δ Opioid Receptor Antagonist, ICI 174,864, Is Suitable for the Early Treatment of Uncontrolled Hemorrhagic Shock in Rats

Liangming Liu, M.D., Ph.D.,* Kunlun Tian, M.S.,† Yu Zhu, M.S.,† Xiaoli Ding, M.S.,† Tao Li, Ph.D.‡

ABSTRACT

Background: Fluid resuscitation is the essential step for early treatment of traumatic hemorrhagic shock. However, its implementation is greatly limited before hospital or during evacuation. The authors investigated whether δ opioid receptor antagonist ICI 174,864 was suitable for the early treatment of traumatic hemorrhagic shock.

Methods: With uncontrolled hemorrhagic-shock rats, the antishock effects of six dosages of ICI 174,864 (0.1, 0.3, 0.5, 1, 3, and 5 mg/kg) infused with or without a small volume of lactated Ringer's solution (LR) before bleeding controlled or bleeding cessation at different times were observed.

Results: ICI 174,864 (0.1–3 mg/kg) with or without 1/4 volume of LR infusion showed dose-dependent increase in the mean arterial blood pressure, and significantly prolonged the survival time and 8-h survival rate, as compared with ICI 174,864 plus 1/2 volume of LR infusion. The best effect was shown with 3 mg/kg of ICI 174,864. Bleeding cessation at 1, 2, or 3 h during infusion of ICI 174,864 (3 mg/kg) plus 1/4 volume of LR improved subsequent treatment (70% 24-h survival rate vs. 50 and 10% 24-h survival rate in hypotensive resuscitation and LR group, respectively). There was significant improvement in hemodynamic parameters, oxygen delivery, and tissue perfusion of hemorrhagic-shock rats with 3 mg/kg of ICI 174,864 plus 1/4 volume of LR infusion.

Conclusion: δ Opioid receptor antagonist ICI 174,864 alone or with small volume of fluid infusion has good beneficial effect on uncontrolled hemorrhagic shock. Its early application can "buy" time for subsequent treatment of traumatic shock.

What We Already Know about This Topic

- Fluid resuscitation is the cornerstone treatment of acute hemorrhagic shock; however, it may not be available in specific situations, such as the battlefield.
- ICI 174,864, an antagonist of the δ opioid receptors, improves hemodynamics in traumatic shock, independent (or slightly dependent) from fluid resuscitation.

What This Article Tells Us That Is New

- ICI 174,864 with or without low volumes of Ringer's lactate, dose-dependently increased blood pressure and prolonged short-term survival in rats subjected to uncontrolled hemorrhagic shock. Survival was markedly higher in rats treated with ICI 174,864 than those with standard fluid resuscitation once bleeding was controlled.

TRAUMATIC hemorrhagic shock is often seen in civilian and military situations. It is the major cause of early death in injured soldiers, accounting for approximately 50% of deaths of battle personnel.[1] Reports have shown that approximately 40% of trauma-induced deaths occur 5–30 min after trauma.[2] Thus, early emergency care for severe trauma or war wounds is very important.

Fluid resuscitation is the essential step for the treatment of traumatic or hemorrhagic shock. Our previous studies

◆ This article is accompanied by an Editorial View. Please see: Duranteau J, Le Manach Y: δ Opioid receptor antagonists: Do they buy time for traumatic hemorrhagic shock patients? ANESTHESIOLOGY 2013; 119:253–5.

* Professor, † Research Assistant, ‡ Associate Professor, State Key Laboratory of Trauma, Burns, and Combined Injury, Second Department of Research Institute of Surgery, Daping Hospital, the Third Military Medical University, Chongqing, P.R. China.

Received from the State Key Laboratory of Trauma, Burns and Combined Injury. Second Department of Research Institute of Surgery, Daping Hospital, Thirol Millitary Medical University, Chongqing, China. Submitted for publication June 4, 2012. Accepted for publication March 5, 2013. This work was supported by the Key Project of the National Natural Scientific Foundation of China (grant number 30830053; Beijing, China), Natural Scientific Foundation of Chongqing for Distinguished Young Scholars (CSTC2011JJJQ0019; Chongqing, China), and Major State Basic Research Program (2012CB518101; Bejing, China).

