Table 1
Characteristics of the experimental and reference areas

	Area size (km^2)	Population (×1000)	Proportion of people aged 65 years and older (%)
Experimental areas			
Funabashi city	85	550	12.6
Cities of Suita, Toyonaka, Minoo	119	860	13.8
Chuwa regional area	166	248	16.0
Wards of Bunkyo and Taito	21	332	19.4
Reference areas			
Sagamihara City	90	605	11.1
Cities of Sakai and Takaishi	148	854	14.9
Konan regional area	206	284	12.0
Sinagawa ward	23	317	17.2

Experimental areas have a two-tiered system combining basic life support (BLS) ambulances and doctor-manned ambulances. Reference areas use the standard Japanese emergency medical services system, a one-tiered system with BLS ambulances.

technique proposed by Dowie et al. [17]. The action tree is shown in Fig. 2; the circular nodes denote chance happenings subsequent to events. In this tree, after basic CPR, the electrocardiogram is monitored, and the heart rhythm is classified as ventricular fibrillation or ventricular tachycardia, pulseless electrical activity, asystole, or the return of spontaneous circulation. Action boxes along the branches show subsequent options that physicians have. For example, defib-

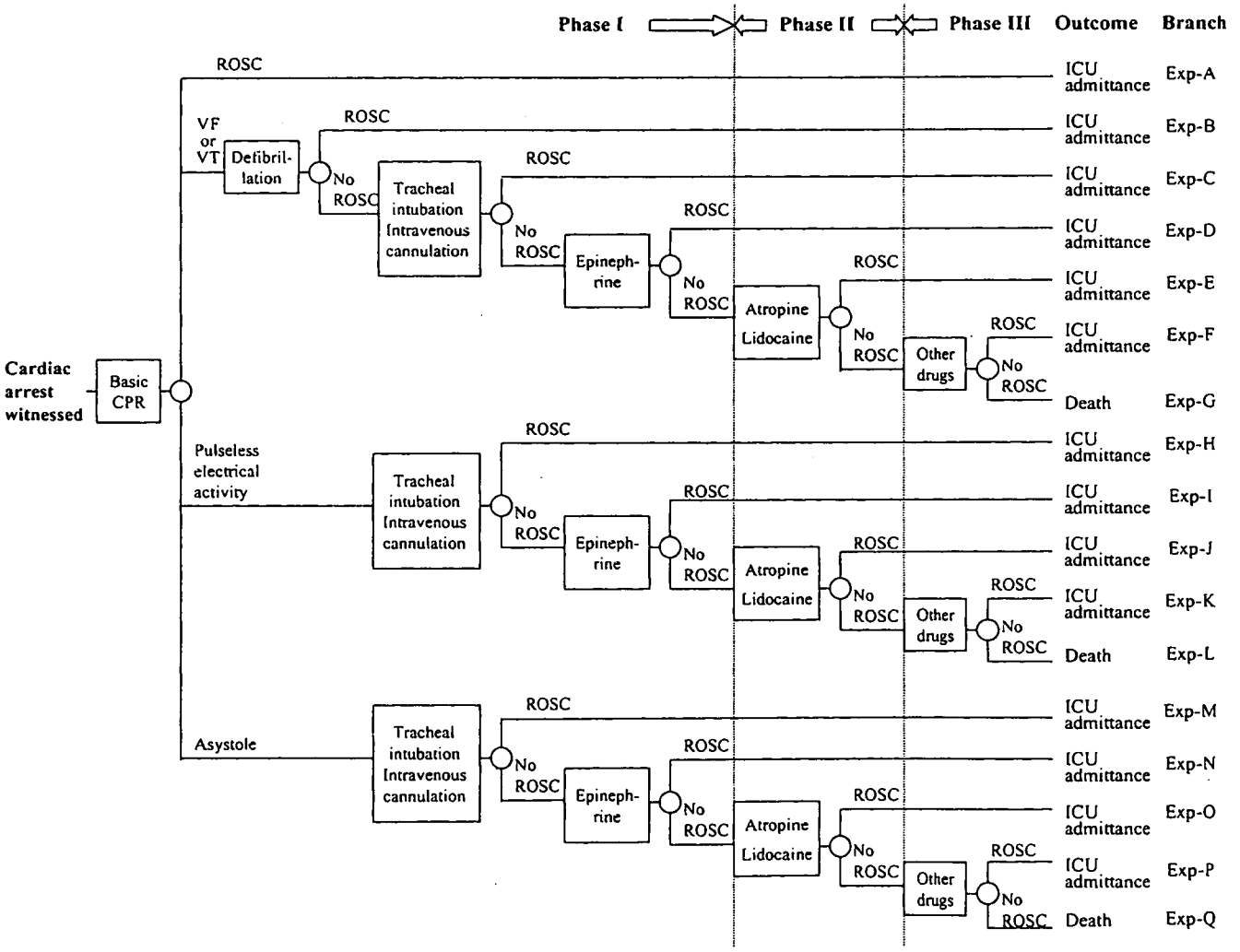

Fig. 2. Action tree of physicians in doctor-manned ambulances. CPR, cardiopulmonary resuscitation; VF, ventricular fibrillation; VT, ventricular tachycardia; ROSC, return of spontaneous circulation; ICU, intensive care unit.

rillation can be done if necessary after epinephrine is administered. Branches from Exp-A to Exp-Q in Fig. 2 show the outcomes, i.e., admittance to the intensive care unit (ICU) or death. Even when the return of spontaneous circulation is observed in the process of resuscitative care, patients are not always admitted to the ICU. Sometimes they die.

The sequence of events was divided into three phases. Phase I included administration of epinephrine. Phase II started with the use of lidocaine or atropine and ended just before the administration of a drug other than one of these three drugs. Phase III began with administration of this other drug and continued until the start of emergency care at the hospital. As shown in Fig. 1, the outcome of phase I was used to evaluate target system-1, out-of-hospital CPR with epinephrine. The outcome of phase II was used to evaluate target system-2, out-of-hospital CPR with epinephrine, lidocaine, and atropine.

The action tree for ELSTs is shown in Fig. 3. This tree was constructed to obtain data on outcomes in accordance with the sequence of events rather than to control the actions of ELSTs.

2.6. Statistical analyses

Difference in mean age between the experimental group and the reference group was analyzed by unpaired *t*-test. Dichotomous data such as sex and cause of cardiac arrest were analyzed by χ^2-test. The time periods from the emergency call to arrival at the scene, from the emergency call to hospital arrival, and from the emergency call to the first defibrillation in cases in which it was performed were compared between the doctor-manned ambulances and BLS ambulances and analyzed by unpaired *t*-test. The outcomes used to evaluate the systems were the percentage of patients with spontaneous circulation admitted to the ICU or an appropriate ward (resuscitation rate) and survival rate 1 month later. Differences in the resuscitation rate and the 1-month survival rate between the experimental group and the reference group were analyzed by χ^2-test. Outcomes through phase II in the experimental group and phase I in the experimental group were compared with the corresponding outcomes of the reference group; these comparisons were by χ^2-test. All tests were two-tailed. *P* values of less than 0.05 were taken as statistically significant.

2.7. Ethics

Data obtained from the study were handled anonymously. The study protocol was approved by the appropriate institutional review board for ethical issues at each participating facility. The study design was approved by the Ministry of Health, Labor, and Welfare of Japan and the Fire and Disaster Management Agency of the Ministry of General Affairs of Japan.

3. Results

Characteristics of the experimental group and reference group are shown in Table 2. There was no statistically signif-

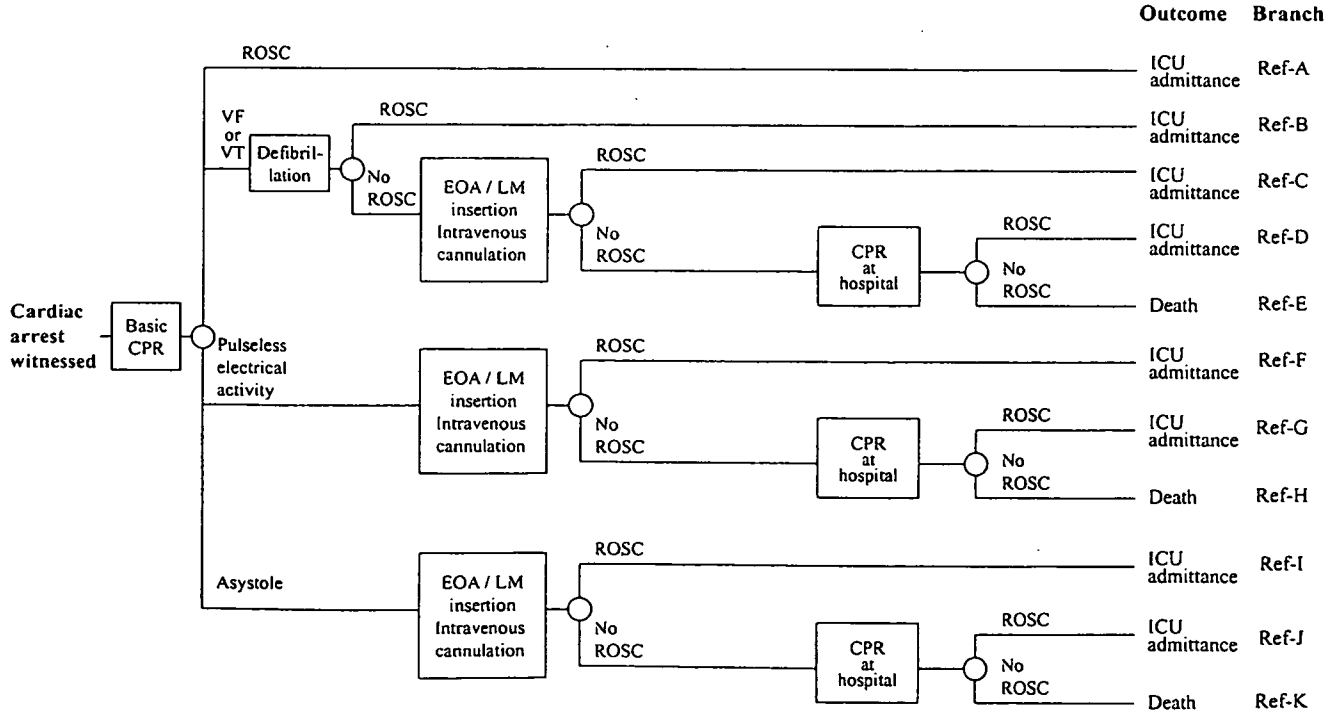

Fig. 3. Action tree of emergency life-saving technicians (ELSTs). CPR, cardiopulmonary resuscitation; VF, ventricular fibrillation; VT, ventricular tachycardia; ROSC, return of spontaneous circulation; EOA, esophageal obturator airway; LM, laryngeal mask airway; ICU, intensive care unit.

