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Experimental Endotoxin-Induced Disseminated Intravascular Coagulation in Rat Model

Seok-Cheol Choi†, Jai-Young Kim, Jin-Bog Koh* and Won-Jae Lee**

Department of Clinical Pathology, Jisan Junior College, Pusan 609-757;
**Department of Biology, Pusan Woman's University, Pusan 617-736;*
***Department of Microbiology, Pukyong University, Pusan 608-023, Korea*

Abstract: In septic patients, disseminated intravascular coagulation (DIC) occurs frequently and is a pathologic condition associated with a variety of critical illness. DIC may complicate the already complex clinical situations and contribute to the high mortality. Nevertheless, its pathogenic mechanisms are not completely elucidated. Present study was prospectively designed to understand the pathogenetic mechanisms involved in the development of DIC. 15 rats were subjected to study and according to the aim, they were divided into three groups: group I, control (not treated-endotoxin, n=5); group II (12 hours after endotoxin injection, n=5); group III (24 hours after endotoxin injection, n=5). Experimental DIC was induced in rats by a bolus injection of endotoxin (1mg/kg, *E. coli* serotype 055:B5). Blood was collected by direct puncture of the heart. Platelet count, fibrinogen and plasminogen concentration, antithrombin III, D-dimer and complement components (C3 and C4) were measured in all subjects. In group II and III, there were apparent signs of DIC, including thrombocytopenia, decreased fibrinogen (but increase in group III), reduced C3 and antithrombin III, and elevated D-dimer. These data indicated that endotoxin might induce the activation of several pathways such as coagulation, fibrinolytic and complement cascade, causing DIC and subsequent multiple organ failures. Ultimately, the increased knowledge of the various pathogenetic mechanisms of coagulation activation and fibrinolysis in endotoxin-induced DIC may have prophylactic or therapeutic implications.

Key Words: Experimental DIC, Endotoxin, Rat

INTRODUCTION

Sepsis is precipitated by the influx of endotoxins or related substances into the blood stream, usually from a site of infection or trauma. Endotoxins, lipopolysaccharide constituents of the outer membrane of gram-negative microorganisms, play a pivotal role in the development of the sepsis syndrom[1]. Sepsis is one of the major causes of morbidity and mortality in hospitalized patients, especially those in intensive care units.

It is estimated that in the United States alone about 400,000 patients encounter annually sepsis, 200,000 patients develop septic shock, and 100,000 die from this complication[2], but not extactly known on its estimation in Korea. In septic patients, disseminated intravascular coagulation (DIC) occurs frequently. DIC may complicate the already complex clin-

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†Corresponding author

ical situations and contribute to the high mortality. DIC is a pathologic condition associated with a variety of critical illness[3]. In the pathophysiology of DIC, systemic microthrombi formation would result in microcirculatory disturbances and organ dysfunction[4]. Although pathophysiology of DIC has been reported by several investigations, its pathogenic mechanisms aren't completely elucidated. The present study was prospectively designed in rats to understand the pathogenetic mechanisms involved in the development of DIC.

MATERIALS AND METHODS

1. Materials

15 female SD (Sprague Dawley) rats aged 6 months were subjected to study. According to the aim, they were divided into three groups (5 animals per group): group I, control (not treated-endotoxin, n=5); group II (12 hours after endotoxin injection, n=5); group III (24 hours after endotoxin injection, n=5). All rats were characterized in Table 1.

Commercialized endotoxin (Lipopolysaccharide; *E. coli* serotype 055:B5, Sigma, Co., U.S.A.) was used for induction of DIC.

2. Methods

1) Experimental DIC

Experimental DIC was induced in rats by a bolus injection of 1 mg/kg of endotoxin through their tails.

2) Sampling and hematologic measurement

All rats were induced general anesthesia using ether and their hearts were exposured through abdominal incision at supine position. To determine platelet counts, and levels of D-dimer, fibrinogen, plasminogen, antithrombin III, and complement components (C3 and C4), about 7mL of blood was collected by direct puncture of the heart.