Address correspondence to Dr. Li: Second Department of Research Institute of Surgery, Daping Hospital, Third Military Medical University, Daping, Chongqing 400042, P.R. China. lt200132@163.com. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

 Anesthesiology 2013; 119:379-88

and other teams demonstrated that hypotensive resuscitation was beneficial to traumatic shock before bleeding controlled. And we further found that 50–60 mmHg of target mean arterial pressure (MAP) was ideal resuscitation pressure for uncontrolled hemorrhagic shock before bleeding controlled in rats.[3] However, a regular and large volume of fluid infusion at an early stage after trauma (especially after a major terrorist incident) or war wound is not available. Hence, an effective antishock agent that is not dependent (or is only slightly dependent) upon fluid resuscitation is needed at this situation. Antishock agents being used currently, such as dopamine, dobutamine, and calcium-channel blockers require volume resuscitation to elicit their effects.[4,5] Therefore, finding an antishock agent that is independent (or only slightly dependent) upon fluid resuscitation is very important for the early treatment of traumatic shock.

Studies have shown that endogenous opioid peptides have very important effects in the development of many types of circulatory shock.[6] The opioid receptor antagonists naloxone, naltrexone, and nalbuphine had a positive effect on circulatory shock. However, these opioid receptor antagonists are not highly selective for particular subclasses of opioid receptors. They may antagonize μ opioid receptors to affect the pain threshold. Thus, their applications in the management of traumatic shock have some content limitations.[7,8] μ, δ, and κ Receptors are the main opioid receptors distributed within the cardiovascular system. We previously found that δ and κ opioid receptors were the main receptors that participated in traumatic shock and that the antagonist of δ opioid receptors (ICI 174,864) had beneficial effects on traumatic shock.[9] However, it is not clear if ICI 174,864 is suitable for the early treatment of traumatic hemorrhagic shock. On the basis of the previous findings that δ opioid receptor antagonist have some inotropic and vasoconstriction effects,[9–11] we hypothesized that ICI 174,864 is suitable for the early treatment of traumatic hemorrhagic shock. Its antishock effects may be not dependent (or only slightly dependent) on fluid resuscitation and can act as a bridge for the subsequent treatment of traumatic shock.

In order to elucidate and testify this hypothesis with a model of uncontrolled hemorrhagic shock in rats, we investigated: (1) if ICI 174,864 is beneficial for hemorrhagic shock that is independent (or slightly dependent) upon fluid resuscitation, (2) if ICI 174,864 could provide time for subsequent treatment of hemorrhagic shock, and (3) the antishock mechanism of ICI 174,864.

Materials and Methods

Ethical Approval of the Study Protocol

This study was approved by the Research Council and Animal Care and Use Committee of the Research Institute of Surgery, Daping Hospital, Third Military Medical University (Chongqing, People's Republic of China). None of the authors are members of this committee.

Animal Management

Sprague–Dawley rats (220–260 g) were fasted for 12 h, but allowed water *ad libitum* before experimentation. Rats were initially anesthetized with sodium pentobarbital (30 mg/kg). This agent was then added until the rats had no response to a needle stimulus, the total amount of sodium pentobarbital was not more than 50 mg/kg. Rats were breathing spontaneously without mechanical ventilation. The right femoral arteries and veins were catheterized with a polyethylene catheter (outer diameter, 0.965 mm; inner diameter, 0.58 mm) for monitoring MAP and drug administration, respectively. The left ventricle was catheterized with the polyethylene catheter described above for hemodynamic measurement *via* the right carotid artery. To prevent clot formation, the carotid artery catheter was filled with normal (0.9%) saline containing 30 U/ml of heparin. A model of uncontrolled hemorrhagic shock was induced by transection of the splenic parenchyma and one of the branches of the splenic artery, as previously described by our research team.[12] Briefly, after the completion of catheterization, the spleen was exposed after laparotomy. A cross-transection was made in the splenic parenchyma between the two major branches of the splenic artery. One of the major branches of the splenic artery was also transected. Blood was allowed to flow into the abdominal cavity. When the MAP decreased to 40 mmHg, uncontrolled hemorrhagic shock was established for subsequent experiments.

