A Selective Thromboxane Synthetase Inhibitor, OKY-046, Fails to Improve Blood Rheology in Endotoxin-shocked Rabbits

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Effects of a selective thromboxane synthetase inhibitor, (E)-3-[4-(1-imidazolylmethyl)phenyl]-2-propenoic acid hydrochloride monohydrate (OKY-046), were studied hemorheologically in endotoxin shocked-rabbits. The animals were intravenously administrated with 0.1 mg of endotoxin 3 times at intervals of 3 days. At 7 days after the last endotoxin injection, endotoxin (0.2 $mg \cdot kg^{-1}$) was intravenously administrated to induce a shock. OKY-046 (30 $mg \cdot kg^{-1}$) was administrated after hypotension was developed by the endotoxin treatment and, then, it was continuously injected at 0.03 $mg \cdot kg^{-1} \cdot min^{-1}$. Blood pressure remained unchanged and hypotensive was maintained during the treatment with OKY-046. Blood was sampled from the femoral artery 15 (before the administration of OKY-046), 45, and 120 minutes after the final administration of endotoxin. Pa_{O_2} increased, and Pa_{CO_2}, arterial pH, and base excess (BE) decreased during the endotoxin shock. The decrease of pH and BE was prevented by the administration of OKY-046. In the endotoxin-shocked animals, hematocrit, whole blood viscosity, erythrocyte deformability, plasma fluidity, and the ratio of hematocrit to whole blood viscosity showed no significant differences between the OKY-046 treated animals and non-treated ones. These data show that a selective thromboxane synthetase inhibitor (OKY-046) does not improve the blood rheology during endotoxin shock, although it seems to prevent the acidosis in some extent. (Key words: endotoxin, selective thromboxane synthetase inhibitor, blood viscosity, erythrocyte deformability, plasma fluidity)

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Septic shock causes a variety of circulatory and metabolic complications. Arterial blood pressure depends on the cardiac output and total peripheral resistance[1,2], the latter of which is determined by the geometry of the resistance vessels and the intrinsic viscosity of the blood. In the normal circulation, blood vessels dilate to compensate for the increase in blood viscosity[3]. In the septic shock, however, the increase in blood viscosity directly induces a decrease in the blood flow due to the impaired vascular autoregulation[4]. Because shock causes anaerobic metabolism and metabolic acido-

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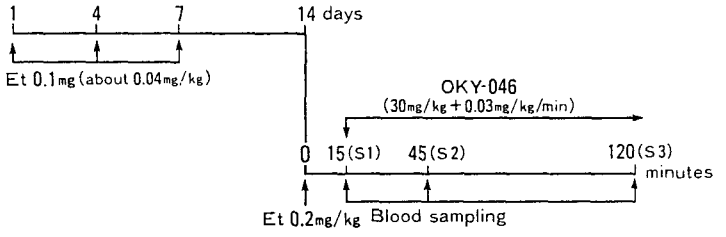

Et : Endotoxin
Lipopolysaccharide B E coli 0111 : B4 (Difco Lab)

Fig. 1. Experimental protocol for Group B. In Group A, no OKY-046 was administrated.

sis due to unsatisfactory tissue perfusion, one of the therapeutic goals for shock is to maintain the hemodynamics and oxygen supply to tissues. Since the erythrocyte diameter is larger than the capillary diameter, erythrocyte deformability is very important to maintain the microcirculatory perfusion and then the oxygen supply to the surrounding tissues[3]. When erythrocyte deformability is enhanced, microcirculatory perfusion would be increased. The increased erythrocyte deformability would prevent the anaerobic metabolism and metabolic acidosis appeared in the case of shock.

If the hemorheological conditions are improved, the vicious cycle of shock might be cut off. For example, fluid therapy is recommended for the increase of oxygen transport in the critically-ill postoperative patients who are in a pre-septic state[5]. The effect of drugs like pentoxyfylline which increases the erythrocyte deformability has been studied in experimentally endotoxin-shocked animals[6,7] as well as in postoperative patients[8]. It has been shown that the inhibitors of cyclo-oxygenase and selective inhibitors of thromboxane improve the hemodynamics and decrease the mortality in endotoxin shock[9–18]. These data indicate that the inhibition of prostaglandin synthesis is effective for the treatment of septic shock.

