[J Tokyo Wom Med Univ
70 (3) 80～91 (2000)]

Comparison of Nitric Oxide (NO) Production in the Tissue Following Hemorrhagic Invasion and Endotoxin Administration in Rats in Relation to the Pathogenesis of Traumatic DIC

Hiroyasu SUGA[1)3)], **Takao NAKAGAWA**[3)], **Norio MIYOSHI**[2)], **Yoshiaki IMAMURA**[2)] and **Masaru FUKUDA**[2)]

[1)]Department of Emergency Medicine (Director: Prof. Toshihiko KUBOTA) and
[2)]Department of Pathology (Director: Prof. Masaru FUKUDA), Fukui Medical University
[3)]Department of Emergency Medicine (Director: Prof. Tadashi SUZUKI),
Tokyo Women's Medical University, School of Medicine

(Received Nov. 12, 1999)

Previouly we confirmed the onset of DIC following hemorrhagic invasion in the rat, and at that time we noticed that nitric oxide (NO) production in the tissue increased rapidly and reached a peak in the early stage. This suggested that the increase in NO production would serve as an indicator for diagnosis of DIC. In the present study, we investigated the NO production in the tissue following hemorrhagic invasion in the rat, in comparsion with the rat following endotoxin administration.

Rats under pentobarbital anesthesia were used. After laparotomy, NO-selective electrode was placed in the kidney for real time recording of NO production in the tissue. The onset of DIC following both hemorrhagic invasion (bleeding of 30% of the predetermined total blood volume) and endotoxin administration (LPS, *E. coli*, 10 mg/kg iv) was confirmed from the changes in the coagulation-fibrinolytic system, cytokine levels in the blood and histopathological findings. NO production in the tissue following hemorrhagic invasion increased rapidly and reached a peak within 1 hr after bleeding. On the contrary, NO production following endotoxin administration was not observed entirely at an early stage, but it began to increase gradually 2 to 3 hr after the administration and reached a peak after 4 to 6 hr. The electron spin resonance (ESR) analysis was performed on the kidney tissues from rats underwent hemorrhagic invasion or endotoxin administrations using N-(dithiocarboxy) sarcosine, disodium salt, hydrate (DTCS Na) as a NO trapping agent. These tissues showed typical ESR signal of NO-Fe-DTCS. NO production following hemorrhagic invasion was not affected by the selective iNOS inhibitor, S-methylisothiourea (SMT, 5 mg/kg iv), and inhibited by the non-selective NOS inhibitor, N^G-monomethyl-L-arginine (L-NMMA, 50 mg/kg iv), which strongly suggested that it was due to cNOS. On the contrary, NO production following endotoxin administration was inhibited clearly by SMT and L-NMMA, which strongly suggested that it was mainly due to iNOS. Thus, the difference in the processes of NO production in the tissue following hemorrhagic invasion and endotoxin administration were confirmed, while their correlation to pathological manifestations of DIC in detail is remained to investigate further.

Introduction

Patients with a serious trauma frequently exhibit coagulation-fibrinolytic system abnormalities and occasionally develop disseminated intravascular coagulation (DIC). The pathological manifestations of DIC vary according to the primary diseases on the basis of very complicated pathophysiology[1]. Although early diagnosis and early treatment are desirable particularly for DIC following a trauma, the mechanism of development of DIC still includes many unknown pathogenic factors, such as the time when the pathological state of trauma changes to DIC.

To investigate the pathogenesis of DIC experimentally, rats were first subjected to hemorrhagic invasion, i.e., the main pathological state following trauma, and the onset of DIC was identified in terms of changes in the coagulation-fibrinolytic system, serum cytokine levels, and histopathological findings in those rats[2]. At that time, it was noticed that nitric oxide (NO) production in the tissue increased obviously in the early stage and the increase lasted for hours. This suggested that the increase in NO production could serve as an indicator for early diagnosis of DIC[2].

In the present study, we investigated the progress of NO production in the tissue following hemorrhagic invasion and analyzed the mechanism of NO production using NO synthetase (NOS) inhibitors in rats, comparing with rats following endotoxin administration, which has been used as an experimental animal model of DIC.

Materials and Methods

The control and treatment of experimental animals for this study was approved by the Institutional Committee of Fukui Medical University.

1. Effects of hemorrhagic invasion

Male Sprague-Dawley rats weighing from 350 to 450 g were purchased from Japan Claer (Jcl: SD Retire, Nihon Kurea Inc., Shiga, Japan). The animals were anesthetized with sodium pentobarbital (50 mg/kg ip). A polyethylene tube (SP 45, Natsume Co., Tokyo, Japan) was inserted in the carotid artery for bleeding and blood sampling. For hemorrhagic invasion, 30% of the predetermined total blood volume (equivalent to 8 % of the body weight) was bled through the carotid artery catheter over 15 min[3]. Blood was sampled at 4 to 6 hr and 24 hr following hemorrhagic invasion, and the blood coagulation-fibrinolytic system activity and cytokine levels were determined.

For assessment of the coagulation-fibrinolytic activity, the platelet count (electric resistance method), fibrin/fibrinogen-degradation product (FDP; FDP-E, MBL Co., Nagoya, Japan), plasma fibrinogen (cyanmethemoglobin method) and antithrombin Ⅲ (ATⅢ; spectrophotometory with coupler, SATIII, Daiichi-Kagaku Yakuhin, Tokyo, Japan) were determined. As for cytokines, interleukin-8 (IL-8; EIA method PRF081, Panapharm Laboratories Co., Kumamoto, Japan) and tumor necrosis factor (TNF; rat TNF-αELISA kit, Biosource International Inc. Camarillo, Cal., USA) levels were determined.