Table 2
Characteristics of the two study groups

	Experimental group (doctor-manned ambulances) $n = 162$	Reference group (basic life support ambulances) $n = 272$	P-value
Age of patients (years)[a]	67.3 (17.5)	69.4 (18.9)	0.275 (t-test)
Sex			
Male	100 (61.7%)	169 (62.1%)	
Female	62 (38.3%)	103 (37.9%)	0.933 (χ^2-test)
Cause of cardiac arrest			
Non-traumatic	120 (74.1%)	222 (81.6%)	
Traumatic	41 (25.3%)	44 (16.2%)	0.025 (χ^2-test)[b]
Unknown	1 (0.6%)	6 (2.2%)	
Time from call to scene arrival (min)[a]	14.1 (6.3)	6.2 (2.7)	<0.001 (t-test)
Time from call to first defibrillation (min)[a]	9.4 (6.7)	10.7 (4.5)	0.360 (t-test)
Time from call to hospital arrival (min)[a]	46.4 (13.6)	28.4 (8.7)	<0.001 (t-test)

[a] Mean (standard deviation).
[b] Excluding unknown causes.

icant difference in age or sex between the two groups. The proportion of cardiac arrests caused by traumatic events in the experimental group was significantly larger than that in the reference group (25.3 and 16.2%, respectively, $P = 0.025$). A significant difference was not observed between the two groups in non-traumatic cardiac arrests of cardiac or non-cardiac origin. The proportion of patients in the experimental group who received CPR from a bystander was smaller than that in the reference group (23.5 and 31.3%, respectively), but the difference was not significant ($P = 0.081$).

The time from the emergency call to arrival at the scene was significantly shorter for the BLS ambulances (mean: 6.2 min, standard deviation: 2.7) than for the doctor-manned ambulances (mean: 14.1 min, standard deviation: 6.3). However, the start of CPR in the experimental group was not later than that in the reference group because, in the experimental group, BLS ambulance crews of the two-tiered system started basic CPR. If the monitored electrocardiogram showed ventricular fibrillation or ventricular tachycardia, defibrillation was performed. The mean time from the emergency call to the first defibrillation did not differ statistically between the two groups (9.4 and 10.7 min, respectively, $P = 0.360$); in the experimental group, the first defibrillation was performed mainly by BLS ambulance crews. Defibrillation was performed one to six times per patient during the out-of-hospital CPR in the reference group (mean: 2.5 times). In the experimental group, defibrillation was performed one to six times per patient (mean: 2.2 times) during phase I and given if needed in phases II and III. The mean time from the emergency call to the time of hospital arrival of the doctor-manned ambulances was 18 min longer than that of the BLS ambulances (Table 2). There was no difference in the mean time from the emergency call to hospital arrival between traumatic arrests and non-traumatic arrest in the experimental group (45.7 and 46.5 min, respectively, $P = 0.812$) or the reference group (27.1 and 28.6 min, respectively, $P = 0.286$).

Immediate outcomes, i.e., resuscitated patients admitted to an ICU, are shown in Table 3. In cases of cardiac arrest without a traumatic event, the proportion of patients admitted to an ICU was significantly larger in the experimental group than in the reference group (40.8 and 24.4%, respectively, $P = 0.001$). Conversely, in cases of cardiac arrest with a traumatic event, the proportion of patients admitted to an ICU was smaller in the experimental group than in the reference group (26.8 and 31.8%, respectively), but the difference was not statistically significant ($P = 0.614$).

For cardiac arrest without a traumatic event, the immediate outcomes are shown according to the action trees in Table 4. The proportion of survivors 1 month later was significantly larger in the experimental group (10.8%) than in the reference group (4.5%) ($P = 0.027$). The phase I and phase II outcomes in the experimental group were significantly better than those in the reference group (ICU admittance rate and 1-month survival rate, odds ratios = 1.815 and 2.551, respectively, $P < 0.05$) (Table 5). The phase I outcome of the experimental group was better, but not statistically better, than that of the reference group (ICU admittance rate and

Table 3
Immediate outcomes in the two study groups

	Experimental group	Reference group
Non-traumatic cardiac arrest		
Admittance to ICU	49 (40.8)	52 (24.4)
Death	71 (51.2)	169 (76.1)
Unknown	0 (0.0)	1 (0.5)
Odds ratio: 2.243 (95% CI: 1.39–3.62; $P = 0.001$)[a]		
Traumatic cardiac arrest		
Admittance to ICU	11 (26.8)	14 (31.8)
Death	30 (73.2)	30 (68.2)
Unknown	0 (0.0)	0 (0.0)
Odds ratio: 0.786 (95% CI: 0.31–2.01; $P = 0.614$)		

The values in parenthesis are given in percentage.
[a] Excluding unknown outcomes. ICU, intensive care unit; CI, confidence interval.

Table 4
Immediate outcomes and number of non-traumatic cardiac arrest patients surviving at 1 month according to the action tree branches in the two study groups

Branch	Immediate outcome	Phase of return of spontaneous circulation	Number of cases (percentage of total)	1-Monsth survival (percentage of total)
Experimental group (n = 120)				
Exp-A	ICU admittance	I	0 (0.0)	0 (0.0)
Exp-B	ICU admittance	I	6 (5.0)	5 (5.0)
Exp-C	ICU admittance	I	4 (3.3)	4 (3.3)
Exp-D	ICU admittance	I	6 (5.0)	0 (0.0)
Exp-E	ICU admittance	II	4 (3.3)	1 (0.8)
Exp-F	ICU admittance	III	0 (0.0)	0 (0.0)
Exp-G	Death		16 (13.3)	
Exp-H	ICU admittance	I	2 (1.7)	0 (0.0)
Exp-I	ICU admittance	I	9 (7.5)	1 (0.8)
Exp-J	ICU admittance	II	3 (2.5)	0 (0.0)
Exp-K	ICU admittance	III	1 (0.8)	0 (0.0)
Exp-L	Death		23 (19.2)	
Exp-M	ICU admittance	I	1 (0.8)	1 (0.8)
Exp-N	ICU admittance	I	5 (4.2)	1 (0.8)
Exp-O	ICU admittance	II	3 (2.5)	0 (0.0)
Exp-P	ICU admittance	III	5 (4.2)	0 (0.0)
Exp-Q	Death		32 (2.7)	
Reference group (n = 222)				
Ref-A	ICU admittance		4 (1.8)	2 (0.9)
Ref-B	ICU admittance		6 (2.7)	2 (0.9)
Ref-C	ICU admittance		2 (0.9)	1 (0.5)
Ref-D	ICU admittance		10 (4.5)	1 (0.5)
Ref-E	Death		26 (11.7)	
Ref-F	ICU admittance		0 (0.0)	0 (0.0)
Ref-G	ICU admittance		15 (6.8)	1 (0.5)
Ref-H	Death		41 (18.5)	
Ref-I	ICU admittance		1 (0.5)	1 (0.5)
Ref-J	ICU admittance		14 (6.3)	2 (0.9)
Ref-K	Death		102 (45.9)	

ICU, intensive care unit.

Table 5
Outcomes of non-traumatic cardiac arrest patients of the reference group and experimental group

		Odds ratio	P-value
Percentage of ICU admittance (number of patients)			
Phases I and II in experimental group (n = 120)	35.8 (43)	1.815 (95% CI: 1.12–2.95)	0.016
Phase I in experimental group (n = 120)	27.5 (33)	1.233 (95% CI: 0.74–2.05)	0.418
Reference group (n = 222)	24.3 (52)	1	
Percentage of 1-month survival (number of patients)			
Phases I and II in experimental group (n = 120)	10.8 (13)	2.551 (95% CI: 1.08–6.01)	0.027
Phase I in experimental group (n = 120)	10.0 (12)	2.333 (95% CI: 0.98–5.57)	0.051
Reference group (n = 222)	4.5 (10)	1	

ICU, intensive care unit; CI, confidence interval.

1-month survival rate, odds ratios = 1.233 and 2.333, respectively, $P > 0.05$) (Table 5).

4. Discussion

4.1. Non-traumatic cardiac arrest

Our study results showed overall that the prehospital EMS system with doctor-manned ambulances is more effective than the BLS ambulance system for non-traumatic cardiac arrest in terms of resuscitation rate and 1-month survival rate; i.e., early advanced cardiac life support is advantageous for resuscitation from non-traumatic cardiac arrest. Study patients treated by physicians in doctor-manned ambulances received advanced life support 14 min earlier on average than patients treated by BLS ambulance crews and not given resuscitative drugs until after arrival at the hospital. Doctor-manned ambulances are in use in many European countries [2,18,19], whereas in the United States, paramedics provide advanced cardiac life support for prehospital cardiac arrest patients because doctor-manned ambulances there are con-

sidered an inefficient use of physician resources [2]. In Japan, it would be difficult to extend the doctor-manned ambulance system to the whole country because of the limited number of physicians committed to emergency medicine. Expanding procedures that ELSTs can perform is an alternative to nationwide introduction of doctor-manned ambulances.

The sequence of events initiated by physicians staffing the doctor-manned ambulance was divided into three phases. From the start of the event to the end of phase II is a simulation of target system-2, i.e., the EMS system in which ELSTs would administer epinephrine, lidocaine, and atropine. Outcomes of CPR from the start of the event until phase II were better than those of the reference group in terms of resuscitation and 1-month survival rates. Thus, target system-2 is likely to be more effective than the reference system. Phase I is a simulation of target system-1, i.e., the EMS system in which ELSTs would administer epinephrine only. The phase I outcomes were better than those in the reference group, but statistical significance was not achieved. This does not necessarily mean that target system-1 is not effective because the outcomes improved gradually as the phase advanced. The results imply that target system-1 is more beneficial than the present standard Japanese system.

Another important result of the study was that the proportion of patients resuscitated before drug administration in the experimental group (branches Exp-A, -B, -C, -H, and -M in Fig. 2) was larger than the proportion in the reference group (branches Ref-A, -B, -C, -F, and -I in Fig. 3), although statistical significance was not achieved (proportions of resuscitated patients were 10.8 and 5.9%, respectively, $P > 0.05$). Not only early drug administration but also the behavioral principle of physicians, attaching importance to early restoration of spontaneous circulation rather than early transport, might benefit prehospital CPR.

