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Mechanism of action of endotoxin on the microcirculation in endotoxin shock

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THE MECHANISM OF ACTION OF ENDOTOXIN
ON THE MICROCIRCULATION IN ENDOTOXIN SHOCK

by

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THE MECHANISM OF ACTION OF ENDOTOXIN
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Introduction

Endotoxins from gram-negative bacteria are of manifold clinical importance. They are involved in the pathogenesis of septicemia, septic abortion, the generalized Schwartzman-Sanarelli phenomenon, septic shock, and the protracted delayed shock following trauma, especially burns.³ In these clinical states the effect of endotoxins on the vessels and the disturbances of the blood coagulation are prominent.

It appears that endotoxin is one of the most common causes of the shock syndrome, both in animal experimentation and in hospitalized patients.²² The syndrome is characterized by a sudden and severe hypotension, decreased renal function, peripheral pooling of blood, and severe acidosis, and are uniformly seen in both hemorrhagic and endotoxin shock. Dogs given the lethal dose of endotoxin showed an initial precipitous fall in arterial, right ventricular, and left ventricular pressures. This was followed by partial return of these pressures to near normal levels, and finally a slow, progressive deterioration terminating in death. At the same time, central venous pressure increased to twenty to thirty mmHg within five minutes after the

introduction of endotoxin. Arterial blood pH and alveolar carbon dioxide were significantly decreased during the initial three hours post endotoxin. This too, was followed by partial recovery at four to six hours with a final irreversible decrease occurring at approximately eight hours.¹ All of these changes are diagnostic of cardiovascular failure, and physicians who treat patients in shock are familiar with them.

Numerous attempts have been made to understand the basic mechanisms underlying the above changes and to define, if possible, the exact mechanisms by which endotoxin shock is produced. The purpose of this paper is to present the various plausible mechanisms that will explain the effect of endotoxin both directly and indirectly on the microcirculation that produces the syndrome present in endotoxin shock.

Structure of the Endothelial Layer

Since small blood vessels and capillaries appear¹ to be the most sensitive organs in a shock situation, one should have a basic knowledge of the structure of these organs in order to understand and interpret the various proposed mechanisms of action of endotoxin. Only by this means, can one make an intelligent appraisal of the various theories proposed.

According to the work of Pappenheimer and his

colleagues, the capillary endothelium behaves as if it were a semipermeable membrane, penetrated by water-filled channels or pores, through which pass water and small, lipid insoluble molecules.¹⁸ Electron microscopists have shown these pores to be the intercellular clefts that lead to the basement membrane. The total cross-sectional area of the pores occupies less than 0.1 percent of the capillary surface with each pore having a radius of 40 to 45 Angstroms.¹⁶ The channels need not actually be cylindrical: slits of equivalent width, 50 to 55 Angstroms, and other geometrical configurations are also possible.¹⁸ Hence, one can see that with increasing molecular size, restriction to diffusion becomes greater, and for large molecules, such as plasma proteins, a high degree of molecular sieving occurs.¹⁸ The residual transport for molecules larger than the small pores may represent either diffusion through a sparse system of large pores with a radius of around 250 Angstroms,¹⁰ or vesicular transport.¹⁹ Moderate restriction to diffusion by the small cylindrical pore occurs in the molecular weight range 1000 to 10,000, and above this the restriction is greater. Theoretically, substances of molecular weight greater than 90,000 would be almost completely restricted, at normal rates of filtration.¹⁸ One can therefore see that small molecules may exchange rapidly between plasma and

interstitial fluid by diffusion through these pores or clefts, and the net volume of flow would therefore be dependent on hydrodynamic forces which I will show later is indirectly affected by the endotoxin in endotoxin shock.