After the end of the experiment, the kidneys, lung, liver and intestines were isolated, fixed in 10 % formalin solution and stored. Thin tissue sections were stained by hematoxylin-eosin (HE) and by phosphotungstic acid hematoxylin (PTAH) for fibrin and then examined histopathologically.

2. Effects of endotoxin administration

Rats administered with endotoxin (LPS, *E. coli*, 10 mg/kg iv; DIFCO Laboratories, Detroit, Michi., USA) have been frequently used as an experimental animal model for DIC[4], and were used as the controls for the hemorrhagic invasion animals in the present experiment. Blood sampling was carried out at time intervals, and the coagulation-fibrinolytic system activity and cytokines levels were measured, as done for the hemorrhagic invasion group. Isolation of the organs and histopa-

thological examination after the experiment were also conducted in a similar manner.

3. Comparison of NO production in the kidney tissue and the mechanism of NO production following hemorrhagic invasion and endotoxin administration

1) Measurement with an NO-selective electrode (ϕ 200 μm, Intermedical Inc., Nagoya, Japan)[5]

Under anesthesia with pentobarbital, the rats underwent a laparotomy and polyethylene tube was inserted in the carotid artery. An NO-selective electrode was introduced into the outer capsule of the kidney and fixed. The NO electrode was connected to an NO measuring instrument (NO-501, Intermedical Inc., Nagoya, Japan)[5] and real-time measurement of NO production was conducted.

The chronological changes in NO production over a period of 4 to 6 hr after the hemorrhagic invasion were continuously measured, using the records prior to hemorrhagic invasion and endotoxin administration as the controls.

2) Examination using electron spin resonance (ESR)

After the sampling of blood at 4 to 6 hr following the hemorrhagic invasion or endotoxin administration, the kidney was isolated and immediately stored in a refrigerator at −70℃. The samples (0.25 g) were homogenized in 500 μl of 14 mM $FeSO_4$, 40 mM N-(dithiocarboxy) sarcosine (DTCS, DOJIN Chem. Inst. Co., Kumamoto, Japan) for the subsequent ESR analysis. ESR was measured within 5 min. ESR spectra of the spin-trapped radicals were recorded on a X-band spectrometer (RE3XR model, JEOL Co. Ltd., Tokyo, Japan) at a modulation amplitude; 0.079 mT, time constant; 0.01 sec, scan speed; 0.083 mT/s[5].

3) Analysis of the mechanism of NO production with NOS inhibitors

There are two NOS isoforms: constitutive NOS (cNOS), which exists in the endothelial cells and nerve tissues and is Ca^{2+}-dependent[7] and inducible NOS (iNOS) which is induced in the macrophages, neutrophils, endothelial cells, liver, lungs, etc by proinflammatory cytokines and endotoxin and is Ca^{2+}-independent[7]. The mechanism of NO production in the rat tissue following hemorrhagic invasion and endotoxin administration was investigated using a selective iNOS inhibitor S-methylisothiourea (SMT) and a non-selective NOS inhibitor N^G-monomethyl-L-arginine (L-NMMA).

4. Statistical analysis

All measurement values are expressed as mean ± SEM (standard error of the mean). For testing of a significance of differences between the groups, analysis of variance (ANOVA) was first carried out using the F-test. After the uniformity was confirmed, the difference in the mean value was tested using Student's t-test with no correspondence. $p<0.05$ was considered to indicate a significant difference.

Results

1. Onset of DIC in the rats following hemorrhagic invasion and endotoxin administration

The changes in the coagulation-fibrinolytic system activity and blood cytokine concentrations following hemorrhagic invasion are shown in Tables 1 and 2. Significantly decreased platelet count, significantly increased FDP, and significantly increased IL-8 and TNF levels strongly suggested the onset of DIC[8]. Histopathological examinations at 4 to 6 hr after treatment revealed fibrin thrombus in the kidney and lung tissues, tubular necrosis in the kidneys, intra-alveolar hemorrhage, and hemorrhage in the hepatic parenchymal cells (Fig. 1). The onset of DIC was identified based on these findings[1)9)].

Significantly decreased platelet count, significantly increased FDP, and significantly increased IL-8 and TNF levels were also noted in the endo-

Table 1 Changes in the platelet count and coagulation-fibrinolytic system

Coagulofibrinolytic system	Control	Hemorrhage			Endotoxin	
		4 ~ 6h	24h	n	4 ~ 6h	n
Plt ($10^3/\mu$l)	90.30 ± 1.55	70.22 ± 2.34 **	71.21 ± 4.47 *	21	21.16 ± 5.99 **	14
FDP (μg/ml)	< 5	> 40 **	> 40 **	21	> 40 **	11
Fibr (mg/dl)	134.89 ± 4.09	182.00 ± 19.44 *	215.33 ± 29.88 *	16	38.81 ± 12.89 *	11
AT Ⅲ (mg/dl)	103.68 ± 3.04	111.87 ± 8.59	134.44 ± 11.51	16	62.09 ± 4.56 **	11

Changes in the platelet count and coagulation-fibrinolytic system after hemorrhagic invasion and endotoxin administration in the rat. Plt : platelet, FDP : fibrin/fibrinogen degradation products, Fibr : fibrinogen, AT III : antithrombin III, n : number of experimental animals. The values are expressed as the mean ± SEM, * $p < 0.05$, ** $p < 0.01$, significantly different from the control.

