood Pressure, Protein Levels and Haematocrit Values Induced by Intravenous Injection of Endotoxin

When a bolus dose of endotoxin in saline (3-30 mg/kg) was injected into the femoral vein of rats, the systemic blood pressure fell gradually (Fig. 1, Table 1).

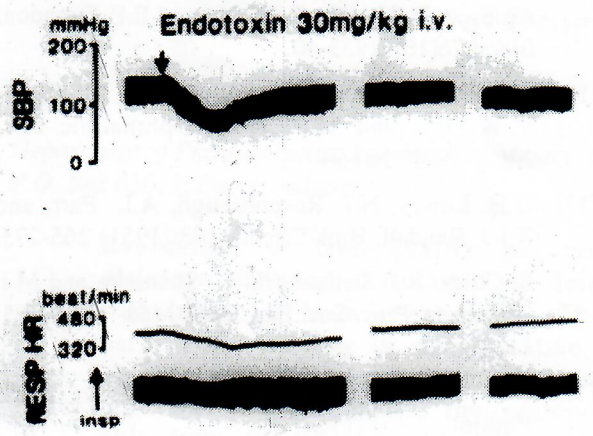
TABLE 1: Changes in Blood Pressure, Protein Levels and Haematocrit Values with Endotoxin

Dose of ET (mg/kg)	0	3	10	30
Change of BP from normal	-	-16.3	-47.4	-49.4
Protein (mg/ml) Mean N=11 (STD DEV)	55.57 (±1.01)	53.33 (±1.25)	50.65 (±1.98)	48.2 (±1.47)
Haematocrit value Mean N=11 (STD DEV)	44.21 (±1.17)	46.53 (±1.82)	48.54 (±1.24)	54.37 (±1.42)

There was a dose-dependent decrease in protein levels expressed in mg/ml of plasma after the treatment of rats with endotoxin. The decreases were 5, 10 and 155% of the control level.

The levels of haematocrit were measured using the micro-method of Chien *et al* [18]. There was a dose-dependent increase in the levels of haematocrit in the blood samples which were collected 15 minutes after the injection of endotoxin which represent increases of 4.99%, 9.78% and 22.9% above the control value for 3, 10 and 30mg/kg endotoxin respectively. These values show that haemo-concentration due to plasma leakage, probably as a result of the formation of kinin, was induced in parallel with the decrease in the blood pressure.

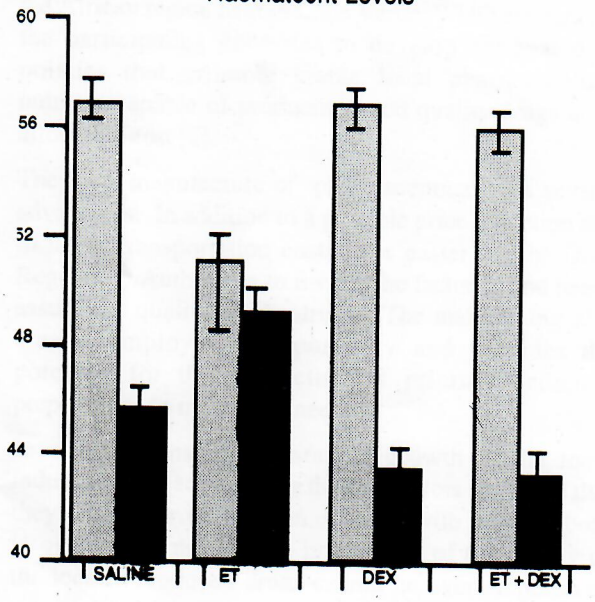
FIG. 1:



Effects of Dexamethasone on Haematocrit and Protein Levels

Dexamethasone administered 20 minutes before the intravenous injection of endotoxin (10 mg/kg) to rats significantly ($p<0.001$) prevented the reduction of protein values by endotoxin Fig. 2). The values were 56.4 ± 0.81, (saline), 50.91 ± 2.87 (endotoxin 10mg/kg), 56.40 ± 1.07 (dexamethasone) and 56.16 ± 1.14 (dexamethasone) 20 mg/kg followed by endotoxin 10mg/kg. Haematocrit levels were modified as follows: 44.67 ± 0.99 saline, 49.22 ± 1.36 endotoxin, 43.68 ± 1.18 dexamethasone, 20 mg/kg and 44.01 ± 1.32 (dexamethasone 20 mg/kg followed by endotoxin 10 mg/kg).

FIG. 2: Effect of Dexamethasone on Protein and Haematocrit Levels



DISCUSSION AND CONCLUSION

The lowering of blood pressure and the development of metabolic and hemodynamic abnormalities are some of the immediate responses to the introduction of endotoxin in an animal [1, 3]. In Table 1 such lowering is demonstrated. There was a dose dependent relationship, with higher than 30 mg/kg body weight causing irreversible hypotension and death to rats [4]. The reasons for this hypotension have been said to be due to the loss of plasma fluid into the interstitial spaces, because of increased vascular endothelial cell permeability and inflammation, resulting in hemoconcentration in the circulation [19, 6, 20]. Olson [5] demonstrated that as haematocrit increases in response to bacterial introduction into an animal, the plasma cell content also increases. These changes have been known for a long time to arise as a result of the release of vasoactive substances, e.g. histamine [21]; 5-hydroxytryptamine [22]; kinins [23], leading to shock status.

The present study demonstrated that plasma protein fell in a dose-dependent manner with endotoxin.. This result contradicts that of Oslon [5] who reported an increase in plasma protein content with endotoxin. However, it should be understood that when the quantity of plasma protein falls, it leads to lowered colloid osmotic pressure with the resultant fluid escape from the vascular spaces, especially the capillaries, into tissue spaces which then causes edema.

The use of steroids for a number of medical emergencies, including shock, is well established. Glucocorticoids have been reported to ameliorate the lethal effects of bacterial endotoxin in animals [7, 8]. Most inflammatory conditions have also been managed by steroids [11].

In this work, dexamethasone (20mg/kg body weight) prevented the increase in hematocrit levels which are brought about by endotoxin (Fig. 2). This same dose also prevented the protein loss into the extra-vascular spaces. Both of these effects were measured in a dose dependant manner. This dual action alone can safeguard against the ulterior effect of endotoxin to an animal.

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