Experimental Protocol

All experiments were carried out in three parts. The first part aimed to investigate if ICI 174,864 is beneficial for hemorrhagic shock that is independent (or slightly dependent) on fluid resuscitation. This was done by observing the effects of ICI 174,864 with or without a small volume of fluid infusion before bleeding was controlled (by ligation of the splenic artery). The second part aimed to investigate if ICI 174,864 could provide time for subsequent treatment of hemorrhagic shock. This was achieved by observing the effects of ligating the splenic artery for different times after administration of ICI 174,864 on subsequent treatment. The third part aimed to investigate the antishock mechanism of ICI 174,864. This was done by observing the effects of ICI 174,864 on hemodynamics, cardiac function, and tissue blood flow during hemorrhagic shock.

First Part of Experiments

Two-hundred ten Sprague–Dawley rats were randomly divided into three groups of 70: ICI 174,864 with 1/2 (17.5 ml/kg) or 1/4 (8.75 ml/kg) volume of blood loss of lactated Ringer's solution (LR) or with no additional fluid infusion (only the solution control, 1 ml/kg of LR). Each group was further divided into seven subgroups of 10 rats (n = 10 per subgroup): 0.1, 0.3, 0.5, 1, 3, and 5 mg/kg of ICI 174,864 and fluid control (1 ml/kg of LR). Experiments were defined in three phases. Phase I was the model stage (uncontrolled hemorrhage period), in which blood was

allowed to flow freely into the abdominal cavity. This phase was achieved if the MAP decreased to 40 mmHg and was maintained at this pressure for 20–30 min. Phase II was the period of ICI 174,864 administration, which was 30 min. Phase III was the observation period, in which the MAP, blood loss, and the number of surviving rats after hemorrhage were observed. The transected spleen was not ligated during the entire process (fig. 1).

Second Part of Experiments

According to the results of the first part of the experiments, 3 mg/kg of ICI 174,864 plus 1/4 volume of LR had the best effects for uncontrolled hemorrhagic shock. The second part of the experiments aimed to investigate the effects of stopping the bleeding at different times during the application of ICI 174,864 on subsequent treatment. The experiment was divided into three groups: ICI 174,864 (3 mg/kg) plus 1/4 volume of LR; pure 1/4 volume of LR; and permissive hypotensive resuscitation (in which the MAP was maintained at 50 mmHg with 6% hydroethyl starch 130 plus LR at the ratio of 1:2). Permissive hypotensive resuscitation is regarded as an "ideal" resuscitation strategy for uncontrolled hemorrhagic shock before bleeding is stopped[3,12] and was adopted as the control group in the present study. Each group was further divided into three time points according to ligation of the splenic artery (1, 2, and 3 h after ICI 174,864 administration). Each group for each ligation time contained 10 rats, and the total number of rats in this experiment was 90. After bleeding was controlled (by ligation of the splenic artery), the rats underwent resuscitation with LR plus whole blood (2:1) to achieve a target MAP of 80 mmHg, which could be maintained for 2 h. Survival time and the number of rats that survived was then noted (fig. 2).

Third Part of Experiments

According to the results of the second part of the experiments, all rats in the hypotensive resuscitation group could not maintain the requisite MAP over 3 h in phase II, so a maintenance time of 2 h was selected in this part of the experiments in phase II. This experiment was also divided into three groups (n = 8 rats per group): ICI 174,864 (3 mg/kg) plus 1/4 volume of LR; permissive hypotensive resuscitation; and 1/4 volume of LR control. Animal care and drug administration were the same as described in the second part of the experiments (fig. 3). MAP, hemodynamic parameters (including left intraventricular systolic pressure, maximal change rate of left intraventricular pressure cardiac output, oxygen delivery, and oxygen utilization), blood gases, tissue blood flow of vital organs (liver, kidney, and brain), liver function, and kidney function were observed at baseline, at the end of phase I (model phase), phase II (before ligation), and phase III (maintaining MAP at 80 mmHg for 2 h), and at the end of phase IV (2-h observation period). This was achieved using a Polygraph Physiologic Recorder (SP844, Power Laboratory; AD Instruments, Castle Hill, NSW, Australia), Cardiomax-III Thermodilution Cardiac Output Computer (Columbus Instruments, Columbus, OH), and a Blood Gas Analyzer (Phox plus L; Nova Biomedical, Waltham, MA), as described in our previous work[3,12] (fig. 3).