Table 2 Changes in the blood cytokine levels

Cytokine	Control	Hemorrhage			Endotoxin	
		4 ~ 6h	24h	n	4 ~ 6h	n
IL8 (ng/ml)	0.22 ± 0.88	0.78 ± 0.84 *	0.09 ± 0.01 **	8	1.52 ± 0.69 *	8
TNF (pg/ml)	< 16.0	21.14 ± 3.38 *	20.14 ± 3.03 *	8	536.56 ± 5.64 **	11

Changes in the blood levels of interleukin-8 (IL-8) and tumor necrosis factor (TNF), after hemorrhagic invasion and endotoxin administration in the rat. n : number of experimental animals. The values are expressed as the mean ± SEM, * $p < 0.05$, ** $p < 0.01$, significantly different from the control.

toxin administration group, which strongly suggested the onset of DIC (Tables 1 and 2). Furthermore, histopathological examinations at 4 to 6 hr after treatment also revealed fibrin thrombus in kidney and lung tissues, tubular necrosis in the kidneys, intra-alveolar hemorrhage, and hemorrhage in the hepatic parenchymal cells (Fig. 2). These findings together with the above-mentioned findings demonstrated the onset of DIC.

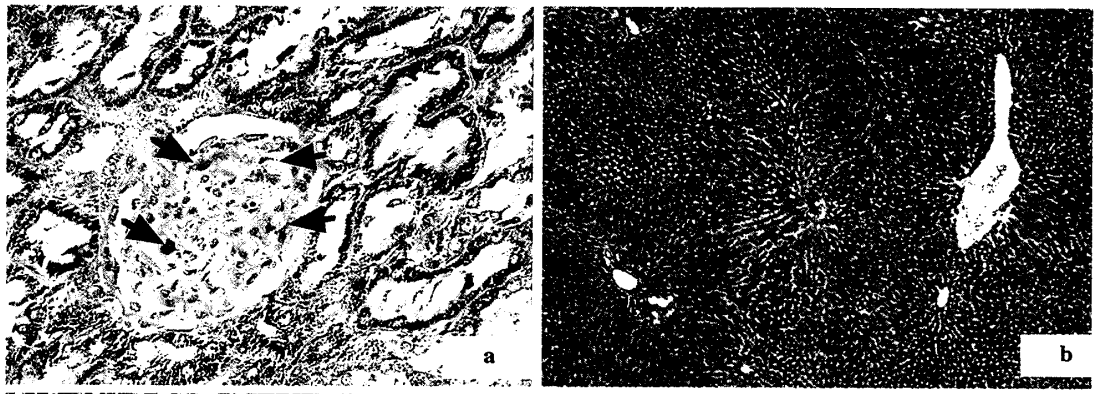
2. NO production in the kidney tissue

1) Measurement with an NO-selective electrode

Figure 3 shows the chronological changes in NO production with an NO-selective electrode placed in the renal tissue in the hemorrhagic invasion and endotoxin administration groups. NO production following hemorrhagic invasion increased early, reaching a peak within 1 hr after bleeding, and this increased state was maintained. On the contrary, no NO production was observed at the early stage following endotoxin administration, but NO production increased slowly at approximately 2 to 3 hr after treatment and attained a peak at 4 to 6 hr, and this state was maintained (Fig. 3). When the NO spin trapping agent DTCS (40 mM 0.3 ml/animal ip) was administered at the peak of NO production, NO production decreased quickly in both the hemorrhagic invasion and endotoxin administration groups, thus, NO production being identified (Fig. 3).

2) Examination of ESR signals

The renal tissue isolated at 4 to 6 hr after the hemorrhagic invasion or endotoxin administration was homogenized with 70 mM $FeSO_4$ solution, and the supernatant was examined for ESR signal using the NO radical spin trapping agent DTCS. An ESR signal (three peaks) of NO-Fe-DTCS radical complex ($g = 2.040$; $aN = 1.27$ mT) was detected in the sample solution of the kidney tissues in both the hemorrhagic invasion and endotoxin administration groups (Fig. 4). In the control without hemorrhagic invasion and endotoxin administration, the ESR signal of NO-Fe-DTCS



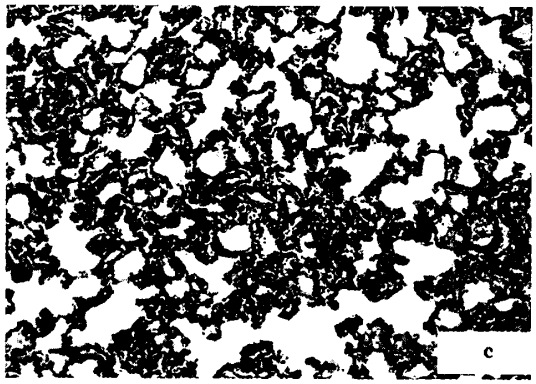
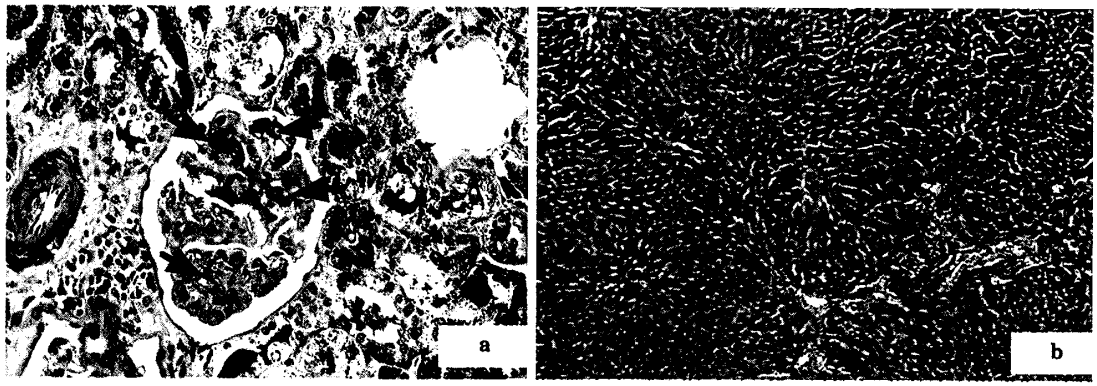