Platelet count was measured with SYSMEX NE-8000 (TOA Medical Electronics, Co. STD), C3 and C4 was measured by Behring Turbitimer (Behring Co.) using commercialized antiserum, Turbiquant (Behring Werke AG, Marburg, Germany), and D-dimer was semi-quantitatively determined by Dimer-test® (AGEN Inc. USA).

Fibrinogen and plasminogen concentration was respectively determined with each testing kit (Kuk Je Co. Japan). Antitrombin III was measured by testing kit (Stachrom AT III, Diagnostica Stago, France).

3) Statistical analysis

Mean values of all measured parameters in the three groups were compared by ANOVA (analysis of variance). When significant values ($p \leq 0.05$) were obtained by ANOVA, multiple post-hoc comparisons were done by Tukey tests. A statistical significance for all tests were accepted for a p-value less than 0.05. Data are presented as mean plus or minus the standard error of the mean. Statistical analysis was performed with the SPSS/PC program.

RESULTS

As showed in figure 1, each platelet counts

Table 1. The characteristics of study population

Parameter	Group			Statistics
	I	II	III	
Population	n=5	n=5	n=5	*
Sex	female	female	female	*
Age	6 months	6 months	6 months	*
Weight (g)	273.0±10.95	264.4±8.32	265.0±12.25	*

Data are expressed as the mean±standard error (SE), Legend: *, p>0.05, not significant among three groups

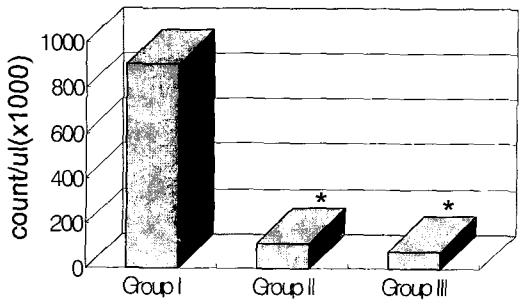

Fig. 1. Platelet counts in each group: platelet count in group II and III was significantly low as compared with that in group I (*: $p<0.05$) and there was also significant difference between group II and III.

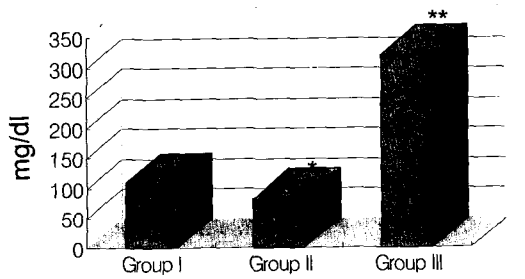

Fig. 2. Fibrinogen concentration in each group: fibrinogen concentration in group II was low, while that in group III was high as compared with that in group I (*, $p<0.05$; **, $p<0.01$).

in group II (112,360±476/μl) and III (78,430±520/μl) were significantly lower than that in group I (907,560±645/μl, $p<0.05$) and there was also significant difference between group II and III. Fibrinogen concentration in group II (82.00±19.14mg/dL) was lower than that in group I (108.00±11.40mg/dL, $p<0.05$), whereas in group III it (319.60±49.12mg/dL) was significantly high as compared with that in group I (Fig. 2, $p<0.05$). Level of plasminogen was not different between group I (1.10±0.18mg/dL) and II (1.34±0.43mg/dL) but lower in group III (0.70±0.09mg/dL, $p<0.05$, Fig. 3). In both group II (83.60±7.00%) and III (92.00±10.97%) level of antithrombin III was respectively lower than that in group I (107.60±1.50%, $p<0.05$, Fig. 4). There was not present D-dimer in group I, whereas was present '++' (1.0~2.0μg/mL) of D-dimer in group II and

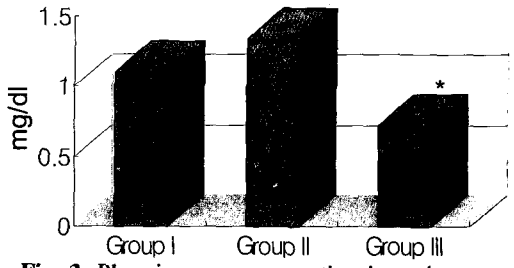

Fig. 3. Plasminogen concentration in each group: plasminogen in group III was significantly lower than that in group I (*, $p<0.05$), whereas there was no significance between group I and II.