The present findings indicate that the preferable plan is to adopt a doctor-manned ambulance system nationwide. If this is difficult to do because of a shortage of physicians, the second best option is likely the target system-2, i.e., that ELSTs administer epinephrine, lidocaine, and atropine. The third best option would be target system-1, i.e., that ELSTs administer epinephrine only. Which system should be adopted will be determined on the basis of several factors: cost of training ELSTs, methods of on-line medical control, and risk of accidents. Additionally, how much the behavioral principles should be shifted is also an important and difficult issue. The reason why doctor-manned ambulances delayed transport of patients was that physicians performed as many resuscitative procedures as possible upon arrival at the scene, including intubation, intravenous cannulation, defibrillation, drug administration, and other procedures they thought necessary. When weight is given to early restoration of spontaneous circulation, inevitably early transport of the patients is sacrificed [20] because there is a trade-off relation between them. Although policymakers must be interested in the optimum balance between the two, the present study did not clarify what that balance should be.

4.2. Traumatic cardiac arrest

In contrast to the outcome of CPR for non-traumatic cardiac arrest patients, the resuscitation rate for patients with traumatic arrest was better in the reference areas was than in the experimental areas, although the difference was not statistically significant. The results may be explained simply by the small sample size. The numbers of cases, 41 and 44, are too small for reliable results. It is also possible that the higher proportion of serious cases in the experimental group introduced a bias into the study results. Third, sacrificing early transport of patients to the hospital might have caused the negative outcome. This third possibility should be considered carefully when the behavioral principle of the ELSTs is being evaluated.

4.3. Study design

Little has been reported on the positive effect of resuscitative drugs such as epinephrine. Direct comparison between survival rates and administration of such drugs occasionally yields misleading results. If, for instance, drugs tend to be used more for persons who are difficult to resuscitate than for persons who are easily resuscitated, drugs can appear to influence survival negatively. To the contrary, if drugs tend not to be used when the probability of resuscitation is poor, the positive effect of drug use is likely overestimated. There is an inherent selection bias when drugs are used with medical judgment. To avoid this selection bias, it is most preferable to perform a randomized-controlled trial in which drugs are administered randomly to patients suffering cardiac arrest. Randomized-controlled trials, however, are extremely difficult to perform on ethical grounds. The second-most preferable approach is a community intervention study in which two different community groups, i.e., areas where ELSTs use drugs en route to the hospital and areas where they do not, are compared for rates of survival from cardiac arrest. However, this was not possible under Japanese law. In addition, for ethical reasons, we could not restrict the prehospital use of other drugs, although use of other drugs masks the real effect of target drugs. Under these restricted circumstances, we abandoned evaluation of the effect of drugs. Instead, we evaluated prehospital EMS systems in which drugs are administered.

The action tree constructed for physicians was medically reasonable. The procedures conducted by emergency physicians were not restricted or controlled; only their action steps were determined.

4.4. Study limitations

The number of patients treated in this study differed between the experimental areas and the reference areas, although the areas were matched in terms of regional characteristics. It is likely that doctor-manned ambulances did not respond to all cardiac arrests in the experimental

areas. Selection bias, however, was unlikely to have occurred for witnessed non-traumatic cardiac arrests.

This comparative study involved two different classes of emergency personnel, physicians and ELSTs. The results of the study, therefore, must be interpreted carefully. If a decision is made that ELSTs should be allowed to administer resuscitative drugs during prehospital EMS, the results of the program should be evaluated because several factors could have distorted the outcomes of this study. Resuscitation techniques of ELSTs could be inferior to those of physicians. This could have caused overvaluation of the target systems. In contrast, this study intrinsically undervalued the target systems by reason of the study design, as shown in Fig. 1. The effect of early drug use could have been underestimated in this study because ELSTs can arrive at the scene as much as 8 min sooner than physicians [21].

5. Conclusion

Early administration of resuscitative drugs in out-of-hospital CPR for witnessed non-traumatic cardiac arrest seemed to be effective in terms of resuscitation rate and 1-month survival rate. This effectiveness was observed when epinephrine, lidocaine, and atropine were administered. Even the system under which ELSTs administered only epinephrine seemed to be more beneficial than the present Japanese EMS system, which prohibits ELSTs from administrating resuscitative drugs. The present findings support a recommendation that ELSTs be allowed to administer resuscitative drugs during prehospital EMS.

Conflict of interest

There are no conflicts of interest.

Acknowledgments

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Epidural Analgesia Prevents Endotoxin-Induced Gut Mucosal Injury in Rabbits

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In the present study, we evaluated the effect of epidural analgesia on the alterations of gut barrier function elicited by endotoxin in rabbits. After the placement of an epidural catheter, 28 male rabbits were randomized into either 0.5% lidocaine (group E) or saline (group C) group. The solutions (0.4 mL/kg) were epidurally injected, followed by continuous infusion (0.1 $mL \cdot kg^{-1} \cdot h^{-1}$) throughout the study period. Under a continuous infusion of lipopolysaccharide (15 $\mu g \cdot kg^{-1} \cdot h^{-1}$), mean arterial blood pressure, intramucosal pH, and plasma thrombomodulin concentrations were measured. At 4 h, mean arterial blood pressure was lower ($P < 0.05$), intramucosal pH was higher ($P < 0.01$), and the progression of hemodilution more profound ($P < 0.05$) in group E versus group C, whereas plasma thrombomodulin levels were increased to a similar extent between the groups. With less wet-to-dry weight ratio of ileum, histopathological injury scores of gut mucosa were significantly less in group E versus group C ($P < 0.01$). In a separate series of experiments ($n = 10$ each group), mucosal permeability in group E was significantly less compared with group C ($P < 0.05$). Collectively, these studies showed that despite a significant decrease of perfusion pressure and arterial oxygen content, epidural analgesia minimized endotoxin-induced functional and structural injury of gut mucosa possibly through endothelium-independent mechanisms.

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Intestinal mucosa is anatomically vulnerable to any type of oxygen deficit because of its low oxygen tension, right angle branching microvessels, and countercurrent blood supply (1). Through the loss of its barrier function, the gut becomes a significant supplier of microorganisms and toxins to the systemic circulation, evoking the discharge of proinflammatory mediators and the development of multiple organ dysfunction syndrome (2). Whereas the preservation of gut integrity has become a therapeutic goal for critically ill patients, few approaches are clinically relevant in preventing the progression of gut injury (2,3).

Epidural analgesia has been demonstrated, in both clinical and experimental examinations, to attenuate the decrease of intramucosal pH (pHi) in patients undergoing major abdominal surgery (4) and to augment intramucosal microcirculation of the gut in rats (5). In addition, epidural analgesia increased splanchnic venous capacitance by depressing sympathetic nerve activity in a rabbit model (6). We previously demonstrated that thoracic epidural anesthesia and analgesia retarded the progression of intramucosal acidosis and prevented endotoxin influx to the portal vein during acute hypoxia in rabbits (7). However, a question remains whether epidural anesthesia and analgesia are still protective for gut mucosa in clinically relevant disease conditions such as sepsis, where tissue hypoxia is more complicated and substantial. Among several pathophysiological profiles, sepsis is characterized by massive discharges of proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α) (8), by pathologic releases of nitric oxide (NO) to counteract oxygen radicals (9), and by considerable liberation of thrombomodulin, which acts as a cofactor of thrombin for protein C activation from injured endothelium (10). With a focus on the alterations of these mediators, we designed the present study to examine whether epidural analgesia preserved functional

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and structural integrity of the gut in a rabbit model of endotoxemia.

Methods

This protocol was approved by the Keio University School of Medicine Council on Animal Care in accordance with the guidelines of the National Institutes of Health.

Fifty healthy rabbits (New Zealand White, male; SEASCO, Saitama, Japan), weighing 2.0–2.5 kg (average, 2.3 kg) and fasted for 24 h, underwent instrumentation under general anesthesia. With sevoflurane 3%–4% inhaled in oxygen (3–4 L/min) via a face mask, the rabbits underwent tracheostomy and IV line access on the marginal ear vein. The rabbits were then mechanically ventilated to maintain normocapnia (fraction of inspiratory oxygen, 0.3; inspiratory pressure, 12–15 cm H_2O; and 10–12 breaths/min) using an intensive care unit type ventilator (New Port E-100; New Port Medical Inc, Newport Beach, CA). An epidural catheter was placed via T11-12 interspace, as previously described (11), and an indwelling arterial catheter (22-gauge) was inserted into the right carotid artery. After a midline abdominal incision, a silastic catheter was inserted through the mesenteric vein to the distal portion of the portal vein. A perivascular probe was attached around the portal vein for measurement of portal blood flow (Transit-Time Ultrasound Flowmeter, T206; Transomic System Inc, Ithaca, NY) (12). A sigmoid tonometer catheter (Tonometrics, Worcester, MA) was surgically inserted into the terminal ileum via the ileocecal portion. To obviate the effects of inhaled anesthesia and to mimic the rabbits the condition of sepsis in the intensive care unit, inhaled anesthesia was discontinued, and a mixture of buprenorphine (0.1 mg/mL) and midazolam (2 mg/mL) was continuously infused at a rate of 1 mL/h. Rectal temperature was monitored and maintained at approximately 37°C. Rabbits were observed for 30 min before baseline measurements were made.

Study Protocol 1

After baseline measurements (baseline), 30 rabbits were randomly assigned to a control (group C; $n = 14$) or epidural (group E; $n = 16$) group using computer-generated random numbers. All rabbits in group E received a 0.4-mL/kg bolus injection of 0.5% lidocaine through the epidural catheter, followed by a continuous infusion of 0.1 $mL \cdot kg^{-1} \cdot h^{-1}$, as described previously (13). Group C received the same doses of normal saline alone epidurally. After an equilibration period, the measurements of systemic and splanchnic variables were described in the measurement of pHi, and the specific measurements and calculations were performed (0 h). Thereafter, both groups received continuous infusion of 15 $\mu g \cdot kg^{-1} \cdot h^{-1}$ of lipopolysaccharide (LPS) (*Escherichia coli* serotype 055:55B5; Sigma Chemical Co, St Louis, MO), accompanied by Ringer's acetate solution infusion at a rate of 25 $mL \cdot kg^{-1} \cdot h^{-1}$, throughout the study periods. We chose the identical fluid volume between the groups to obviate a confounding factor for edema formation. In our pilot study using periaortic flow measurements, as previously described (12), the animals demonstrated a hyperdynamic circulatory pattern by manifesting an approximately 25% increase of cardiac output for 5 h, indicating that the animal model could be clinically relevant to septic patients throughout the 4-h study periods. The measurements of systemic and splanchnic variables were repeated at 2- and 4-h time periods. At the completion of the experiment, 0.4 mL/kg of indocyanine green was injected through epidural catheter, and if the cephalic spread of dye did not cover the range between the T4 and L1 level of the spine, the rabbit was excluded from the data collection. After tissue sampling was performed to determine wet-to-dry weight ratio and histological analysis of terminal ileum, the rabbits were killed with an IV pentobarbital overdose.