Fig. 1



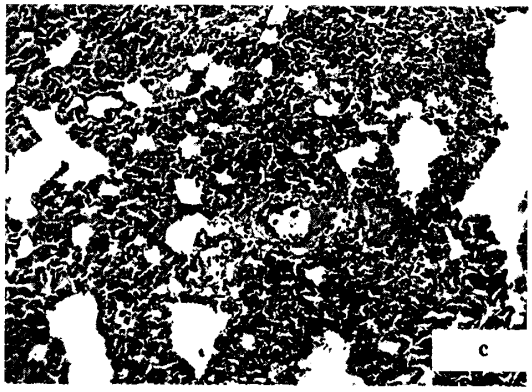


Fig. 2

Fig. 1 The histopathological findings at 6 hr after hemorrhage
a: kidney (PTAH stain ×80), b: liver (HE stain ×20), c: lung (HE stain ×40). Arrows indicate fibrin thrombi.

Fig. 2 The histopathological findings at 6 hr after endotoxin administration
a: kidney (PTAH stain ×80), b: liver (HE stain ×20), c: lung (HE stain ×40). Arrows indicate fibrin thrombi.

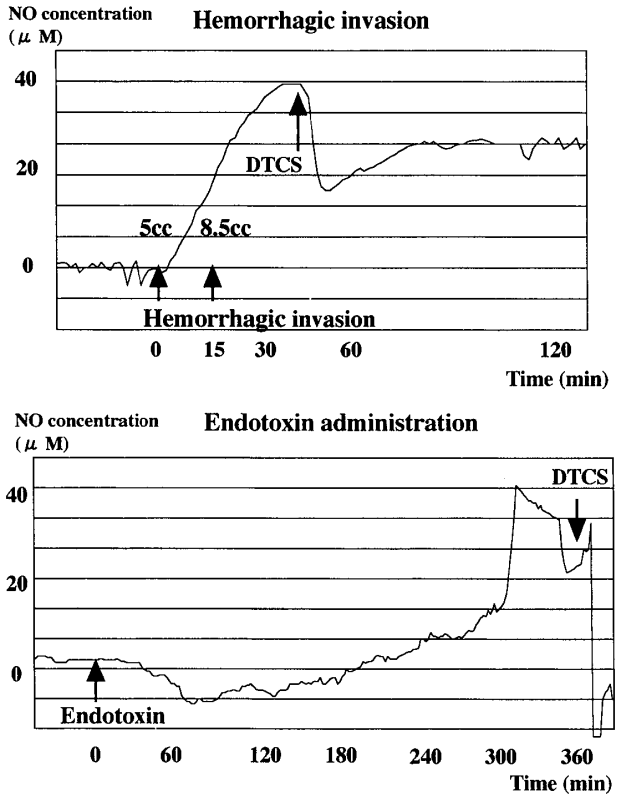


Fig. 3 Measurement of NO production in the renal tissue with NO-selective electrode
A difference in time course of NO production between hemorrhagic invasion and endotoxin administration was observed. The increased NO production recordings exhibited a drop promptly after administration of NO trapping agent DTCS (a typical recording).

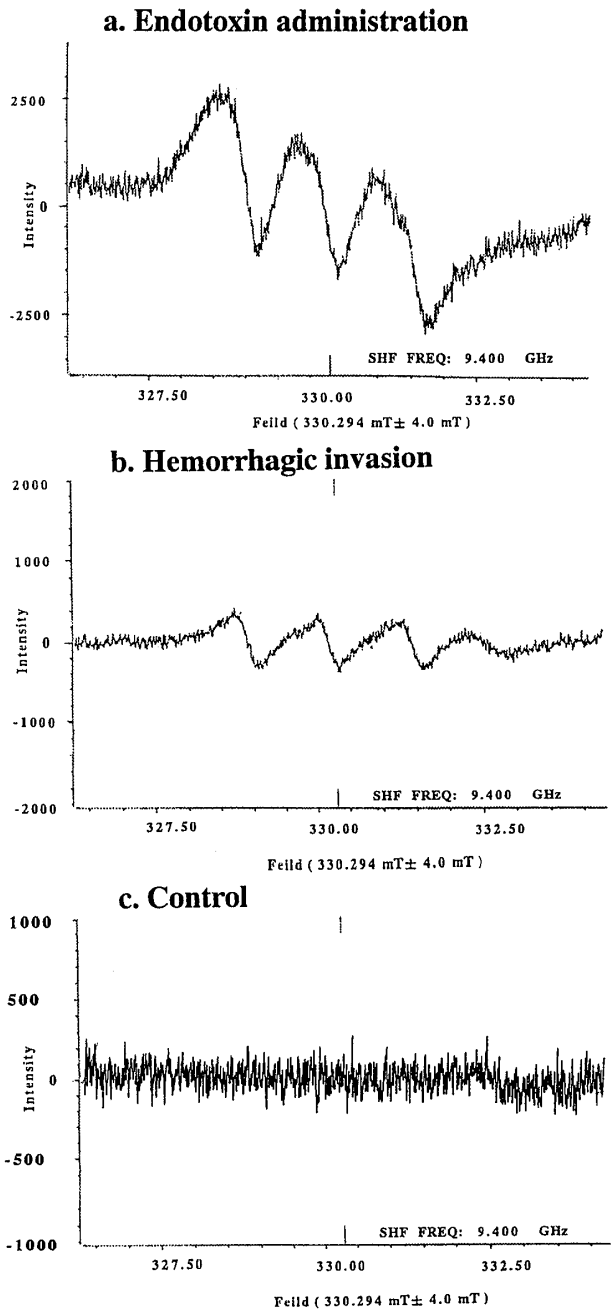


Fig. 4 ESR signal in the renal tissue
An ESR signal (three peaks) of NO-Fe-DTCS radical complex was demonstrated in the sample solutions of renal tissues after endotoxin administration and hemorrhagic invasion (a and b). In the control without endotoxin administration and hemorrhagic invasion, the ESR signal was not detected (c). Moduration width: 0.79 × 0.1 mT, Modulation frequency: 100 kHz, Time constant: 0.01 sec.

was not detected (Fig. 4).