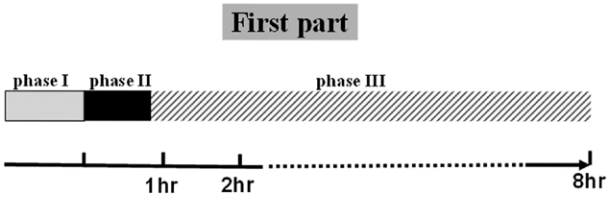

Fig. 1. Experiment protocol (schematic) of part I. Phase I: establishment of a model of uncontrolled hemorrhagic shock; phase II: administration of ICI 174,864 (30–40 min); phase III: observation period.

Statistical Analyses

Data are the mean ± SD of n observations. The parametric data, such as mean arterial blood pressure and left intraventricular systolic pressure, and so on were analyzed by two- or three-way ANOVA, followed by the *post hoc* Tukey test (SPSS 15.0; SPSS Inc., Chicago, IL). The animal survival time was analyzed by median and interquartile ranges. The sample size calculation was mainly based on our previous study and expected treatment effects (increased about 100% for general effect) by power analysis (power set at 80%, α at 0.05, β at 0.20). *P* value less than 0.05 was considered significant.

Results

Part I

Effects of different dosages of ICI 174,864 with or without infusion of a small volume of fluid on uncontrolled hemorrhagic shock.

MAP. ICI 174,864 dose-dependently increased the MAP after hemorrhagic shock in rats with or without infusion of a small volume of LR. ANOVA analyses showed MAP in all ICI 174,864 groups was significantly higher than in control group. Of the dosages of 0.1, 0.3, 0.5, 1, 3, 5 mg/kg of ICI 174,864 given, 3 mg/kg of ICI 174,864 had the best effect for maintaining the MAP for hemorrhagic-shock rats with or without a small volume of LR infusion. MAP in the group of ICI 174,864 at 3 mg/kg without LR infusion could be maintained at approximately 60 mmHg for 3 h (fig. 4A). MAP in the group of ICI 174,864 at 3 mg/kg with 1/4 volume of LR infusion could be maintained at 70 mmHg or lesser for 3 h (fig. 4B). In the group of ICI 174,864 at 3 mg/kg with 1/2 volume of LR infusion, MAP could increase to 50 mmHg after administration, but rapidly decreased to less than 40 mmHg at 2 h, and the maintenance time was shorter than that for ICI 174,864 at 3 mg/kg with 1/4 volume of LR infusion or without LR infusion (fig. 4C).

Blood Loss. Blood loss during the entire treatment period was not significantly different when different doses of ICI 174,864 were used in the same fluid volume group (no