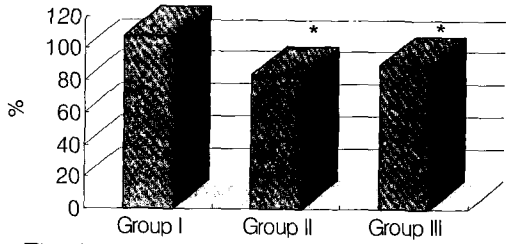

Fig. 4. Percent of antithrombin III in each group: antithrombin III level in group II and III was respectively lower than that in group I (*, $p<0.05$).

Table 2. *Individual level of D-dimer in each group*

Individual rat	Group		
	I	II	III
1	−	++	++
2	−	++	++
3	−	++	++
4	−	++	+
5	−	++	++

Legend: −, <0.25μg/mL (normal range).
+, 0.5~1.0μg/mL.
++, 1.0~2.0μg/mL.

III ($p<0.05$, Table 2). C3 levels in both group II and III were very low (10.00±00mg/dL, respectively) as compared with 674.20±20.15mg/dL of group I, whereas C4 level was not changed and not different among 3 groups (Fig. 5).

DISCUSSION

In an experimental DIC model of rats, we found severe thrombocytopenia with time de-

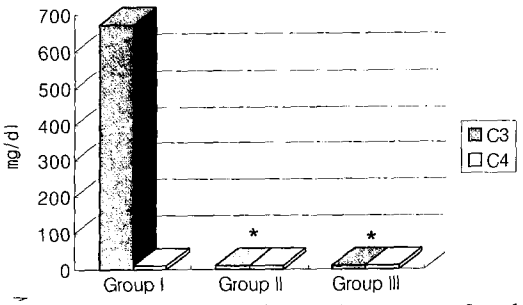

Fig. 5. C3 and C4 level in each group: profound decrease of C3 level group II and III was observed but not changed in C4, indicating complement activation via alternative pathway by endotoxin (*, $p<0.001$).

pendent-fashion. Endotoxin activates platelets[5] and induce platelet aggregation, resulting in profound thrombocytopenia. Endothelial injury, either by endotoxin directly or by TNF-α or any other mediator leads to an activation of the coagulation system and finally the development of disseminated intravascular coagulation (DIC). Endothelial injury leads to adhesion, aggregation and activation of platelets, to activation of the contact phase of clotting (factor XII-Hageman factor, prekallikrein and high molecular weight kininogen[6]) and to a release of tissue factor[4]. In this process, both the intrinsic and extrinsic pathways of coagulation are activated, leading ultimately to the generation of thrombin and subsequent conversion of fibrinogen to fibrin. In the present study, decreased antithrombin III level in group III is also considered as a result from generation of thrombin. Interestingly, fibrinogen concentration in group III was very higher than that in group I, reflecting an acute phase protein response by endotoxin[7].

Another effect of endotoxin may be the effect on the fibrinolytic system. In septic patients with DIC, low plasma levels of the fibrinolytic proteins and inhibitors and increased plasma levels of fibrinogen/fibrin degradation products (FDP) or D-dimer indicate extensive activation of the fibrinolytic system, which is traditionally believed to be secondary to the activation of coagulation[8].

In this study we also observed fibrinolytic activation with '++' level (1.0~2.0μg/mg) of D-dimer in group II and III. In addition to present of D-dimer, diminished plasminogen level in group III was found, suggesting a result from consumption by fibrinolytic activation. Nevertheless, we could not determined whether the increased levels of D-dimer in group II and III were due to primary activation of fibrinolysis to be independent on the activation of coagulation system or due to secondary activation of fibrinolysis following clot formation.

Potential answer on this question may include studies by Levi et al[9,10]. Levi and associates reported that the endotoxin-induced effects on fibrinolysis were unaffected in experiments in which the endotoxin-induced activation of coagulation was blocked by monoclonal antibodies that inhibit activation of extrinsic route of coagulation, which further indicated that the fibrinolytic response to endotoxin can be uncoupled from the activation of coagulation.