3) Investigation of the mechanism of NO production using NOS inhibitors

We investigated the effects of selective iNOS inhibitor, SMT (5 mg/kg iv), and non-selective NOS inhibitor for iNOS and cNOS, L-NMMA (50 mg/kg iv), on NO production in the tissue. Application of SMT at the time of increased NO level with the NO-selective electrode in the hemorrhagic invasion group had little effect on NO production. Application of L-NMMA, however, led to a clear decline in NO level (decrease rate = decrease in NO level induced by NOS inhibitor /NO level before NOS inhibitor application, 28.87 ± 6.85%, n = 6, Fig. 5). NO level decreased following application of SMT in the endotoxin administration group (decreas rate = 18.80 ± 5.66%, n = 4) with further decrease following application of

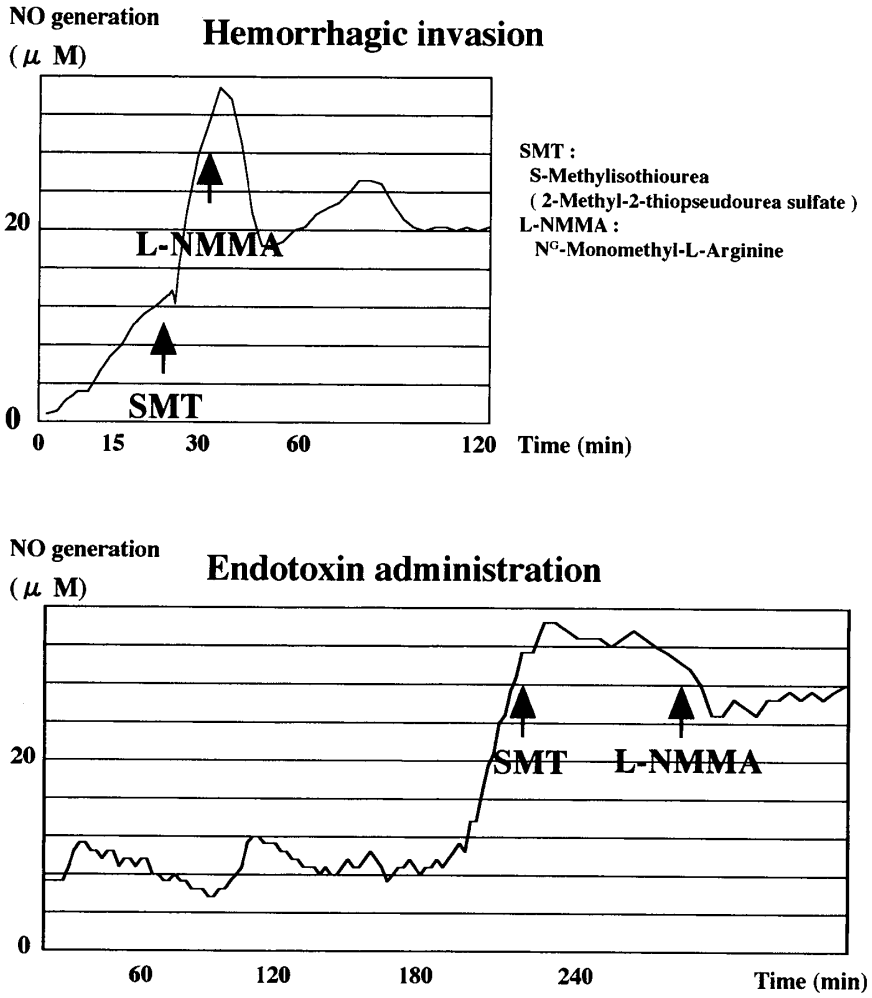


Fig. 5 Effects of application of NOS inhibitors on NO production in the renal tissue
The early increase of NO production after hemorrhagic invasion and the late increase after endotoxin administration are thought to be due to cNOS and iNOS, respectively.
SMT: S-methylisothiourea, L-NMMA: N^G-monomethyl-L-arginine

L-NMMA (decrease rate = 11.39 ± 2.21%, n = 4, Fig. 5).

Discussion

1. Onset of DIC and NO production in the tissue under various invasions

It was possible to identify the onset of DIC from the findings, such as the increased coagulation-fibrinolytic system activity[8] and the resultant histopathological findings[9] in the hemorrhagic invasion and endotoxin administration rat groups. Increased inflammatory cytokine levels were also identified. The cytokines released from the monocytes activated by various invasions act on the endothelial cells to release tissue factor and induce activation of the exogenous coagulation system, and moreover, inhibit the protein C-protein S coagulation inhibition system via down regulation of thrombomodulin production as well as production and release of plasminogen activator inhibitor type 1, leading to amplification of coagulation[10)~13)]. The cytokines further activate the neutrophils and macrophages to induce the release of esterase and free radicals including active oxygen, NO, etc, and these result in tissue injuries and affect the coagulation-fibrinolytic system[11)~16)]. In the present experiment, the time course of NO production was recorded with an NO-selective electrode placed in the tissue.