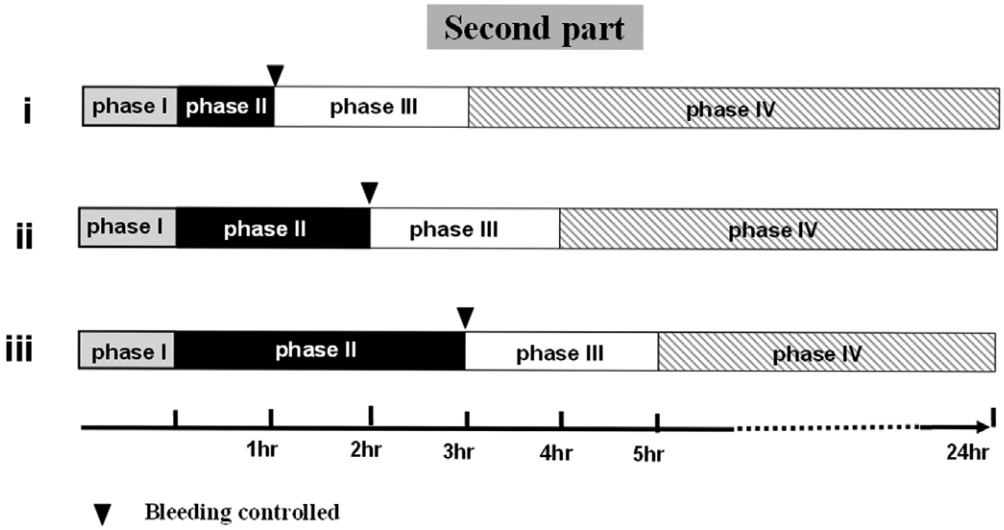

Fig. 2. Experiment protocol (schematic) of part II. i, ii, and iii: Ligation of the splenic artery was undertaken 1, 2, and 3 h after administration of ICI 174,864; phase I: establishment of a model of uncontrolled hemorrhagic shock; phase II: administration of ICI 174,864, LR or permissive hypotensive resuscitation; phase III: resuscitation to a mean arterial pressure of 80 mmHg for 2 h; phase IV: survival observation; *arrow*: bleeding controlled (by ligation of the splenic artery).

fluid group, 1/4 volume of LR group, and 1/2 volume of LR group). However, there were significant differences when different fluid volumes were used. Blood loss in the 1/2 volume of LR infusion group was more than 60% of the total estimated blood volume of a rat, was 55–58% in the 1/4 volume of LR infusion group, and was 47–50% in the group without LR infusion (fig. 4, D–F).

Survival. ICI 174,864 significantly prolonged the survival time and 8-h survival rate in uncontrolled hemorrhagic-shock rats as compared with the control group. ICI 174,864 plus 1/4 volume LR infusion or without LR infusion had the same effect on survival time. However, with respect to prolonging survival time, ICI 174,864 with 1/2 volume LR infusion was inferior to ICI 174,864 with 1/4 volume LR or without LR infusion. The number of rats surviving over 8 h when ICI 174,864 was given at 0.1, 0.3, 0.5, 1, 3, and 5 mg/kg without LR was 1/10, 2/10, 3/10, 3/10, 5/10, and 2/10, respectively. The number was 1/10, 2/10, 3/10, 4/10, 5/10, 3/10, respectively, in the ICI 174,864 plus 1/4 volume LR infusion group. The number was 2/10, 2/10, 3/10, 3/10, 4/10, and 4/10, respectively, in the ICI 174,864 plus 1/2 volume LR infusion group. Of the six doses of ICI 174,864 tested, the 3 mg/kg ICI 174,864 plus 1/4 volume LR group or without LR infusion group had the best 8-h survival rate (both 5/10). The results shown above are shown in figure 4, G–L.

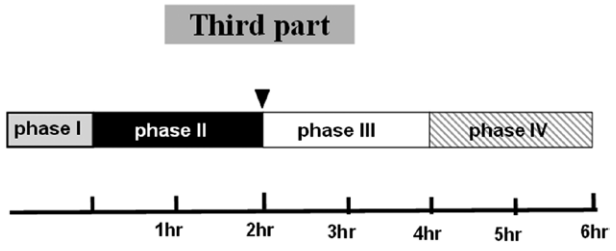

Fig. 3. Experiment protocol (schematic) of part III. Phase I: establishment of a model of uncontrolled hemorrhagic shock; phase II: administration of ICI 174,864, lactated Ringer's solution or permissive hypotensive resuscitation; phase III: resuscitation to a mean artery blood pressure of 80 mmHg for 2 h; phase IV: observation period; *arrow*: bleeding controlled (by ligation of the splenic artery).

Part II

Effects of bleeding cessation at different times after administration of ICI 174,864 upon hemorrhagic shock.

Animal Survival. Median survival time in the ICI 174,864 group at 1, 2, and 3 h of bleeding cessation (by ligation of the splenic artery) was 24, 15.7, and 9.7 h, respectively. In the LR control group (1/4 volume LR) it was 15.1, 5.5, and 0 h, respectively. In the permissive hypotensive resuscitation group it was 6.9, 5.4, and 0 h, respectively. All rats in the LR control and permissive hypotensive resuscitation groups died before the 3-h ligation time point (fig. 5, A and B).

Blood Loss. Blood loss in the hypotensive resuscitation group up to 2-h ligation was 101.23% of the estimated total blood volume. In the ICI 174,864 and LR control group it was only 50.3–56.8%, which was significantly less than hypotensive resuscitation group ($P < 0.01$; fig. 5C).