Gut pHi was monitored using automated air tonometry (Tonocap, Datex Ohmeda, Helsinki, Finland) (14). The measured regional Pco_2 ($Prco_2$), together with simultaneously obtained arterial [HCO_3^-], were applied in the Henderson-Hasselbalch equation for calculation of pHi according to the manufacture's instruction:

$$pHi = 6.1 + \log[HCO_3^-]/0.03 \times PrCO_2$$

[HCO_3^-] being the arterial bicarbonate concentration, 6.1 the dissociation constant of HCO_3^-, and 0.03 the solubility of CO_2 in plasma.

Arterial TNF-α plasma concentrations were measured using enzyme-linked immunosorbent assay that was developed in our laboratory. The assays were performed by using a combination of purified polyclonal goat anti-rabbit TNF antibody as a capture antibody and biotinylated polyclonal goat anti-rabbit TNF antibody for detection (15). Standard material, which was used in the rabbit TNF-conditioned medium (PharMingen, San Diego, CA), and obtained samples were run in duplicate. The detection limit in this assay was 13.7 pg/mL, and linear standard curves were obtained that ranged from 123 to 10,000 pg/mL.

The arterial thrombomodulin plasma level was measured by using enzyme-linked immunosorbent assay, as described elsewhere (16). Briefly, each well of a microtiter plate was coated with 1 μg/mL of goat

anti-rabbit thrombomodulin immunoglobulin G dissolved in 0.1 mol/L of $NaHCO_3$ (pH value of 9.6), and the plate was incubated overnight at 4°C. After blocking with Block Ace (Dainippon Seiyaku, CO, Ltd, Osaka, Japan), rabbit standard thrombomodulin (American Diagnostica Inc, Greenwich, CT) and test specimens were loaded into the wells, and the plate was incubated for 60 min at room temperature. Biotylated anti-thrombomodulin immunoglobulin G was added to the wells, followed by a 30-min incubation. After a 15-min incubation with avidin-peroxidase complex, a substrate solution containing 0.01% H_2O_2 and 0.4 mg/mL of o-phenyl-enediamine was added. The reaction was stopped using 4.5 N of H_2SO_4, and the absorbance at 490 nm was measured using the plate analyzer (ETY-3A, Toyo Sokki, Zama, Japan).

At the completion of experiments in Study Protocol 1, mucosal edema and microstructure of the terminal ileum were examined. Wet weights of five 2-cm parts of ileal tissues were measured and then dried in a vacuum oven (DP22; Yamato Scientific, Tokyo, Japan) at 95°C and −20 cm H_2O for 48 h. The dry weights were determined, and the wet-to-dry weight ratio was calculated (12). Ileal samples for microscopic examination were obtained from the terminal ileum, which was distant from the tonometer catheter placement. Histological sections were evaluated in a blinded manner using light microscopy. Twenty-five random fields from each tissue were examined, and the degree of mucosal damage was graded on a scale of 0–4, with a modification of grading system previously described (17). In this classification, normal villi were graded as 0, mucosal edema limited to the apex of the villous tip and development of the subepithelial space as 1, extension of subepithelial space as 2, localized area of mucosal destruction or extensive submucosal edema was 3, and severe cell disruption was grade 4.

Arterial and portal pH, Pco_2, Po_2, and lactate concentrations were determined by using a blood gas analyzer (Chiron 860 series, Chiron Diagnostics Corp, East Walpole, MA). Hemoglobin and hemoglobin oxygen saturation were measured using a co-oximeter (OSM3, Radiometer, Copenhagen). Splanchnic oxygen extraction ratio was calculated using standard formulae: splanchnic oxygen extraction ratio (%) = $100 \times (CaO_2 - CpO_2)/CaO_2$. Arterial and portal blood samples were centrifuged, and the plasma was stored at −80°C until analysis. NO release was assessed by the determination of stable NO metabolites (NO_2^- + NO_3^-; NOx) in plasma using spectrophotometric assay (Cayman, Detroit, MI). Plasma lidocaine concentrations at 4 h in both groups were determined by fluorescence polarization immunoassay (TDX system, Abbot, North Chicago, IL). Measurements of all variables were performed in duplicate, and mean values were used for results.

Study Protocol 2

In a separate series of experiments, we determined the changes of gut permeability using fluorescein isothiocyanate-conjugated dextran with a molecular weight of 4000 Da (FD4) (13). After the same preparatory surgery, excluding the placement of the tonometer catheter and perivascular flow probe, 20 rabbits (New Zealand White, male; SEASCO) were randomized into groups C or E (n = 10 each group). The rabbits received the same dose of endotoxin infusion as those in Study Protocol 1. At 4 h, the abdomen was opened for preparation of an *in situ* loop of the gut. Briefly, double ligatures at both ends were made on the 10-cm length of the terminal ileum. Through a cannula placed into this segment of terminal ileum, FD4 (50 mg) was injected. After 30 min, blood samples from both the portal vein and artery were taken and centrifuged, and plasma FD4 concentrations were measured using fluorescence spectrometry (Spectrofluorophotometer:RF-1500; Shimadzu, Kyoto, Japan). Results were corrected for the plasma protein contents measured by the Lowry method.

Data are expressed as mean ± SD unless otherwise specified. Analysis of variance with repeated measures was used to evaluate the differences as shown using SPSS/11.0J for Windows (SPSS Inc, Chicago, IL). Separate analysis was performed if the interaction was statistically significant. When $P < 0.05$, the Scheffe multiple-comparison test was applied to distinguish differences between measurement variables. If the data were not normally distributed, the Friedman test was used to evaluate pair-wise comparisons. The histological scoring data were analyzed by χ^2 test. Differences were considered statistically significant if $P < 0.05$.

Results

Plasma lidocaine concentrations in both groups were all less than the undetectable level (<1.0 μg/mL). Because of misplacement of the epidural catheter, 2 rabbits in group E in Study Protocol 1 were excluded from the data collection.

Compared to the 0-h period, LPS infusion decreased mean arterial blood pressure (MAP) in both study groups ($P < 0.05$). The extent of hypotension in group E was significantly more than group C ($P < 0.05$) (Table 1). Heart rate was significantly depressed to a similar extent in both study groups compared with 0 h ($P < 0.05$). Whereas arterial Po_2 remained constant throughout the study periods in both study groups, arterial oxygen content was significantly depressed to a larger extent in group E compared with group C ($P < 0.05$), mainly because of the progressive reduction of hemoglobin levels. Macroscopic hemolysis and persistent hemorrhage were not observed in either study

Table 1. Effects of Epidural Analgesia on Systemic Circulatory Variables in Endotoxemic Rabbits

	Group	Baseline	0 h	2 h	4 h
Mean arterial blood pressure (mm Hg)	C	92 ± 14	93 ± 14	83 ± 18	81 ± 15
	E	90 ± 14	85 ± 15	59 ± 11*†	59 ± 11*†
Heart rate (bpm)	C	279 ± 25	278 ± 28	257 ± 37†	248 ± 41†
	E	277 ± 34	275 ± 37	245 ± 43†	240 ± 51†
Arterial Po_2 (mm Hg)	C	179 ± 20	182 ± 25	184 ± 17	187 ± 22
	E	181 ± 17	176 ± 28	206 ± 33	187 ± 26
Arterial oxygen content (mL O_2/L)	C	13.9 ± 1.2	13.2 ± 1.2	11.7 ± 1.2†	10.3 ± 1.3†
	E	12.9 ± 1.9	11.6 ± 1.5*	9.6 ± 1.4*†	8.0 ± 0.9*‡
Arterial hemoglobin (g/dL)	C	9.8 ± 0.9	9.3 ± 0.9	8.2 ± 0.9	7.1 ± 1.0‡
	E	9.1 ± 1.3	8.1 ± 1.1	6.6 ± 1.0*†	5.5 ± 0.7*‡
Arterial pH	C	7.39 ± 0.09	7.41 ± 0.06	7.33 ± 0.06	7.32 ± 0.05
	E	7.36 ± 0.05	7.39 ± 0.05	7.41 ± 0.07	7.34 ± 0.05
Arterial lactate (mmol/L)	C	3.6 ± 1.5	3.1 ± 1.5	4.7 ± 2.2	5.9 ± 2.4†
	E	3.5 ± 1.6	3.3 ± 1.7	4.5 ± 2.5	4.8 ± 2.4†

Data are expressed as mean ± SD. The number of rabbits examined is 14 in each group.
* $P < 0.05$ versus Group C; † $P < 0.05$; ‡ $P < 0.01$ versus 0 h.

Table 2. Effects of Epidural Analgesia on Splanchnic Circulation Variables in Endotoxemic Rabbits

	Group	Baseline	0 h	2 h	4 h
Portal blood flow (mL/min)	C	126 ± 32	93 ± 24	116 ± 18	111 ± 29
	E	97 ± 21	82 ± 26	102 ± 33	97 ± 20
Portal oxygen content (mL O_2/L)	C	11.4 ± 1.9	10.6 ± 1.7	10.3 ± 1.5	9.3 ± 2.0
	E	11.0 ± 2.2	9.1 ± 2.2	8.1 ± 1.5*	6.6 ± 1.2*†
Oxygen extraction ratio (%)	C	18 ± 14	20 ± 11	14 ± 10	17 ± 16
	E	16 ± 7	23 ± 12	17 ± 6	22 ± 12
Portal lactate (mmol/L)	C	3.6 ± 1.5	3.1 ± 1.4	4.8 ± 2.2	5.9 ± 2.4†
	E	3.4 ± 1.6	3.3 ± 1.7	4.3 ± 2.2	4.7 ± 2.3†
Portal pH	C	7.39 ± 0.07	7.41 ± 0.06	7.31 ± 0.07†	7.30 ± 0.05†
	E	7.37 ± 0.05	7.39 ± 0.04	7.36 ± 0.09	7.31 ± 0.04†
pHi	C	7.36 ± 0.05	7.36 ± 0.05	7.21 ± 0.09†	7.20 ± 0.07†
	E	7.36 ± 0.06	7.38 ± 0.08	7.36 ± 0.11**	7.33 ± 0.08**

Data are expressed as mean ± SD. The number of rabbits examined is 14 in each group.
Hb = hemoglobin; pHi = intramucosal pH.
* $P < 0.05$; ** $P < 0.01$ versus Group C; † $P < 0.05$; ‡ $P < 0.01$ versus 0 h.

group. Arterial pH values remained unchanged, whereas arterial lactate showed a mild increase at 4 h of LPS infusion in both groups ($P < 0.05$).