2. NO production under invasions

NO production in the tissue was observed in both the hemorrhagic invasion and endotoxin administration groups, but there was a marked difference in the chronological change of NO production between the two groups. Records with an NO-selective electrode placed in the kidney tissue showed that NO production increased early following hemorrhagic invasion, reaching a peak at 30 to 60 min after invasion with a gradual decrease thereafter, but this increased NO production was maintained until a few hours later. For the endotoxin administration group, however, NO production showed no increase at the early stage, with a tendency to increase slowly at 2 to 3 hr after treatment. This tendency to increase gradually continued until NO production reached a peak at 4 to 6 hr, showing a great increase. Application of NO radical spin trapping agent DTCS at the peak in the respective groups led to a rapid decline in NO level, and consequently, NO production was ascertained. In ESR on the renal tissue using DTCS as well, ESR signal typical of NO was detected (Fig. 4). We attributed the aforementioned difference in the chronological changes of NO production between the hemorrhagic invasion and endotoxin administration groups to the different mechanisms of NO production. NO production was investigated using NOS inhibitors.

1) NO production following hemorrhagic invasion

The increased NO production early following hemorrhagic invasion was little affected by the selective iNOS inhibitor SMT but was clearly inhibited by L-NMMA, an inhibitor having almost equal affinities for iNOS and cNOS (Fig. 5). Consequently it was strongly suggested that this early NO production was attributed mainly to activation of cNOS, while iNOS does not participate in it.

It has been demonstrated that iNOS was induced by cytokines released at the invasion, and that peripheral circulatory failure was caused by this NO production because this peripheral circulatory failure was improved following administration of cytokine antagonists[17)~19)]. There have been some reports on NO production observed in the early stages of hemorrhagic invasion as well[20)21)]. Early NO production was attributed to cNOS for the following reasons: a) complete recovery from vascular hyporeactivity to catecholamines at the early stages of hemorrhagic shock was achieved following administration of non-selective NOS inhibitor L-NMMA, but the vascular hyporeactivity was not affected by dexamethasone which inhibits iNOS induction[20)], b) no iNOS can be demonstrated in various organs, including the lung, liver, aorta, etc, at 60 min after the hemorrahage, a time when near-maximal vascular hyporeactivity is already present[21)].

The mechanism of early NO production was assumed to be as follows: cathecholamines, vasopressine, angiotensin II, platlet agglutinating factor (PAF) released into the blood at an early stage of hemorrhagic shock were transferred by blood and activated cNOS via intimal receptors to produce NO[21)]. Another opinion proposed that ischemia induced in the local microcirculation by some invasions provoke Ca^{2+} influx in the cell membrane, thus increasing the intracellular Ca^{2+}, activate cNOS in the endothelial cells, and produce NO[22)].

In any case, however, we consider this report to be the first to demonstrate that early NO production in the tissue following hemorrhagic invasion was attributed to cNOS by the real-time recording of the progress of NO production in the tissue.

2) NO production following endotoxin administration

NO production was not observed at the early

stage following endotoxin administration in the present experiment. NO production increased gradually 2 to 3 hr after treatment and markedly at 4 to 6 hr, reaching a peak, and tended to decrease gradually thereafter. Application of SMT at this peak led to a clear reduction in NO level; and NO level then further decreased following application of L-NMMA (Fig. 5). These findings suggested that NO production was ascribable to iNOS.

Induction of iNOS following endotoxin administration has been reported to take several hours after administration[23], but there have been a few reports on NO production by cNOS activation early after administration. That is, it has been reported that administration of endotoxin to rats under anesthesia led to hypotension at 5 to 20 min after treatment, which was prevented or reversed by NOS inhibitors[24)25]. Dexamethasone, which blocks iNOS induction by endotoxin in vitro and in vivo, did not change the vascular hyporeactivity to norepinephrine early (at 60 min) after endotoxin administration[26]. Since recovery from this vascular hyporeactivity early after endotoxin administration was achieved with L-NMMA application, the vascular hyporeactivity has been reported to be due to NO production resulting from cNOS activation[27)28)]. Regarding these findings, a theory that several mediators released by endotoxin were involved in the mechanism of early cNOS activation has been proposed. That is, it has been assumed that endotoxin released bradykinin[29], PAF[30], catecholamines, 5-hydroxytryptamine, angiotensin II, and histamine[31], which stimulated the endothelium to release NO[21].

On the other hand, it has been described that early hypotension following administration of endotoxin to rats was not inhibited by NOS inhibitor but was attributed to a mechanism other than NO production[32]. Another argument went that PAF released by endotoxin activated cNOS in the endothelial cells to produce NO[33]. However, inhibition of PAF increase by dexamethasone was inconsistent with that cNOS activation was not inhibited by dexamethasone[34]. Moreover, depression of the vascular reactivity induced by endotoxin was not prevented by a PAF antagonist[35]. Arachidonic acid metabolites such as prostacycline is released by endotoxin and induces depression of the vascular reactivity, and the vascular reactivity induced by endotoxin was prevented by an inhibitor of cycloxygenese or arachidonic acid metabolite synthetase. These findings suggested the involvement of arachidonic acid metabolites as well[36].

NO production induced by iNOS at the late stages following endotoxin administration has been established. Concerning early NO production resulting from cNOS activation, however, there have been some negative views as mentioned above, and the differences in the experimental conditions had something to do with these discrepancies.

It was shown in the present study that there was no early NO production following endotoxin administration and that NO production in the late stage was mainly due to induction of iNOS based on the real time NO recording obtained through the NO-selective electrode set in the kidney tissue.

Thus, the differences in the processes of NO production in the tissue following hemorrhagic invasion and endotoxin administration were confirmed. Pathological manifestations of DIC vary according to the primary diseases. The correlation of these differences in the NO production following invasions with pathological manifestations of DIC in detail is remained to investigate further.

Conclusions

1. Assessment of changes in the coagulation-fibrinolytic system activity and blood cytokine

levels and histopathological findings following hemorrhagic invasion in rats made it possible to identify the onset of DIC. NO production in the kidney tissue following hemorrhagic invasion was examined with the NO-selective electrode, in comparison with the endotoxin administration rat group.