Structure of the Basal Lamina

Sections of the mosquito midgut epithelial basal lamina parallel to the epithelial base reveal an extensive, well-ordered grid structure. This has not yet been shown in vertebrates, however. The grid components are moderately dense, parallel lines intersecting one another approximately at right angles. Often, the lines intersect at an angle close to but different from 90 degrees. The moderately dense lines are about 75 Angstroms wide and surround a roughly circular space of low density about 150 Angstroms in diameter. In longitudinal sections, the epithelial basal lamina consists of stacked lamellae, filamentous areas of moderate density, and irregular areas of low density. The stacked lamellae often follow an undulating course, but can be roughly parallel to the epithelial base. A single lamella contains the grid structure seen in sections parallel to the epithelial base. The lamellae have a minimum width of about 100 Angstroms. Basal laminae are noted for their amorphous composition and of the close similarity of the grid

arrangement to that of a Millipore ultrafilter.²⁰

Because of the arrangement of the basal lamina to that of a grid with approximately each circular space having a diameter of 150 Angstroms, one can see that the basal lamina probably plays a secondary role in capillary permeability; as once the molecules get past the intercellular clefts, they may pass quite freely through the grid structure.¹⁶

Histamine Release and Its Effects

Reviews of the ultrastructure of capillaries and of the effects of pharmacologic agents on increasing capillary permeability point out that several naturally occurring substances present in tissues are capable of affecting vascular permeability. Bradykinin is one of these; as is histamine.⁸ It is a well-established fact that endotoxins can release histamine in the organism.³ This might be explained by the present investigation of Branemark which revealed degranulation and disruption of the tissue mast cells, periendothelial granular cells and intravascular granulocytes containing histamine when endotoxin was introduced.² From ten to fifteen minutes after local or intravascular application of endotoxin the first changes in the microcirculation were observed. The corpuscular flow of blood began to slow down and

after ten minutes the blood flow was reduced to 90 percent of the original value.³ There was arteriolar dilatation and venular constriction of varying degree.¹⁵

The pericytes and endothelial cells increased in volume. The plasticity of the cells was reduced to varying degrees. Depending on the dose of the drug administered, the permeability of the vessel wall was increased and the damage resulted in functional or structural disruption of the endothelial lining with varying degrees of subsequent diapedesis of cells, edema, and micro-bleedings.³

The lag period between application of endotoxin and the first appearance of microcirculatory disturbance corresponds in time with the occurrence of degranulation of intravascular and extravascular granular cells.² This time sequence points toward the participation of the mediator histamine in the microcirculatory effects of endotoxin rather than endotoxin itself causing the microcirculatory effect. Hence, endotoxin causes a release of histamine which causes arteriolar dilatation and venular constriction which therefore causes increase in hydrostatic pressure at the capillary level, and this favors the passage of fluid from capillaries to extravascular space. Also, endotoxin may affect capillary permeability directly by causing structural disruption of the endothelial lining which allows for free passage of fluid to extravascular

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space. Another thing to keep in mind is that histamine is increased in its effect by endotoxins. The resulting high activity of histamine has a response in the reaction on the blood vessels and also in the increased permeability of the gut which allows more endotoxins to enter the circulation. The high amount of endotoxins now develops a fatal action by liberating more histamine and thus creating a vicious cycle.²¹

Another possible mechanism has been postulated by Fine and Minton.⁶ They have shown that the amount of endotoxin required to produce fatal shock by the intracerebral route in rabbits is roughly one-twentieth of that required by the intravenous route. They have postulated, therefore, that the shock which followed the injection of endotoxin into the brain was the result of an action on the peripheral vessels from a distance, via the sympathetic nervous system. They have also shown that by blocking the coeliac plexus the survival rate was then increased by 50 percent with the same dosage of injected endotoxin. Fine and Milton, therefore, came to the conclusion that while endotoxin appears to be capable of acting directly on certain tissues, the lethal effects of endotoxin appear to be mediated by the catecholamines released in the splanchnic tissues which caused the loss of integrity of the blood vessels in the splanchnic area.

Effects on Coagulation

Studies of the components of the blood coagulation system and histologic and immunologic studies of tissues have demonstrated that one of the major effects of bacterial endotoxin in the circulating blood is to trigger the blood coagulation system.¹² Ultrastructural studies have demonstrated that disseminated intravascular coagulation occurred in the early stages of endotoxin shock in the rhesus monkey.¹³ The type of intravascular clotting was manifested by the formation of short strands of fibrin which was first noted after two hours which appeared to float free in the plasma. They were sometimes found in close association with platelet aggregates and in general did not form occlusive masses even in the capillary vessels. The sole exception was the liver where platelet--leukocyte--fibrin masses were found occluding the lumens of hepatic sinusoids two to four hours after exposure to endotoxin. The fibrin demonstrated by electron microscopy was not visualized by the light microscope.¹³ These studies were illustrative that a considerable amount of intravascular clotting may occur without formation of gross or microscopic thrombosis.