Portal blood flow remained constant during the study periods in both study groups (Table 2). Portal pH values decreased, and lactate concentration increased in both groups ($P < 0.05$). Splanchnic oxygen extraction ratio remained constant during LPS infusion despite a slight increase of portal lactate at 4 h ($P < 0.05$). The pHi values of group E were preserved within the normal range (>7.32), whereas those of group C decreased significantly after LPS administration ($P < 0.05$) (Table 2). The wet-to-dry weight ratio of terminal ileum in group E was significantly smaller versus group C (3.40 ± 1.99 versus 6.15 ± 1.27; $P < 0.01$), and the plasma FD4 concentrations in group E were also significantly less (3.26 ± 0.47 versus 4.13 ± 0.98 mg/mg-protein; $P < 0.05$), indicating that the increase of endothelial and intestinal wall permeability induced by LPS infusion were significantly reduced by epidural analgesia.

Figure 1 illustrates representative microscopic pictures of villi of distal ileum in normal rabbits ($n = 3$), group C rabbits, and group E rabbits using light microscopy. The villi of the rabbits in group C showed definitive evidence of mucosal injury, such as disruption of microvilli, lifting of the epithelium from the basal lamina, and submucosal edema (Panel B), whereas those in group E seemed near normal (Panel C). The mucosal injury of distal ileum observed in group C was significantly attenuated in group E (Fig. 2) ($P < 0.01$).

Figure 3 illustrates the changes of plasma NOx and TNF-α concentrations at the 2- and 4-h study periods. Both arterial and portal NOx levels were not changed during endotoxin infusion in either study group (Fig. 3A). The level of TNF-α in group C was significantly reduced in group E after 2 h of endotoxin infusion (Fig. 3B). The difference of TNF-α levels found at the 2-h period between the groups disappeared at 4 h. The portal thrombomodulin level was comparable at 0 h and increased significantly to the similar extent in both group C

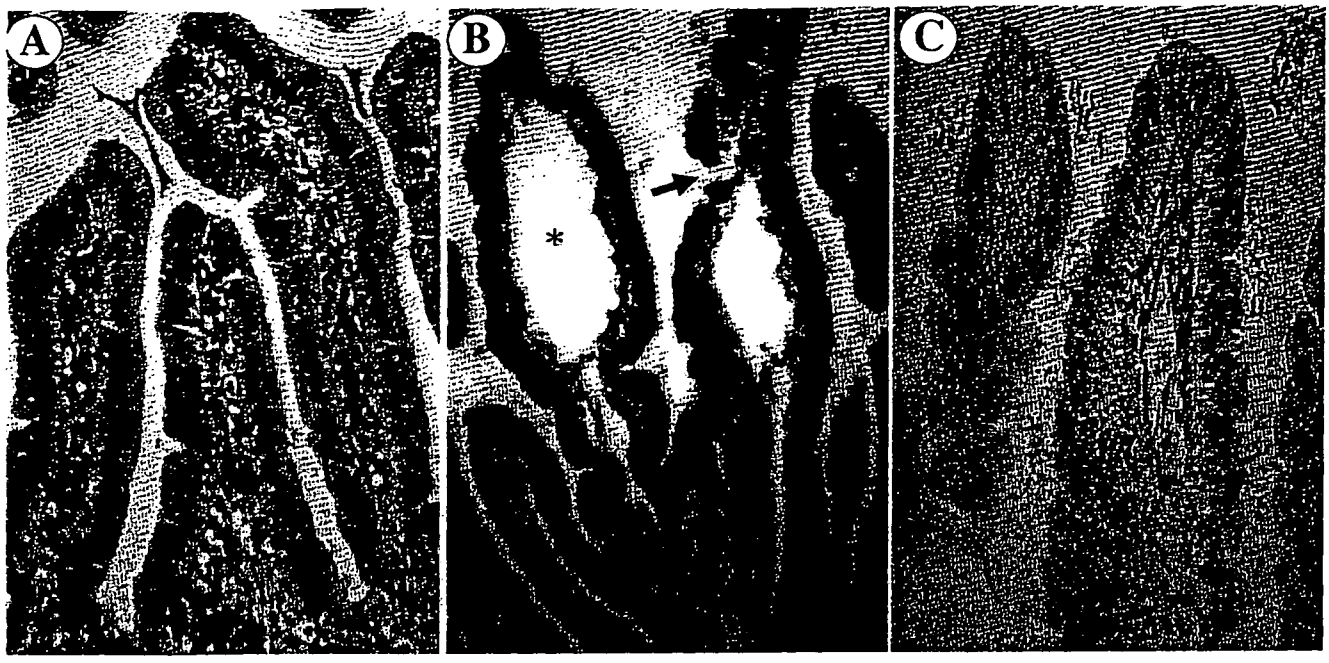

Figure 1. Representative pictures showing villi of distal ileum captured by light microscopy (×100) from rabbits with toluidine blue staining. (A) A representative villi of distal ileum from a normal rabbit. Bar = 50 μm. (B) A representative villi of distal ileum from a rabbit in group C. Note that the epithelial lifting to denuded tips of villi (arrow) and increased cellularity of lamina propria (asterisk) are evident in the ileum. (C) A representative villi of distal ileum from a rabbit in group E. Note near norma-appearing structure of ileal mucosa.

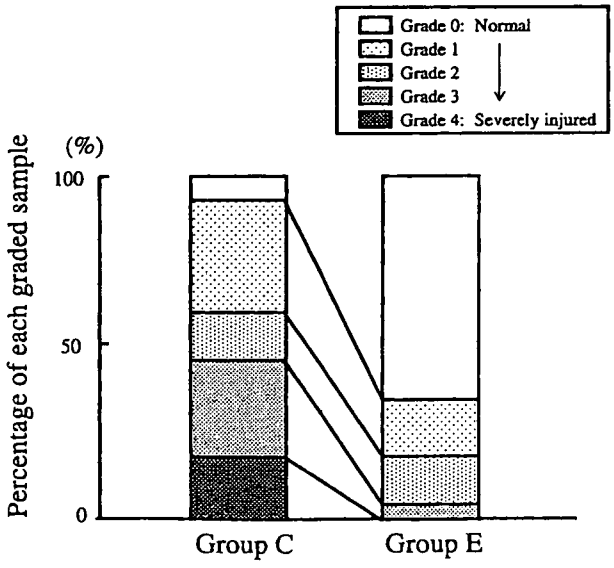

Figure 2. Effects of epidural analgesia on the severity of mucosal injury during endotoxemia. The degree of mucosal injury was graded on a scale of 0–4, with 0 considered normal and 4 representing severe cell disruption. Data are expressed as percentage of 25 fields for each rabbit (n = 14 each group). χ^2 test showed a significant difference between the groups ($P < 0.01$).

and E at 4 h after LPS infusion (213 ± 46 ng/mL to 1037 ± 991 ng/mL versus 225 ± 28 ng/mL to 1395 ± 878 ng/mL, respectively; $P < 0.01$), indicating that endotoxin-induced endothelial injury was not modulated by epidural analgesia.

Discussion

The current study indicates that the application of epidural analgesia with lidocaine in endotoxemic hosts attenuates the progression of intramucosal acidosis, the increase of intestinal permeability, and the structural alterations of intestinal villi, possibly through the restoration of microcirculation. In addition, the protective effects are independent from modulation of endotoxin-induced endothelial cell activation to liberate thrombomodulin. These beneficial effects were noted, even though the epidural block was accompanied by a significant decrease of perfusion pressure and arterial oxygen content, which could be potent confounding factors to deteriorate gut mucosal oxygenation.

Our previous study using an acute hypoxia model showed the similar progression of moderate hemodilution (arterial hemoglobin, 7.7 ± 0.8 g/dL) under approximately one-fifth fluid volume resuscitation compared with this endotoxemia model (7). Because persistent bleeding was not observed after the preparatory surgery, aggressive fluid resuscitation and frequent blood sampling were the primary contributors to this progressive hemodilution in both groups. Thus, the augmentation of hemodilution only observed in group E could be attributed to the presence of epidural analgesia *per se*. A previous study demonstrated that thoracic epidural anesthesia with extra intravascular fluid administration induced significant hemodilution but not with basic fluid infusion in healthy pigs

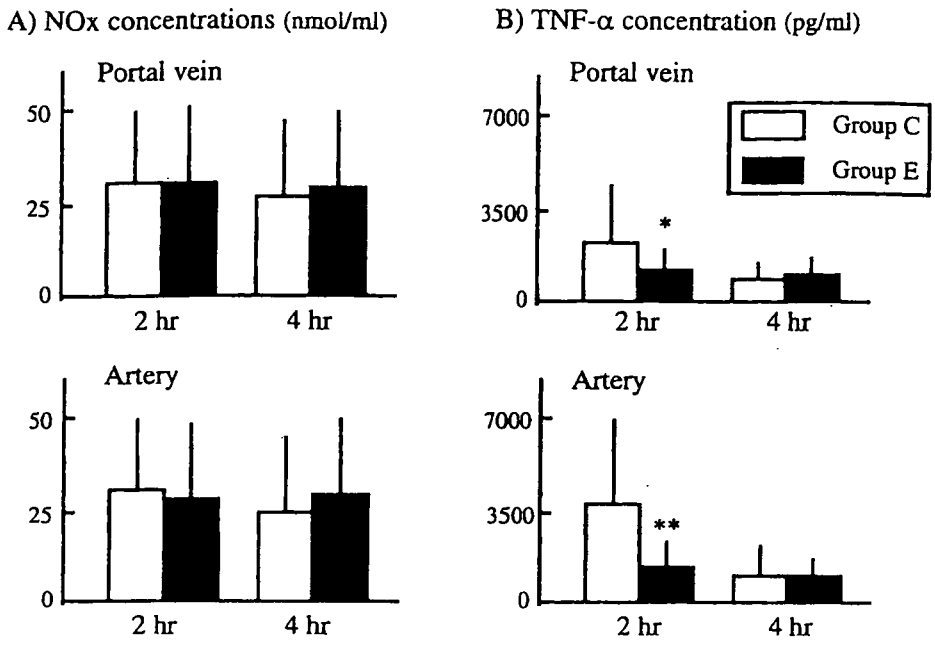

Figure 3. Changes of plasma NOx (A) and tumor necrosis factor (TNF)-α (B) concentrations during endotoxin infusion. Data are expressed as mean ± SD (n = 14 in each group). *P < 0.05; **P < 0.01 versus group C. No significant differences were found in NOx data between the groups.