2. NO production in the tissue following hemorrhagic invasion increased early and attained a peak in the early stage. Following endotoxin administration, however, NO production showed no increase at the early stages, and gradually increased at 2 to 3 hr, reaching a peak at 4 to 6 hr. The NO production following both invasions was confirmed by ESR investigation of kidney tissue using trapping agent DTCS.

3. Early NO production following hemorrhagic invasion was not affected by the selective iNOS inhibitor, SMT, but was inhibited by the non-selective NOS inhibitor, L-NMMA. This strongly suggested that mainly cNOS induced the increased NO production. As for the endotoxin administration group, however, NO production was clearly inhibited by SMT and by L-NMMA. It was also strongly suggested that the increased NO production was induced mainly by iNOS.

4. Thus, the differences in the processes of NO production in the tissue following hemorrhagic invasion and endotoxin administration were confirmed. However, their correlation with pathological manifestations of DIC in detail is remained to investigate further.

The auther thanks Prof. Tadashi Suzuki for his thonghtful review of this manuscript.

This study was partly supported by Grant-in-Aid from the Ministry of Education of Japan (Monbu-sho), for Scientific Research (Research Project (C) (2) 10671108). An abstract of this paper was presented at the Forum Session of the 26th General Meeting of the Emergency Medical Society of Japan (1998, November, Matsuyama).

References

1) **Bick RL**: Disseminated intravascular coagulation: pathophysiological mechanisms and manifestations. Semin Thromb Hemost **24**: 3–18, 1998
2) **Suga H, Nakagawa T, Yokoyama T et al**: Experimental study on the pathogenesis of traumatic DIC; changes in cytokine levels in blood and coagulofibrinolytic system in the hemorrhage. Procceding of 4th International Congress on the Immune Consequences of Trauma. Shock and Sepsis: 143–147, 1997
3) **Abraham E, Richmond NJ, Change YH**: Effects of hemorrhage on interleukin-1 production. Circ Shock **25**: 33–40, 1988
4) **Schoendort TH, Rosenberg M, Beller FK**: Endotoxin-induced disseminated intravascular coagulation in nonpregnant rats: A new experimental model. Am J Pathol **65**: 51–58, 1971
5) **Ichimori K, Ishida H, Fukahori M et al**: Practical nitric oxide measurement employing a nitric oxide-selective electrode. Rev Sci Instrum **65**: 2714–2718, 1994
6) **Furusawa N, Baba H, Miyoshi N et al**: Herniation of cervial intervertebral disc: Immunohistochemical examination and measurement of nitric oxide production. Spine: in press, 1999
7) **Moncada S**: The L-arginine-nitric oxide pathway. Acta Physiol Scand **145**: 201–227, 1992
8) **Matsuda T, Asakura E**: Problems of DIC diagnostic standards: Significance of fibrinogen and prothrombin time. Ministry of Health and Welfare Special Research, *In* Study Report by Blood Coagulation Disorder Task Force, pp 24–30, Tokyo (1992) (in Japanese)
9) **Iseki K, Takeuchi M**: Pathology of DIC. Clin Stud Jpn **51**: 30–36, 1993 (in Japanese)
10) **Wada H, Ohiwa M, Kaneko T et al**: Plasma level of tumor necrosis factor in disseminated intravasucular coagulation. Am J Hemol **37**: 145–151, 1991
11) **Wada H, Tanaka S, Tamaki M et al**: Plasma level of IL-1 β in disseminated intravascular coagulation. Thromb Haemost **65**: 364–368, 1991
12) **Wada H, Tanigawa M, Wakita Y et al**: Increased plasma level of interleukin-6 in disseminated intravascular coagulation. Blood Coagul Fibrinolysis **4**: 583–590, 1993
13) **Levi M, Cate H, van der Poll T et al**: Pathogenesis of disseminated intravascular coagulation in sepsis. JAMA **270**: 975–979, 1993