It has been reported that prostaglandin I_2 (PGI_2) increases in the erythrocyte deformability[19]. In the case of thromboxane synthetase inhibitors, some of their beneficial effects result from their redirection of endoperoxide substrate to PGI_2[11,16,17]. Thus inhibition of thromboxane synthesis may ameliorate hemodynamics and tissue metabolism by improving the hemorheological property of blood although little information is available on hemorheological effect of thromboxane synthetase inhibitors. This paper deals with the hemorheological effects of a selective thromboxane synthetase inhibitor, (E)-3-[4-(1-imidazolylmethyl)phenyl]-2-propenoic acid hydrochloride monohydrate (OKY-046)[20], in endotoxin-shocked rabbits.

Materials and Methods

Figure 1 shows the experimental protocol. Twelve male Japanese white rabbits weighing 2.6 ± 0.3 (mean ± SD) kg were used in this study. Endotoxin (Difco Lab. Lipopolysaccharide B E. coli 0111:B4) of 0.1 mg was dissolved in 1 ml of physiological saline solution and given intravenously through the auricular vein 3 times at intervals of 3 days for immunization[21]. Seven days after the final injection of endotoxin, anesthesia was induced by pentobarbital sodium (20–30 $mg \cdot kg^{-1}$). One percent lidocaine was used for local anesthesia during surgical procedures. Each rabbit was subjected to tracheotomy and was allowed to breath room air spontaneously during the experiment. A catheter was inserted into the right femoral artery for recording the blood pressure and sampling blood, and another catheter was inserted into the right femoral vein for the infusion of lactate Ringer's solution at the rate of 2.5 $ml \cdot kg^{-1} \cdot hr^{-1}$. Six endotoxin pretreated rabbits were administered 0.2 $mg \cdot kg^{-1}$ of endotoxin alone intravenously (Group A). Another six rabbits

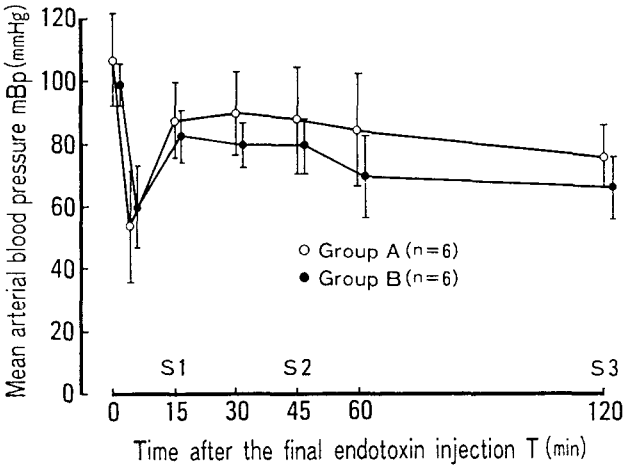

Fig. 2. Time course of the mean arterial blood pressure. Group A is non-OKY-046 group (n=6); Group B is OKY-046 group (n=6); S1, S2, and S3 indicate the time of blood sampling. Values are expressed as means ± standard deviations.

(Group B) which were also administered the same amount of endotoxin, at 15 min later were administered 30 $mg \cdot kg^{-1}$ of OKY-046 (Ono Pharmaceutical Co. Ltd., Osaka) and, then, OKY-046 was continuously infused at the rate of 0.03 $mg \cdot kg^{-1} \cdot min^{-1}$ during the experiment.