(18). Interestingly, such additional fluid volume did not restore epidural anesthesia-induced hypotension, which was consistent with our findings. Another study showed that intravascular fluid administration with crystalloid solution resulted in more pronounced hemodilution in patients who developed hypotension during epidural anesthesia (19). Although the precise mechanisms remain unclear (19), either hypovolemia or hypotension caused by epidural anesthesia is able to recruit a considerable quantity of fluid from skin and skeletal muscle (20,21) in combination with its restoring effects of increased vascular permeability during endotoxemia. Some may argue that moderate hemodilution *per se* rather than epidural lidocaine protects mucosal microcirculation of the gut. In a porcine model, gut mucosal oxygen supply was well maintained to systemic hematocrit values of approximately 10%, whereas serosal tissue oxygen supply decreased (22). Conversely, another study reported that acute severe hemodilution from 20% to 14% of hematocrit, close to the level observed in group E, exhausted the compensatory mechanisms of splanchnic circulation (23). In normal rats, the critical hematocrit for intestinal tissue oxygenation was approximately 16% (24). Therefore, the hemoglobin concentration observed in group E seems close to the level of critical hematocrit, not providing further beneficial aspects of hemodilution on the microcirculation.

To explain the initial host response against endotoxin exposure, it is postulated that prototypic cytokines like TNF-α are discharged first from macrophages and monocytes, subsequently initiating a second level of inflammatory cascades such as interleukin-6 and damaging gut mucosa by modulating splanchnic oxygen transport and intramucosal microcirculation (25). The TNF-α levels in endotoxemia are characterized by a rapid increase within 30 min, followed by a one-hour peak and a decrease during the next two hours (26). Considering these kinetics, epidural analgesia may have been able to modulate the process to discharge TNF-α at the early stage of endotoxemia. Simultaneously, the decrease of plasma TNF-α levels at a later stage, accompanied by a loss of significant difference between the groups, seems to be rational, although the serum TNF-α profile in human and animal sepsis models is an issue of much debate regarding its time course and magnitude (8,9,26–28). However, a local increase of such cytokines and oxygen radicals could, in turn, upregulate inducible NO synthase expression, leading to a prolonged increase of NOx levels in tissue and plasma. A previous study showed that endogenous production of NO played an important role in the modulation of gut permeability in rats (29). Although we were unable to show any significant alterations of the NOx levels in this model (Fig. 3), it was consistent with a previous animal study that demonstrated a species-specific modulation of NO pathway after endotoxin injection in rabbits (9). Previous studies showing a significant increase of NOx in endotoxemia were applied to a shock model with a single injection of large-dose endotoxin (9,30). However, in the present study, we intended to mimic a more clinically relevant model of sepsis by using small-dose endotoxin infusion, presenting a normotensive hyperdynamic circulatory state. Another important finding of this study was to show a marked increase of plasma thrombomodulin in both study groups. Thrombomodulin, which plays a crucial role in hemostasis by binding thrombin and subsequently converting protein C to its active form, has been recognized as a sensitive marker of endothelial injury

(10). In the present study, such endothelial injury was not modulated by epidural analgesia. However, intestinal edema through an increased vascular permeability was significantly reduced by application of epidural analgesia. These conflicting results suggest that (a) upregulation of NO in this model might not be involved so significantly as is detected, (b) protein leakage through separation of endothelial tight junctions, rather than endothelial cell activation liberating thrombomodulin, is a major element to develop tissue edema, and (c) plasma thrombomodulin does not always mirror the whole profile of endothelial cell injury.

The clinical implications of the present study should be interpreted with caution because endotoxemia or severe sepsis is likely accompanied by the risk of infection such as meningitis or coagulation abnormalities such as low platelet count and coagulation factors that limit the indication of epidural analgesia. Furthermore, application of epidural anesthesia and analgesia may worsen the stability of systemic hemodynamics in critically ill patients who are treated with aggressive fluid resuscitation and vasoactive drugs. Indeed, MAP in group E was significantly depressed versus MAP in group C. Even with such a confounding factor, epidural analgesia shows a protective property on gut mucosa. Although it remains to be determined whether epidural analgesia can restore gut barrier dysfunction when it has already been established, epidural analgesia may be indicated for patients with functional obstruction of gastrointestinal tract or major vascular surgery who may develop increased mucosal permeability and subsequent bacterial translocation (31,32). In addition, potent analgesic effects of epidural blockade in this acutely instrumented model might provide more optimal analgesia and subsequently blunt stress responses, resulting in the reduction of discharges of vasoconstrictive mediators and the preservation of gut mucosal microcirculation. Finally, small plasma concentrations of lidocaine, absorbed from the epidural space in group E, could have modified the results, although they were all less than the detectable limit of the assay we applied. A previous study showed that lidocaine (plasma concentration, 1.4–2.5 μg/mL) reduced the extravasation of albumin in the lungs of endotoxemic rabbits (33). Another study reported that IV pretreatment with the same dose of lidocaine in rats attenuated endotoxin-induced increases in leukocyte adhesion and transvascular leakage against albumin (34). However, there is a possibility that small-dose lidocaine infusion, inducing plasma lidocaine at less than the detectable range, via the parenteral route might have produced the similar results.

In conclusion, the present study demonstrated that epidural analgesia prevented endotoxin-induced functional and structural alterations of gut mucosa in rabbits without modulating injured endothelial cells. Although further investigation is warranted, this study suggests that epidural anesthesia may be given to critically ill patients who are at risk of gut barrier dysfunction as a therapeutic option to preserve functional integrity.

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SIVELESTAT, A NEUTROPHIL ELASTASE INHIBITOR, ATTENUATES NEUTROPHIL PRIMING AFTER HEPATOENTERIC ISCHEMIA IN RABBITS

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ABSTRACT—Neutrophils play an important role in ischemia-reperfusion injury. The neutrophil elastase not only causes tissue damage, but also mediates neutrophil priming. In the present study, we use a rabbit model of hepatoenteric ischemia-reperfusion to test the hypothesis that neutrophil elastase inhibition ameliorates an ischemia-reperfusion injury by attenuating neutrophil priming and suppressing enzymatic activity. Twenty-four Japanese white rabbits underwent 30 min of supraceliac aortic cross-clamping and 180 min of reperfusion under isoflurane anesthesia. The rabbits randomly received the neutrophil elastase inhibitor, sivelestat (n = 10), or saline (n = 14). Neutrophil priming was then assayed with luminol-dependent neutrophil chemiluminescence. Hepatic, intestinal, renal, and pulmonary damages were assessed with serum transaminase, lactate dehydrogenase concentrations, urinary *N*-acetyl glucosaminidase activity, and protein concentration in *post mortem* bronchoalveolar lavage fluid. We discovered that neutrophil elastase inhibition suppressed plasma neutrophil elastase, and that lipid peroxide concentrations increased after reperfusion. It improved ischemia-reperfusion injuries in the liver, intestine, kidney, and lung. Furthermore, inhibition of neutrophil elastase with sivelestat significantly attenuated post-reperfusion neutrophil priming. The results of this study demonstrate that neutrophil elastase inhibition could effectively attenuate an ischemia-reperfusion injury caused by supraceliac aortic cross-clamping, most likely from the attenuation of neutrophil priming.

KEYWORDS—Reperfusion, priming, chemiluminescence, liver, kidney, lung

INTRODUCTION

Hepatoenteric ischemia and reperfusion can trigger injuries in reperfused and remote tissues, and can frequently cause multiple organ dysfunction syndrome (1–3). Reperfusion of the intestine and liver elaborates various inflammatory mediators such as xanthine oxidase (4) and inflammatory cytokines (5). These mediators may promote neutrophil priming, which is defined as the amplification of a neutrophil response to a given stimulus after prior exposure to a different agonist (6). Primed neutrophils adhere endothelial cells on the reperfused and remote vascular beds and elicit oxygen free radical and protease release (7). Thus, neutrophil priming is a crucial step in the pathophysiology of reperfusion injury after hepatoenteric ischemia.

Among the damaging substances released from primed neutrophils, neutrophil elastase (NE) has been regarded as a key mediator because it degrades the basement membrane and other important intercellular matrixes to cause severe tissue damage. Recent investigations revealed that NE could enhance cytokine production (8), adhesion molecule expression (9), and superoxide release (10). Therefore, it is plausible to hypothesize that NE inhibition attenuates reperfusion injury by suppressing neutrophil priming. However, this possibility has not been extensively studied.

Sivelestat (ONO-5046; *N*-[2-(4-[2,2-dimethylpropionyloxy] phenylsulphonyl-amino) benzoyl] amino acetic acid; Ono Pharmaceuticals, Osaka, Japan) is a specific and competitive NE inhibitor in various species, including rabbits (11). This agent has several advantages over the natural α1-protease inhibitor, such as its low molecular weight and its good resistance to inactivation by oxygen free radicals (11). Several previous animal studies discovered that sivelestat mended various types of tissue injuries, including ischemic as well as endotoxin-induced liver injury (12, 13), aspiration pneumonia (14), acute lung injury (15–18), and hemorrhagic shock (19).

We hypothesized that NE inhibition by sivelestat significantly attenuated ischemia-reperfusion injury by modulating neutrophil priming. Using a rabbit model of hepatoenteric ischemia-reperfusion, we investigated the effects of NE inhibition on neutrophil priming and hepatic, intestinal, renal, and pulmonary injuries.

MATERIALS AND METHODS

This study was performed in compliance with the Guide for the Care and Use of Laboratory Animals and was approved by the Animal Review Committee of Keio University.

Surgical preparation

Twenty-four male Japanese white rabbits weighing between 2 and 2.5 kg were fasted for 24 h, but were allowed free access to water before the experiment. Each rabbit was anesthetized with intramuscular ketamine (50 mg/kg) and xylazine (5 mg/kg), and was subsequently anesthetized with 1.5% isoflurane in 100% oxygen. After a tracheostomy, the rabbit was mechanically ventilated to maintain $PaCO_2$ between 30 and 45 mmHg. Lactated Ringer's solution was infused at a rate of 20 mL/kg/h, and the rabbit's right carotid artery was cannulated for blood pressure monitoring.

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Intervention

After a 15-min stabilizing period, the rabbits were randomly assigned to a treatment group (n = 10) or a control group (n = 14). The rabbits in the treatment group intravenously received 10 mg/kg sivelestat followed by a 10 mg/kg/h infusion throughout the study period. The dose of sivelestat described above was based on the previous report (14). Those in the control group received the same amount of saline. Thereafter, each rabbit underwent a midline laparotomy and had its supraceliac abdominal aorta clamped for 30 min. During the cross-clamp, nitroglycerin (0.5 mg/h) was continuously infused to control the rabbit's blood pressure, and 10 mg of lidocaine was intravenously administered if ventricular arrhythmia occurred. After removing the clamp, 50 μg of phenylephrine was intravenously administered if the mean arterial pressure was below 50 mmHg. The rabbits were observed for 180 min after reperfusion and were then killed by pentobarbital overdose.