Fluid Requirement before Ligation of the Splenic Artery. The amount of fluid infusion in the ICI 174,864 and LR control group, irrespective of the ligation time point, was 8.75 ml/kg (1/4 volume of blood shed during the model stage). The fluid requirement in the permissive hypotensive resuscitation group (maintenance of MAP at 50 mmHg) for 1, 2, and 3 h was 53, 72.2, and 81.8 ml/kg, respectively, which was significantly higher than that in the ICI 174,864 and LR control group (fig. 5D).

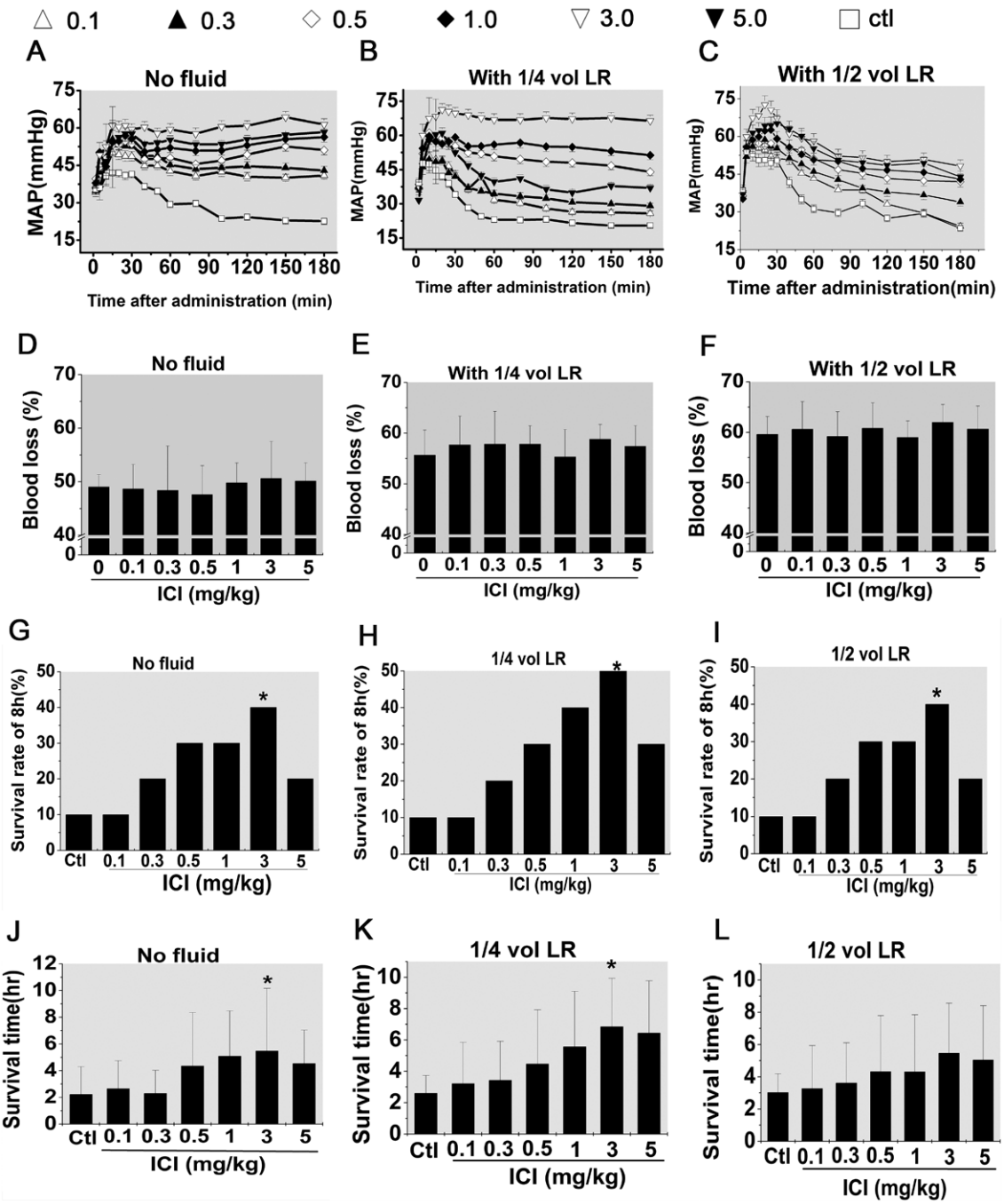

Fig. 4. Effects of different doses of ICI 174,864 with or without 1/2 and 1/4 volume of lactated Ringer's solution (LR) on uncontrolled hemorrhagic shock in rats (n = 10 per each group). Data are mean ± SD. (*A–C*) Changes in mean arterial pressure (MAP) in ICI 174,864 with no infusion of LR, with 1/4 volume of LR infusion, and with 1/2 volume of LR infusion; (*D-F*) blood loss; (*G* and *J*) survival number and survival time in ICI 174,864 without infusion of LR; (*H* and *K*) survival number and survival time in ICI 174,864 with 1/4 volume of LR infusion; (*I* and *L*) survival number and survival time in ICI 174,864 with 1/2 volume of LR infusion. 0.1, 0.3, 0.5, 1, 3, 5 in the figure indicates 0.1, 0.3, 0.5, 1, 3, and 5 mg/kg of ICI 174,864; Ctl = control; ICI = ICI 174,864, delta-opioid receptor antagonist. *$P < 0.05$ as compared with or without infusion LR group.

Part III

Effects of ICI 174,864 on hemodynamics, cardiac function, and tissue blood flow after hemorrhagic shock.