The blood sampling was performed 15 (S1 in figure 1, just before the administration of OKY-046), 45 (S2), and 120 (S3) minutes after the final administration of endotoxin. At each time 7 to 8 ml blood was sampled for the measurement of hematocrit, blood viscosity, and passage time through microporous membranes. Blood viscosity was measured at the shear rate of 150 sec^{-1} at 37°C using a cone plate viscometer (Biorheolizer, Tokyo Keiki, Tokyo). The passage time of 0.5 ml of 5% red cell suspension diluted with physiological saline solution and that of plasma were measured by the filtration method using polycarbon membranes (Nuclepore membrane, Nuclepore Co, California)[22]. The membranes having the pore sizes of 5 and 3 μm were used for the measurement of passage time of 5% red cell suspension and that of plasma, respectively. The passage time of red cell suspension is related to the erythrocyte deformability and that of plasma represents the plasma fluidity. The relative passage time which is defined as the ratio of the passage time of 5% red cell suspension or plasma to that of the physiological saline solution was used in this study because the distribution of pores is not always uniform in all membranes[23]. Blood gas, pH, and base excess (BE) were measured at S1 and S3 by ABL2 blood gas analyzer (Radiometer, Copenhagen). The normal values of these hemorheological parameters were determined in non-endotoxin-treated rabbits.

Statistical analysis of the data was performed using the unpaired Student's t-test for the difference between the groups. Two-way analysis of variance or paired Student's t-test was used for the intragroup comparison. Differences were considered statistically significant when P was less than 0.05. All values were expressed as means ± standard deviations (SD).

Results

Figure 2 shows the change in the mean arterial blood pressure in each group. The blood pressure decreased to less than 60 mmHg 3 to 4 min after the injection of endotoxin and, then, increased to over 80 mmHg for the next ten minutes, followed by a gradual decrease thereafter. No significant effect on the hypotension induced by the endotoxin was observed following the administration of OKY-046.

The changes in pH, BE, and gas tension in the arterial blood are shown in table 1. At each time, there were no significant differences of pH and gas tension between the

Table 1. Change in blood gas and pH

			Blood sample		
			S1	S3	
pH		A	7.51 ± 0.09	7.42 ± 0.07	$P < 0.01$
		B	7.47 ± 0.06	7.45 ± 0.04	n.s.
Pa_{CO_2}	(torr)	A	27 ± 3	25 ± 2	$P < 0.05$
		B	27 ± 5	24 ± 3	n.s.
Pa_{O_2}	(torr)	A	89 ± 20	105 ± 6	n.s.
		B	91 ± 5	112 ± 15	$P < 0.05$
BE	($mEq \cdot l^{-1}$)	A	0.3 ± 5.5	−6.8 ± 2.8	$P < 0.01$
		B	−3.0 ± 3.9	−5.9 ± 2.2	$P < 0.05$

See the main text or figure 2 for the grouping and the time of blood sampling. pH in the non-endotoxin normal rabbits was 7.42 ± 0.04 (n=12). Pa_{CO_2} and Pa_{O_2} in the normal rabbits were 31 ± 3 (n=12), and 91 ± 7 torr (n=12), respectively. BE (Base excess) in the normal rabbits were −3.1 ± 2.9 $mEq \cdot l^{-1}$. All values are expressed as means ± standard deviations. Paired Student's t-test was used for the comparison between S1 and S3. Unpaired Student's t-test was used for the comparison between two groups.

Table 2. Changes in hematocrit, whole blood viscosity, and relative passage times of 5% red blood cell suspension

		Blood sample				
		S1	S2	S3		
Ht (%)	A	38.8 ± 2.5	37.2 ± 2.2	34.8 ± 2.2	$P < 0.05$	$P < 0.001$
	B	39.7 ± 2.1	39.1 ± 2.2	36.1 ± 2.4		$P < 0.001$
η_b (mPa·s)	A	3.44 ± 0.57	3.48 ± 0.59	3.00 ± 0.48		$P < 0.05$
	B	3.72 ± 0.46	3.63 ± 0.48	3.20 ± 0.32		$P < 0.001$
$(\tau_{5\%})_{rel}$	A	1.71 ± 0.04	1.74 ± 0.05	1.74 ± 0.06		n.s.
	B	1.78 ± 0.13	1.72 ± 0.09	1.77 ± 0.07		n.s.