14) **Okajima K, Yang WP, Okabe H et al**: Role of leukocytes in the activation of intravascular coagulation in patients with septicemia. Am J Hematol **36**: 265–271, 1991
15) **Abe H, Okajima K, Okabe H et al**: Granulocyte proteases and hydrogen peroxide synergistically inactivate thrombomodulin of endothelial cells in vitro. J Lab Clin Med **123**: 874–881, 1994
16) **Fukuyama N, Nakazawa H**: Diversified mode of action of NO. J Clin Exp Med (Igaku no Ayumi) **172**: 529–534, 1995 (in Japanese)
17) **Perbeck L, Hedquist P**: Blood pressure responses associated with hemorrhagic shock in anesthetized rats. Acta Chir Scand **148**: 3–8, 1982
18) **Ertel W, Morrison MH, Ayala A et al**: Chloroquine attenuates hemorrhagic shock induced suppression of Kupffar cell antigen presentation and major histocompatibility class II antigen expression through blockade of tumor necrosis factar and prostaglandin release. Blood **78**: 1781–1788, 1991
19) **Pellicane JV, De Meria EJ, Abd-Elfattah A et al**: Interleukin-1 receptor antagonist improves survival and preserves organ adenesine-5'-triphosphate after hemorrhagic shock. Surgery **114**: 278–284, 1993
20) **Thiemermann C, Szabo S, Mitchell JA et al**: Vascular hyporeactivity to vasoconstrictor agents and hemodynamic decompensation in hemorrhagic shock is mediated by nitric oxide. Proc Natl Acad Sci USA **90**: 267–271, 1993
21) **Szabo C**: Alterations in nitric oxide production in various forms of circulatory shock. New Horiz **3**: 2–32, 1995
22) **Tansei K, Kurosu Y**: Biological defense mechanism and multiple organ failure. J Nihon Univ Sch Med **50**: 457–463, 1994 (in Japanese)
23) **Salter M, Knowles RG, Moncado S**: Widespread tissue distribution, species distribution and changes in activity of Ca^{2+}-dependent and Ca^{2+}-independent nitric oxide synthesis. FEBS Lett **291**: 145–149, 1991
24) **Thiemermann C, Vane J**: Inhibition of nitric oxide synthesis reduces the hypotension induced by bacterial lipopolysaccharides in the rat in vivo. Eur J Pharmacol **182**: 591–595, 1990
25) **Kilbourn RG, Jubran A, Gross SS et al**: Reversal of endotoxin-mediated shock by N^G-methyl-L-arginine, an inhibitor of nitric oxide synthesis. Biochem Biophys Res Commun **172**: 1132–1138, 1990
26) **Radomski MW, Palmer RMJ, Moncada S**: Glucocorticoids inhibit the expression of an inducible, but not the constitutive nitric oxide synthase in vascular endothelial cells. Proc Natl Acad Sci USA **87**: 10043–10047, 1990
27) **Szabo C, Mitchell JA, Thiemermann C et al**: Nitric oxide-mediated hyporeactivity to noradrenaline precedes the induction of nitric oxide synthase in endotoxin shock. Br J Pharmacol **108**: 786–792, 1993
28) **Paya D, Gray GA, Fleming I et al**: Effect of dexamethasone on the onset and persistence of vascular hyporeactivity induced by E. coli lipopolysaccharide in rat. Circ Shock **41**: 103–112, 1993
29) **Fleming I, Dambacher T, Busse R**: Endothelium-derived kinins account for the immediate response of endothelial cells to bacterial lipopolysaccharide. J Cardiovasc Pharmacol **20** (Suppl 12) : S135–S138, 1992
30) **Doebber TW, Wu MS, Robbins JC et al**: Platelet activating factor (PAF) involvement in endotoxin-induced hypotension in rat. Studies with PAF-receptor antagonist kadsurenone. Biochem Biophys Res Commun **127**: 799–808, 1985
31) **Parrot JR**: Neurohumoral agents and their release in shock. *In* Handbook of Shock and Trauma (Altura BM ed), pp 311–336, Raven Press, New York (1983)
32) **Wright CE, Rees DD, Moncada S**: Protective and pathological roles of nitric oxide in endotoxin shock. Cardiovasc Res **26**: 48–57, 1992
33) **Moritoki H, Hisayama T, Takeuchi S et al**: Involvement of nitric oxide pathway in the PAF-induced relaxation of rat thoracic aorta. Br J Pharmacol **107**: 196–201, 1992
34) **Braquet P, Touqui L, Shen TY et al**: Perspectives in platelet-activating factor reseach. Pharmacol Rev **39**: 97–145, 1987
35) **Gray GA, Furman BL, Parratt JR**: Platelet activating factor and endotoxin induced depression of vascular reactivity. Br J Pharmacol **97** (Suppl) : 526, 1989
36) **Gray GA, Furman BL, Parratt IR**: Endotoxin-induced impairment of vascular reactivity in pithed rat: role of arachidonic acid metabolites. Circ Shock **31**: 395–406, 1990

外傷性DICの病態発生との関連におけるラットの出血性侵襲およびエンドトキシン適用後の組織NO産生の比較

[1]福井医科大学　救急部（主任：久保田紀彦教授）
[2]福井医科大学　第一病理（主任：福田　優教授）
[3]東京女子医科大学　医学部　救急医学（主任：鈴木　忠教授）

須賀（スガ）　弘泰（ヒロヤス）[1)3)]・中川（ナカガワ）　隆雄（タカオ）[3)]・三好（ミヨシ）　憲雄（ノリオ）[2)]・今村（イマムラ）　好彰（ヨシアキ）[2)]・福田（フクダ）　優（マサル）[2)]

先に，ラットにおいて出血性侵襲後のDICの発症を確認し，その際組織一酸化窒素（NO）産生の早期の増加を認めた．これは，NO産生の増加はDICの早期診断の指標としての可能性を示唆した．今回の研究では，ラットにおける出血性侵襲後の組織NO産生を，エンドトキシン適用後のラットと比較しながら検討した．Pentobarbital麻酔下のラットを用いた．開腹の後，腎にNO選択性電極を設置して組織NO産生をリアルタイムで記録した．ラットにおいて出血性侵襲（推定全血量の30%量の放血）およびエンドトキシン投与（LPS，*E. coli*，10 mg/kg iv）により，凝固線溶系，血中サイトカインレベルおよび組織病理学的所見から，DICの発症を確認した．出血性侵襲後のNO産生は，早期（侵襲後1 hr以内）に速やかに増加してピークに達した．これに対し，エンドトキシン投与後のNO産生は，早期には全く認められず，2～3 hr後より緩徐な増加が認められるようになり，4～6 hr後にピークに達した．出血性侵襲およびエンドトキシン投与後のN-(dithiocarboxy)sarcosine, disodium salt, dihydrate（DTCS Na）を用いた電子スピン共鳴スペクトラム分析（ESR）によりNO-Fe-DTCSのシグナルを検出した．出血性侵襲による早期のNO産生は選択的iNOS阻害剤S-methylisothiourea（SMT，5 mg/kg iv）では殆ど影響をうけず，非選択的NOS阻害剤N^G-monomethyl-L-arginine（L-NMMA，50 mg/kg iv）により抑制され，cNOSの活性化によるものであり，iNOSの関与のないことが強く示唆された．これに対し，エンドトキシン投与による後期のNO産生はSMTおよびL-NMMAの添加により抑制され，主としてiNOSの誘導によることが強く示唆された．かくして，出血性侵襲およびエンドトキシン適用後の組織NO産生の過程の差異が確認された．これらとDICの病態との詳細な関連性は今後の検討に残されている．