Hemodynamic Parameters. At baseline and at the end of the model stage, hemodynamic parameters (MAP, left intraventricular systolic pressure, and the maximal change rate of left intraventricular pressure) showed no differences in all groups. At 2-h ligation, MAP in the ICI 174,864 and hypotensive resuscitation group was higher than that in the LR control group, and the maximal change rate of left intraventricular pressure in the ICI 174,864 group was higher than that in the hypotensive resuscitation group and LR control

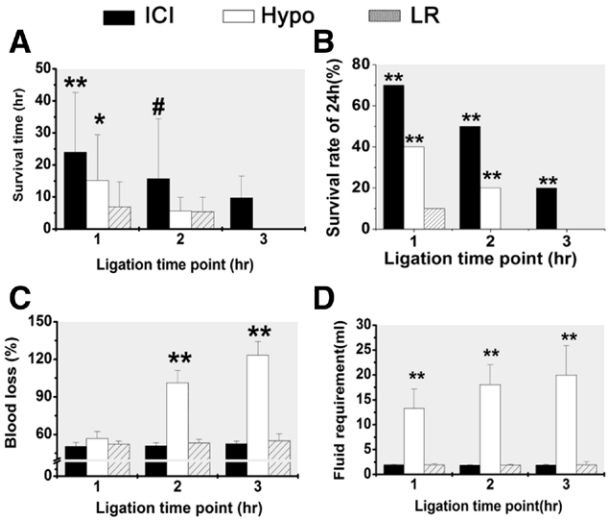

Fig. 5. Effects of different times of ligation of the splenic artery to stop bleeding after administration of ICI 174,864 on hemorrhagic shock in rats (n = 10 per group). (*A*) Survival time after ligation; (*B*) survival number; (*C*) blood loss; (*D*) volume of fluid infusion. Hypo = permissive hypotensive (50 mmHg) resuscitation group; ICI = ICI 174,864, delta-opioid receptor antagonist; ICI-1, -2, -3, hypo-1, -2, LR-1, -2 = different ligation time points; LR = lactated Ringer's solution. *P < 0.05, **P < 0.01, as compared with the LR group; #P < 0.05, as compared with the 50-mmHg group.

group. At the end of phase III and phase IV, left intraventricular systolic pressure and the maximal change rate of left intraventricular pressure in the ICI 174,864 group were higher than those in the 50 mmHg hypotensive resuscitation group, and left intraventricular systolic pressure, and the maximal change rate of left intraventricular pressure in the 50 mmHg hypotensive resuscitation group were higher than those in the LR control group (table 1).

Blood Gases. At baseline and at the end of the model stage, blood pH, Pa_{O_2}, and PCO_2 did not show significant differences in all groups. At the end of phases II, III, and IV, Pa_{O_2} in the ICI 174,864 group was higher than that in the hypotensive resuscitation group and LR control group. Blood pH and PCO_2 did not show significant differences in all groups (table 2).