See the main text or figure 2 for the grouping and the time of blood sampling. Ht = hematocrit, the normal value of which was 39.8 ± 4.4% (n=18) in non-endotoxin normal rabbits. η_b = whole blood viscosity measured at the shear rate of 150 sec^{-1}, the normal value of which was 3.28 ± 0.44 mPa·s (n=18). $(\tau_{5\%})_{rel}$ = relative passage time of 5% red blood cell suspension (the ratio of passage time of 5% red blood cell suspension to that of physiological saline solution), normal value of which was 1.76 ± 0.09 (n=18). All values are expressed as means ± standard deviations. Two-way analysis of variance was used for the comparison between the rabbits in the same group. Unpaired Student's t-test was used for the comparison at the same time between two groups.

two groups. The change in pH (pH at S1 − pH at S3) was 0.09 ± 0.04 in the Group A, and 0.02 ± 0.04 in the Group B. The change in BE was −7.1 ± 3.4 $mEq \cdot l^{-1}$ in the Group A, and −5.9 ± 2.2 $mEq \cdot l^{-1}$ in the Group B. The changes in pH and BE were significant ($P < 0.05$, respectively). This significant decrease in Group A might reflect the enhancement of anaerobic metabolism in this group. Pa_{CO_2} significantly decreased in group A, but did not change in Group B, while Pa_{O_2} increased in Group B, but did

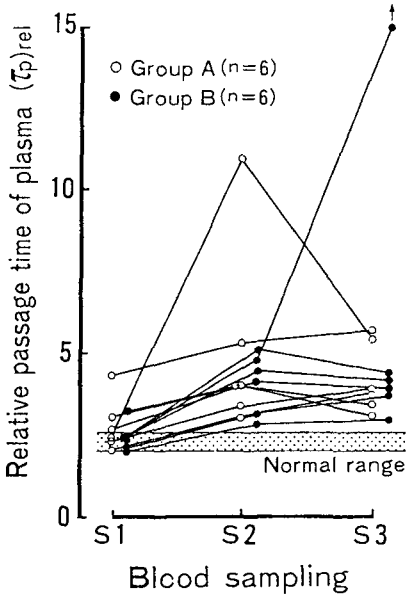

Fig. 3. Change in plasma fluidity represented by relative passage time of plasma. See the main text or figure 2 for the grouping and the time of blood sampling. Shaded area shows the normal range (2.30 ± 0.3, n=18). Values are expressed as means ± standard deviations.

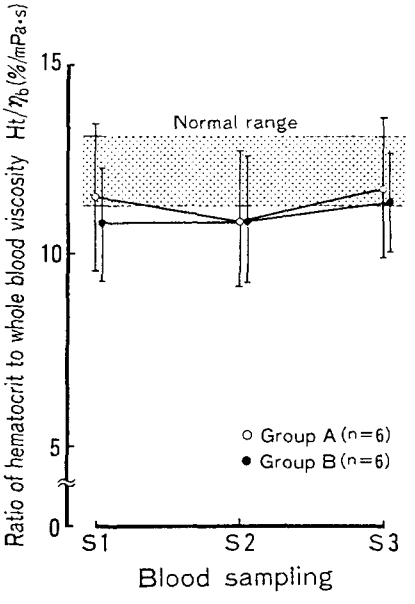

Fig. 4. Change in the ratio of the whole blood viscosity to hematocrit. See the main text or figure 2 for the grouping and the time of blood sampling. Shaded area shows the normal range (12.2 ± 0.9 %/mPa·s, n=18). Values are expressed as means ± standard deviations.

not change in Group A.

Table 2 summarizes the data on hematocrit, whole blood viscosity, and the relative passage time of 5% red blood cell suspension. The hematocrit and whole blood viscosity decreased gradually and significantly in the both groups within 120 min. No significant difference in those changes was noted between the groups, except in the hematocrit values at S3. Erythrocyte deformability represented by the relative passage time of 5% red cell suspension showed no change with time and no difference between the two groups.

Change in the plasma fluidity represented by the relative passage time of plasma is shown in figure 3. At S2 and S3, the relative passage time of plasma was significantly higher in the endotoxin-treated groups than in the normal rabbits, although there were no significant differences between Groups A and B.