Arterial blood samples were corrected at the following time points: after the stabilizing period (baseline), at the end of aortic occlusion (I30), and at 60, 120, and 180 min after reperfusion (R60, R120, and R180, respectively). Arterial blood gas and blood lactate were also measured. Neutrophils were counted with a blood cell counter and by using Diff-Quik stain (Baxter Diagnostics, Issaquah, WA).

Neutrophil priming

Blood samples obtained form the rabbits were used for the neutrophil priming assay. This measures the release of reactive oxygen species from *in vitro*-stimulated neutrophils with luminol-dependent (LD) neutrophil chemiluminescence (CL) (20). The reaction mixture consisted of 1.7 mL of diluted whole luminol solution and 0.2 mL of a 1 mM luminol solution. After preincubation at 37°C, 5 mg/mL opsonized zymosan was added to the mixture and the peak CL value during the 20-min assay time was used as a neutrophil-priming indicator.

NE and lipid peroxide (LPO)

The plasma NE concentration was photometrically determined using a UV-160 Spectrophotometer (Shimadzu Corp., Kyoto, Japan) (21). Plasma LPO concentration was assayed photometrically (LPO-586; Bioxytech, Portland, OR).

Ischemia-reperfusion organ damage

Organ damage caused by aortic occlusion-reperfusion was assessed by serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and urinary *N*-acetyl glucosaminidase (NAG) activities. These measurements were conducted at baseline and at 180 min after reperfusion.

Pulmonary damage was assessed by the protein concentration in the bronchoalveolar lavage fluid (BALF). After authorizing the animals, 20 mL of warm saline was intratracheally infused and gently aspirated. This procedure was repeated three times and the protein concentration was assayed.

Statistical analysis

The results are expressed in the form of mean ± SD. Serially measured parameters were compared with two-way analysis of variance (ANOVA). If the difference was statistically significant, an unpaired *t* test was used. To analyze the difference within each group, a repeated-measure ANOVA and a *post hoc* Bonferroni test were used. Other data between the two groups were compared with an unpaired *t* test. A *P* value less than 0.05 was considered statistically significant.

RESULTS

Four rabbits in the control group died during the reperfusion period due to hypotension that was unresponsive to the vasoconstrictor and fluid loading. Ten animals in each group completed the study protocol and were included in the analyses.

Hemodynamics and blood gas data

The hemodynamic parameters and blood gas data are respectively summarized in Tables 1 and 2. The doses of phenylephrine administered after aortic declamping were not significantly different between the two groups (a total of 1.2 mg in four control animals vs. 0.6 mg in three treatment animals). At 180 min after reperfusion, A-aDO_2 significantly increased in the control group compared with baseline (191 ± 82 mmHg vs. 123 ± 39 mmHg, $P < 0.05$). However, the A-aDO_2 of the treatment group did not change much after reperfusion compared with baseline (161 ± 53 mmHg vs.131 ± 50 mmHg). In both groups, the base deficit remained elevated when compared with the baseline value throughout the study period. However, the base deficit was relatively lower in the treatment group after reperfusion and achieved a statistical significant level at 180 min after reperfusion. The blood lactate significantly increased during occlusion in both groups. However, it steadily decreased after reperfusion in the treatment group, and was significantly lower than that of the control group at 180 min after reperfusion.

Neutrophil parameters

The blood neutrophil count slightly decreased after 30 min of hepatoenteric ischemia in both groups (8500 ± 2600/mm^3 vs. 4700 ± 2900/mm^3 in the control group and 7800 ± 2300/mm^3 vs. 4800 ± 2200/mm^3 in the treatment group). In the control group, the neutrophil count quickly reversed the trend and increased during the reperfusion period (5600 ± 2300/mm^3 at R60 and 6200 ± 2600/mm^3 at R180). In the treatment group, it further decreased 60 min after reperfusion, and then turned back (3500 ± 2800/mm^3 at R60 and 5400 ± 1600/mm^3 at R180). However, the difference between these groups did not reach a statistically significant level.

Figure 1 displays the peak LDCL value, which reflects the neutrophil priming degree. In the control group, this parameter gradually increased after reperfusion and reached a statistically significant level 120 min after reperfusion when compared with baseline. In the treatment group, it remained unchanged throughout the study period and was significantly different from the control group during three stages of reperfusion.

Plasma NE activity significantly increased 180 min after reperfusion in the control group, as shown in Figure 2. Sivelestat suppressed NE activity after reperfusion. However, the plasma LPO concentration failed to achieve a statistical difference between the two groups after reperfusion ($P = 0.061$).

TABLE 1. Mean arterial pressure and heart rate during the study period

	Baseline	I 30	R 60	R 120	R 180
Mean arterial pressure (mmHg)					
Control	72 ± 35	109 ± 40*	61 ± 16	55 ± 16	65 ± 18
Treatment	69 ± 11	129 ± 14*	70 ± 16	66 ± 16	66 ± 24
Heart rate (bpm)					
Control	261 ± 37	213 ± 28*	262 ± 19	270 ± 25	281 ± 28
Treatment	256 ± 45	198 ± 32*	272 ± 19	277 ± 25	281 ± 29

Values are expressed as mean ± SD.
*$P < 0.05$ versus baseline. I 30, at the end of aortic occlusion; R 60, 60 min after reperfusion; R 120, 120 min after reperfusion; R 180, 180 min after reperfusion.

TABLE 2. Blood gas analysis and lactate concentrations

	Baseline	I 30	R 60	R 120	R 180
PaO_2 (mmHg)					
Control	549 ± 36	567 ± 23	519 ± 48	464 ± 94	481 ± 81
Treatment	508 ± 49	553 ± 58	530 ± 67	525 ± 51	545 ± 47#
$PaCO_2$ (mmHg)					
Control	39 ± 7	23 ± 5*	37 ± 6	41 ± 11	39 ± 7
Treatment	42 ± 10	23 ± 3*	35 ± 6	37 ± 6	35 ± 4
Base deficit (mEq/L)					
Control	2.4 ± 2.6	9.0 ± 3.6*	10.5 ± 3.8*	10.3 ± 2.9*	10.3 ± 3.1*
Treatment	1.3 ± 4.0	6.3 ± 3.3*	5.9 ± 3.7*	6.1 ± 3.9*	5.9 ± 3.6*#
Lactate (mM)					
Control	1.9 ± 3.6	9.5 ± 2.4*	6.9 ± 2.2*	5.0 ± 2.8	5.2 ± 3.5
Treatment	1.7 ± 3.0	8.2 ± 2.3*	4.0 ± 1.3	2.7 ± 1.8	2.4 ± 0.8#

Values are expressed as mean ± SD.
#$P < 0.05$ versus control.
*$P < 0.05$ versus baseline. I 30, at the end of aortic occlusion; R 60, 60 min after reperfusion; R 120, 120 min after reperfusion; R 180, 180 min after reperfusion.

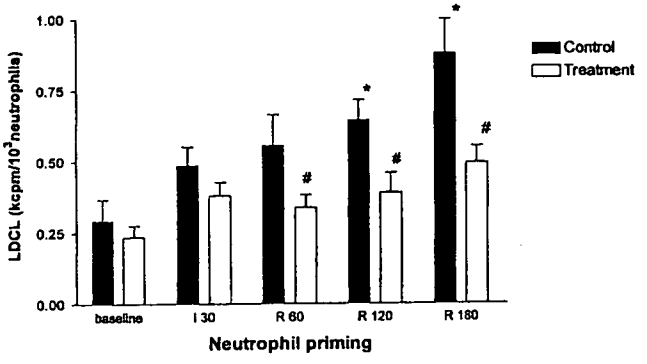

FIG. 1. **Neutrophil priming assessed with the peak value of luminol-dependent neutrophil chemiluminescence in the control group (shaded bar, n = 10) and treatment group (nonshaded bar, n = 10).** Data were corrected with a blood neutrophil count and are expressed in the form of mean ± SD. The unit of peak CL was kcount per minute per 10^3 neutrophils. *$P < 0.05$ versus baseline; #$P < 0.05$ versus control. I 30, At the end of aortic occlusion; R 60, 60 min after reperfusion; R 120, 120 min after reperfusion; R 180, 180 min after reperfusion.

Organ damage

The organ damage results are summarized in Table 3. Baseline values were not much different between the two groups. Sivelestat significantly attenuated the increase of the enzymes, and also significantly suppressed the increase of urinary NAG activity after reperfusion. Two animals in the control group became anuric after reperfusion and were thus excluded from the NAG analysis. *Post mortem* BALF analysis revealed a high protein concentration in the BALF after hepatoenteric ischemia-reperfusion. The rabbits that received the sivelestat showed lower protein concentrations in BALF 180 min after reperfusion.

DISCUSSION

In the present study, we found that 30 min of hepatoenteric ischemia caused progressive increases of zymosan-activated LDCL and plasma NE concentrations after reperfusion. Reperfused organs such as the liver, intestine, and kidney were significantly damaged. Additionally, the high protein concentration in BALF 180 min after reperfusion implies that pulmonary vascular permeability was increased. These results convincingly suggest that the circulating neutrophils are primed and that they mediate reperfusion injuries in the hepatoenteric region and lung. Administration of sivelestat, a specific NE inhibitor, throughout the ischemic and reperfusion periods significantly attenuated neutrophil priming and ameliorated reperfusion injuries in the liver, intestine, kidney, and lung. These results indicate that the inhibition of NE is able to extend an ischemia-reperfusion injury by suppressing neutrophil priming.

The primed neutrophils adhering to the vessel walls release toxic products, such as reactive oxygen metabolites and proteases, to promote endothelial damage (22–24). Conner et al. (7) reported that there was a strong positive relation between the neutrophil priming state and the degree of pulmonary capillary leakage after gut ischemias in rats. There is a natural defense system, such as the α1-protease inhibitor, that protects the endothelium from neutrophil elastase. However, the protective mechanism is conceivably overwhelmed by pathophysiologic processes, including the firm adhesion of the neutrophil to the endothelial cells and the inactivation of the α1-protease inhibitor by concomitantly released oxygen free radicals (25). Several therapeutic approaches, such as the xanthine oxidase inhibitor (4) and the oxygen free radical scavenger (26), have been investigated to block the aforementioned pathophysiologic processes in ischemia-reperfusion injuries. Previous investigations have concluded that the suppression of released NE contributed to tissue injury attenuation and improvement of the outcome. However, neutrophil elastase was reported to promote neutrophil priming (27). Our findings suggest another therapeutic potential of NE inhibition that suppresses neutrophil priming in ischemia-reperfusion injuries.