The response of fibrinolytic system to endotoxin may be mediated by TNF-α and appears to be in imbalance with the activation of coagulation. Further study, however, will be need to fully explain mechanisms of fibrinolytic activation by endotoxin.

Endotoxin has a direct activating effects on the complement system and both, the classical and alternative pathways are involved[11]. Fragment C3a and especially C5a injury endothelial cells through their effects on neutrophils[12]. Activated neutrophils by endotoxin release a serine elastase which directly acts on the endothelium[13]. C5a also enhances the generation of oxygen-derived free radicals via neutrophil activation[14] and furthermore induces the release of IL-1 from monocytes[15]. It also leads to mast cell degranulation with its release of histamine and subsequent vasodilation.

We support complement activation via alternative pathway as profound reduction of C3

concentration following endotoxin injection (group II and III) was observed in present study.

In above all data, we confirmed some signs of DIC by thrombocytopenia, reduced level of fibrinogen, plasminogen and antithrombin III, and elevated level of D-dimer following endotoxin injection. Endotoxin may induce the activation of several pathways such as coagulation, fibrinolytic, and complement cascade, causing DIC and probably subsequent multiple organ dysfunction. Ultimately, the increased knowledge of the various pathogenetic mechanisms of coagulation activation and fibrinolysis in endotoxin-induced DIC may have prophylactic or therapeutic implications.

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=국문초록=

쥐 모델에 있어 내독소에 의한 실험적인 범발성 혈관내 응고증

지산전문대학 임상병리과, *부산여자대학 생물학과, **부경대학교 미생물학과

최석철† · 김재영 · 고진복* · 이원재**

범발성 혈관내 응고증은 패혈증 환자들에 있어 빈번히 발생하며 여러 가지 위급한 질병 상태에 관계하는 병리학적 상황이다. 범발성 혈관내 응고증은 기존의 복잡한 임상 상황을 더욱 어렵게 만들어서 높은 사망률의 원인이 된다. 그럼에도 불구하고 그것의 병인적 기전들은 완전히 규명되지 않았다. 본 연구는 범발성 혈관내 응고증의 발생에 관여하는 병인적 기전들의 이해를 위해 전향적으로 계획되었다. 15마리의 쥐를 대상으로 해서 연구목적에 따라 세 군으로 나누었다: I 군은 대조군으로서 내독소를 투여하지 않은 쥐들이고 (n=5), II 군은 내독소 투여 후 12시간이 경과한 쥐들이며 (n=5), III 군은 내독소 투여 후 24시간이 경과한 쥐들이었다 (n=5). 실험적 범발성 혈관내 응고증은 일정량의 내독소를 한번에 투여하여 유도하였다 (1mg/kg, *E. coli* serotype 055:B5). 실험대상 쥐들의 심장으로부터 직접 채혈하여 혈소판수, 섬유소원 농도, plasminogen 농도, 항트롬빈 III 농도, D-dimer, 보체성분 (C3 및 C4)을 측정하였다. 내독소를 투여한 II 군과 III 군에 있어 혈소판수, 섬유소원 (III 군의 경우는 오히려 증가), plasminogen, 항트롬빈 III, 그리고 C3 등의 혈중 농도들이 대체로 감소하였고 D-dimer 농도는 증가함으로써 명백한 범발성 혈관내 응고증이 관찰되었다. 본 연구 결과들은 내독소에 의해 응고계, 섬유소용해계, 그리고 보체계와 같은 여러경로의 활성화가 유도될 수 있으며, 이로해서 범발성 혈관내 응고증 및 이차적인 중복 장기기능 부전이 발생하리라는 점을 시사하고 있다. 결국, 이와같은 실험적인 내독소 유도 범발성 혈관내 응고증에 있어 응고계 및 섬유소 용해계의 활성을 일으키는 다양한 기전에 관한 축적된 지식들은 그와같은 질병의 예방 혹은 치료방법을 제공해 줄 것이다.

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†별책요청 저자