The ratio of hematocrit to whole blood viscosity is roughly related to the degree of oxygen delivery[1]. As shown in figure 4, these ratios in the endotoxin-treated groups did not change with time, and were somewhat lower than the normal range. There was no significant difference in this value between Groups A and B.

Discussion

Human septic shock is associated with a gradual and/or repeated release of endotoxin. To simulate the clinical septic shock with animals, hypotension is induced by a small dose of endotoxin following the repeated administration of extremely small dose. Watanabe[21] showed that this model mimics the human septic shock in regards to hemodynamics and immunology. The authors[24] studied this model from the hemorheological point of view and pointed out the importance of plasma fluidity in endotoxin shock. Since clinical therapy for shock is usually started after we recognized a symptom like hypotension, a thromboxane synthetase inhibitor (OKY-046) was given in this study after hypotension was established.

In the present study, the authors did not study the dose-dependent response of OKY-046, because it was reported that OKY-046 has therapeutic effects with the dose of over 10 $mg \cdot kg^{-1}$ [14,15].

Prostaglandins play an important role in the hemodynamical and humoral changes observed with endotoxin shock[9–18,25,26]. Thromboxane A_2 (TxA_2) induces platelet aggregation and constricts blood vessels, whereas PGI_2 inhibits platelet aggregation and dilates blood vessels[10,17]. Cook et al.[11] observed that the administration of a cyclo-oxygenase inhibitor (indomethacin), a thromboxane synthetase inhibitor (imidazole), or a thromboxane antagonist (13-azaprostanoic acid) significantly reduced the mortality of rats from lethal endotoxin shock. The administration of such a cyclo-oxygenase inhibitor as indomethacin and ibuprofen to the endotoxin-shocked dogs returned the systemic blood pressure and cardiac index to the preendotoxin levels[9,13]. The treatment with indomethacin prior to the administration of endotoxin prevented the increases in the renin activity and catecholamine level in plasma[18]. Fukumoto and Tanaka[14] observed that the administration of thromboxane synthetase inhibitors (OKY-046 and OKY-1581) suppressed microthrombus formation and improved the survival rate of endotoxin-shocked rats. Kubo and Kobayashi[15] reported that OKY-046 prevented the pulmonary hypertension in endotoxemic sheep. Thromboxane synthetase inhibitors prevented the disseminated intravascular coagulation in endotoxin-shocked rats[12] and significantly improved organ perfusion in the early stage of endotoxin shock[16]. In contrast, Fletcher and Ramwell[25] showed that the continuous administration of PGI_2 increased the survival rate of endotoxin-shocked dogs. The infusion of exogenous PGI_2 significantly attenuated the cardiorespiratory embarrassment caused by endotoxemia[26].

These results imply that the inhibition of thromboxane synthesis partially improves the pathophysiological alternations induced by endotoxin. The increase of Pa_{O_2} and the decrease of Pa_{CO_2} appear commonly in endotoxemic rabbits to compensate for the metabolic acidosis[27]. Similar phenomena were observed in this study, although the decrease of pH and BE was prevented by the administration of OKY-046. This result suggests that anaerobic metabolism is suppressed by the administration of OKY-046. However, the whole blood viscosity, plasma fluidity, and erythrocyte deformability remained unchanged with this treatment, which means that the prevention of acidosis by OKY-046 does not result from the improvement of hemorheological properties. The authors observed that OKY-046 improves the mechanical properties of the renal artery and its reactivity to norepinephrine in the same endotoxin-shocked rabbits as used in the present study[28]. It has been reported that OKY-046 inhibits the arteriolar constriction caused by norepinephrine in the rat mesentery[29] and also prevents the increase of pulmonary arterial pressure in endotoxemic sheep[15]. It seems to be likely that the effects of OKY-046 on endotoxin shock are not attributable to the hemorheological improvement but to the vascular function.

The inhibition of thromboxane synthesis improves many alternations induced by endotoxin shock[9–18]. In the present study, it has been shown that OKY-046 prevents the decrease of pH induced by endotoxin. However, the authors failed to demonstrate a therapeutic effect of OKY-046 on the blood viscosity and erythrocyte deformability.

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