The findings of this study confirm several pervious reports that circulating neutrophils are primed for superoxide anion release after hepatoenteric ischemia-reperfusion (28, 29). Additionally, we also observed that plasma NE activity and LPO concentrations were significantly increased after reperfusion. Because the release of reactive oxygen species and

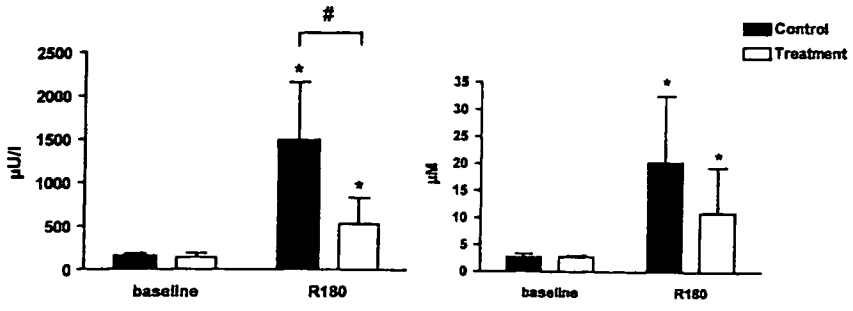

FIG. 2. **Plasma NE (left) and LPO (right) concentrations in the control group (shaded bar, n = 10) and treatment group (nonshaded bar, n = 10).** Data are expressed as mean ± SD. *$P < 0.05$ versus baseline; #$P < 0.05$ versus control. R 180, 180 min after reperfusion.

various proteases from neutrophils depends upon neutrophil-endothelial adhesion (30), neutrophils adhering to the endothelium are supposed to actively release toxic substances while circulating neutrophils are primed. There are several possibilities why NE inhibition can attenuate reperfusion injury. First, NE has been implicated in the conversion from xanthine dehydrogenase to xanthine oxidase during ischemia (31). Therefore, inhibition of NE may attenuate neutrophil priming by suppressing xanthine oxidase release and subsequent superoxide generation after reperfusion. Alternatively, suppression of inflammatory cytokines may also be a possible mechanism of the NE suppression beneficial effect. This speculation may be supported from the previous study that NE inhibition significantly reduces cytokine-induced neutrophil chemoattractant production, which is equivalent to human IL-8, after hepatic ischemia-reperfusion in rats (8).

Supraceliac aortic cross-clamping also caused prolonged lactic acidosis (32). Although the exact pathophysiologic mechanisms are not yet clearly defined, these changes are commonly noticed in a clinical setting and supposedly reflect ischemic damage in the intestine. The base excess and plasma lactate concentrations during the ischemic period were comparable between the two groups in this study. These parameters returned toward baseline in the animals treated with sivelestat, but stayed elevated in the control animals. Previous reports indicate that the gut and liver play significant roles in lactate production and uptake. Therefore, the attenuation of intestinal and hepatic injuries after reperfusion by sivelestat supposedly accounts for this difference. Additionally, postischemic tissue perfusion is reduced by endothelial dysfunction and neutrophil plugging, which cause a no-reflow phenomenon. The beneficial effects of sivelestat on lactate metabolism may be attributed to the protection of parenchymal and endothelial cells. Other data, such as transaminases, LDH, and urinary NAG activities, also reflect reperfusion-induced tissue damage and indicate that sivelestat significantly attenuated ischemia-reperfusion injuries.

Our experiment demonstrated a pulmonary injury after hepatoenteric ischemia-reperfusion. Many clinical and experimental studies also demonstrated that hepatoenteric ischemia-reperfusion caused significant pulmonary injuries (2,5,33–36). Activation of neutrophils and their sequestration and adherence to the pulmonary endothelium are important steps in the development of a pulmonary microvascular permeability increase. Therefore, sivelestat could significantly attenuate pulmonary capillary damage after reperfusion, possibly by reducing neutrophil accumulation and subsequent endothelial damage in pulmonary microcirculation.

Limitations of the study

The present study has several limitations. We did not measure the effects of NE inhibition on the neutrophil accumulation in tissues and the neutrophil-endothelial interaction. Previous reports have documented that sivelestat attenuated the release of chemoattractant (8) and the expression of adhesion molecules (9). Thus, it is highly possible that sivelestat attenuated reperfusion injury by inhibiting neutrophil adhesion to the endothelium and accumulation into tissues. Another limitation is that we did not conduct postreperfusion blood flow determination at both the systemic and regional levels. In the microcirculation level, activated neutrophils and released substances may participate in the pathophysiology of the postischemic no-reflow phenomenon. NE inhibition may maintain general and regional perfusion states. This possibility needs further investigation. The third limitation is that the circulating neutrophil count slightly decreased in the treatment group after reperfusion, and this result was unexpected because neutrophil priming and activation are believed to trigger upregulated adhesion response and neutrophil accumulation in tissues.

TABLE 3. **Hepatic, renal, and pulmonary damage**

	Control		Treatment	
	Baseline	R 180	Baseline	R 180
Serum aspartate aminotransferase (U/L)	37 ± 12	417 ± 263	36 ± 11	149 ± 91*
Serum alanine aminotransferase (U/L)	17 ± 11	202 ± 88	15 ± 9	80 ± 38*
Serum lactate dehydrogenase (U/L)	236 ± 34	1084 ± 656	229 ± 44	446 ± 133*
Urine *N*-acetyl glucosaminidase (U/L)	3.2 ± 2.9	70.5 ± 42.9	2.5 ± 2.1	36.1 ± 23.1*
BALF protein (mg/dL)	N/A	39.9 ± 37.4	N/A	7.3 ± 1.7*

Values are expressed as mean ± SD. Data were statistically analyzed with an unpaired *t* test.
N/A, not available.
*$P < 0.05$ versus control group.

Possible clinical implications

Clinically, hepatoenteric ischemia-reperfusion carries a significantly high mortality. Although the beneficial effect of allopurinol or tungsten by suppressing xanthine oxidase has been reported in experimental and clinical situations (4, 37), these measures usually require some pretreatment. On the contrary, this study shows that sivelestat is effective when it is administered just before the establishment of ischemia. This is advantageous in many clinical situations, and further study is warranted to determine whether NE inhibition after reperfusion is effective for attenuating reperfusion injuries.

In conclusion, we found that a specific NE inhibitor suppressed postreperfusion neutrophil priming and subsequent intestinal, hepatic, and renal reperfusion injuries in rabbit models of descending aortic occlusion-reperfusion. It also attenuated pulmonary microvascular leaks, as evidenced by the reduced protein concentration in *post mortem* BALF.

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Infusion of the β-adrenergic blocker esmolol attenuates myocardial dysfunction in septic rats*

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Objective: Since β-blocker therapy is known to be effective in patients with an injured heart, such as infarction, we designed the present study to examine the protective effects of infusion of the β1-selective blocker esmolol on myocardial function in peritonitis-induced septic rats using an isolated working heart preparation.

Design: Randomized animal study.

Setting: University research laboratory.

Subjects: Thirty-one rats treated with cecal ligation and perforation to evoke peritonitis.

Interventions: After cecal ligation and perforation, rats were randomly allocated to the control group (normal saline 2 mL/hr, n = 11), low-dose esmolol group (10 mg/kg/hr, n = 10), or high-dose esmolol group (20 mg/kg/hr, n = 10). After obtaining blood samples for measurement of arterial lactate and tumor necrosis factor-α at 24 hrs, we assessed cardiac output, myocardial oxygen consumption, and cardiac efficiency (cardiac output × peak systolic pressure/myocardial oxygen consumption) at various preloads in an isolated perfused heart preparation.

Measurements and Main Results: Esmolol infusion did not cause an elevation of arterial lactate levels but reduced tumor necrosis factor-α concentrations vs. the control group ($p < .05$). Both cardiac output and cardiac efficiency in the esmolol-treated rats were significantly higher throughout the study periods vs. the control group ($p < .05$).

Conclusions: Esmolol infusion in sepsis improved oxygen utilization of myocardium and preserved myocardial function. (Crit Care Med 2005; 33:2294–2301)

KEY WORDS: β1-adrenergic receptor; hyperdynamic sepsis; tumor necrosis factor-α; working heart preparation

Sepsis is a clinical syndrome that arises from an inappropriate and excessive systemic inflammatory response against infection (1). With adequate fluid resuscitation and pharmacologic interventions, systemic hemodynamics of sepsis is characterized by a hyperdynamic circulatory state, resulting in augmentation of oxygen supply to tissues. The importance of sufficient tissue oxygenation was recently addressed in a large-scale clinical trial in which patients at the early stage of sepsis were treated by aggressive management to optimize hemodynamic function (2). During the progression of sepsis, however, regional tissue dysoxia becomes evident and organ dysfunction including heart dysfunction develops (3). Several mechanisms are considered to be responsible for myocardial dysfunction in sepsis (4, 5). For example, proinflammatory cytokines like tumor necrosis factor (TNF)-α have been shown to play a consequential role in the pathogenesis of myocardial dysfunction in sepsis (6, 7). In addition, the number of β-adrenergic receptors was reduced in critically ill patients involving sepsis (8). Few specific strategies, however, have been demonstrated to restore myocardial dysfunction effectively in sepsis.

There is increasing evidence that β-blocker therapy during perioperative periods improves morbidity and mortality in high-risk patients with ischemic heart disease (9). Another study showed a tenfold decrease in the 30-day perioperative incidence of death from cardiac causes and nonfatal myocardial infarction in β-blocker-treated patients undergoing vascular surgery (10). Such protective effects of β-blocker for ischemic myocardium could be accounted for by an improved balance between oxygen supply and consumption (11), regulation of cytokine release (12, 13), and/or restoration of down-regulated β-receptor (14). A question remains, however, whether β-blocker therapy would improve myocardial dysfunction at a hyperdynamic stage of sepsis where cardiac work is augmented. We therefore designed the present study to examine if infusion of esmolol, a β1-selective adrenergic blocker, suppressed the progression of myocardial dysfunction in a normotensive, hyperdynamic model of septic rats.

METHODS

This study protocol was approved by the animal care and use committee of Keio University School of Medicine.

Animal Preparation. Thirty-one male Wistar rats, weighing 320–360 g, were studied after 3- to 7-day acclimatization periods in our laboratory. With sevoflurane anesthesia in oxygen, the jugular vein and carotid artery were cannulated with a catheter (PE50; Intermedic, Sparks, MD) under sterile conditions. Then a laparotomy was performed and a ligature was placed around the cecum immediately distal to the ileocecal valve. The cecum was then punctured twice with an 18-gauge needle. Fol-

*See also p. 2433.

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