From the standpoint of electron microscopy, the initial change, when the blood stream was exposed to bacterial endotoxin, was the aggregation and sequestration of platelets in the pulmonary microcirculation which was

noted as early as fifteen minutes. This aggregation of platelets probably accounted for the thrombocytopenia, and the intravascular formation of fibrin for the decrease in plasma fibrinogen concentration.¹³

The second major event in endotoxemia was the aggregation of polymorphonuclear leukocytes in the pulmonary capillaries which accounts in part for the leukopenia, a prominent feature in the early reaction to endotoxin. This is also contributed to by the intravascular destruction of a few neutrophils. The leukocyte destruction resulted in the release of nuclear fragments and the specific granules of eosinophils as well as neutrophils. Leukocyte destruction was observed only in the pulmonary vascular bed and was first noted only one hour after injection of the endotoxin.¹³

As one can see, the above observations account in large part for the development of fibrinogenopenia, neutropenia, and thrombocytopenia which takes place in the early stages of experimental endotoxin shock in primates.

Even though it is clear that lethal doses of endotoxin in monkeys trigger the clotting mechanism, one of the unsolved problems is the exact manner in which endotoxin acts by activating the intrinsic prothrombin activator. Such a mechanism would require that the Hageman

factor (factor XII) be activated also.¹¹ An alternative possibility is that bacterial endotoxin in the blood stream damages the endothelium and causes release of tissue thromboplastin (extrinsic prothrombin activator) into the circulation. However, it appears by McKay's¹³ microscopic studies that the endothelial damage is secondary in time to the ischemia produced by the platelet and fibrin aggregations. However, the limitation of electron microscopy must be recognized, and it is entirely possible that functional alterations of the endothelium could occur without a structural change.

Even though lethal doses of endotoxin in monkeys trigger the clotting mechanism, this cannot be the main cause of death, because prophylactic injection of heparin does not cause a decrease in the number of deaths in the monkeys.¹⁴ However, it does decrease the number of deaths in rats.

Effect on Polymorphonucleocytes

It was shown by McKay¹³ previously that one of the effects of endotoxin injection was the aggregation of polymorphonuclear leukocytes in the pulmonary capillaries. It has therefore been postulated that polymorphonuclear leukocytes may cause injury in the vessels by many investigators, especially in the immunologic diseases as the Arthus phenomenon⁹ and acute nephrotoxic nephritis.⁵

Considerable speculation exists as to the mechanism by which polymorphonuclear leukocytes may injure blood vessel walls. In addition, little is known about the critical target in the vessel walls attacked by the polymorphonucleocytes. The vascular basement membrane was disrupted in the presence of polymorphonucleocytes and was not disrupted when polymorphonucleocytes were not present during the Arthus phenomenon. This disruption was evidenced by the inability of the basement membrane to retain circulating carbon and by the electron microscopic examination showing actual gaps in the structure of the vascular basement membrane.⁴ Several investigators have described permeability factors either released during incubation of polymorphonucleocytes in normal saline at 37 degrees or contained within the cytoplasmic granules.⁷ The factors within polymorphonucleocytes responsible for the damage to isolated glomerular basement membrane in vitro were found by isolation procedures to be cathepsins D and E. One can, therefore, see that the polymorphonucleocytes probably play a major role in the microcirculatory effects of endotoxin.

Summary

An attempt has been made to show that endotoxin exerts both an indirect and a direct effect on the capil-

laries in endotoxin shock. Endotoxin shock is probably not the result of a single causative factor alone, but rather the result of the accumulation of all the physiologic changes that are produced both directly and indirectly by the endotoxin: 1. The release of histamine which causes arteriolar dilatation and venular constriction resulting in vascular pooling, 2. The disruption of the endothelial lining which allows for free passage of fluid into the extravascular space, 3. The increase in the effectiveness of histamine, 4. The release of catecholamines in the splanchnic system which causes further loss of integrity of the peripheral blood vessels, 5. The triggering of the coagulation system with the resultant aggregates of platelets and polymorphonucleocytes, and 6. The release of cathepsin D and E with disruption of the basement membrane allowing large molecules to pass freely.

All of the above mechanisms predispose to blood stasis that results in peripheral pooling of blood, decreased cardiac output, hypotension, decreased renal function, severe acidosis, and eventually death.

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