Cardiac Output, Cardiac Index, and Stroke Index. At baseline, at the end of the model stage and at 2-h ligation, cardiac output, the cardiac index, and the stroke index did not show significant differences in all groups. At the end of phases III and IV, cardiac output, the cardiac index, and the stroke index in the ICI 174,864 group were significantly higher than those in the 50 mmHg hypotensive resuscitation group and LR control group (P < 0.05 or P < 0.01; table 3).

DO_2 and Oxygen Uptake. At baseline and at the end of the model stage, oxygen delivery (DO_2) and oxygen uptake did not show significant differences in all groups. At 2-h ligation and at the end of phases III and IV, DO_2 and oxygen uptake in the ICI 174,864 group were slightly higher than those in the hypotensive resuscitation and LR control group, and the differences among them were not significant (table 3).

Tissue Blood Flow in the Liver, Kidney, and Brain. Tissue blood flow in the liver, kidney, and brain at baseline and at the model stage did not show significant differences in all groups. ICI 174,864 significantly increased blood flow in the liver and kidney, which were significantly higher than in the hypotensive resuscitation and LR control group at 2-h

Table 1. Changes in Hemodynamic Parameters

Group	Baseline	End of Phase I	End of Phase II	End of Phase III	End of Phase IV
MAP, mmHg					
ICI	109.3 ± 9.8	39.6 ± 2.3	54.3 ± 8.9**	78.6 ± 9.5**	87.3 ± 10.9**#
Hypo resus	113.2 ± 12.7	38.7 ± 1.2	53.8 ± 3.2**	76.2 ± 8.6*	74.8 ± 11.7*
1/4 LR	111.4 ± 13.2	40.4 ± 1.3	21.5 ± 9.8	60.2 ± 8.5	65.96 ± 15.6
$+dp/dt_{max}$, mmHg/s					
ICI	6,074.0 ± 635.2	1,929.8 ± 487.8	4,676.6 ± 1,022.2*#	4,652.5 ± 665.2**#	4,775.6 ± 766.7**#
Hypo resus	6,278.4 ± 502.9	2,015.7 ± 367.3	3,243.8 ± 443.6	3,365.5 ± 586.2	3,424.9 ± 590.4
1/4 LR	6,282.9 ± 1,617.2	2,122.3 ± 3,34.6	3,283.6 ± 1,097.4	2,255.6 ± 801.2	2,093.8 ± 909.3
$-dp/dt_{max}$, mmHg/s					
ICI	5,046.9 ± 742.8	1,896.8 ± 345.3	3,372.7 ± 446.0*	3,865.7 ± 562.4*#	3,939.8 ± 520.5*#
Hypo resus	5,351.8 ± 420.7	1,813.3 ± 245.3	2,631.3 ± 642.2	2,856.2 ± 302.5	3,080.1 ± 294.9
1/4 LR	5,145.6 ± 580.3	1,958.2 ± 328.3	2,084.1 ± 814.9	1,925.6 ± 1,023.2	1,882.8 ± 1,405.8
LVSP, mmHg					
ICI	133.9 ± 16.4	86.3 ± 11.1	94.02 ± 12.65	100.2 ± 9.5**	104.6 ± 12.7**
Hypo resus	137.3 ± 5.7	87.0 ± 10.9	90.18 ± 20.28	90.5 ± 5.6*	91.0 ± 21.6*
1/4 LR	133.3 ± 29.8	90.2 ± 11.2	83.68 ± 35.11	74.5 ± 12.3	71.3 ± 32.0

Data represent the mean ± SD of eight observations. Two-way ANOVA analysis showed that these hemodynamic parameters had significant differences among all treated groups (ICI, hypo res, and 1/4 LR group).

*P < 0.05, **P < 0.01, as compared with the LR group, #P < 0.05, as compared with the 50-mmHg group.

$\pm dp/dt_{max}$ = maximal change rate of left intraventricular pressure; Hypo resus = hypotensive resuscitation; ICI = ICI 174,864; LR = lactated Ringer's solution; LVSP = left intraventricular systolic pressure; MAP = mean arterial pressure.

Table 2. Changes in Arterial pH, Pa_{O_2}, and Pa_{CO_2} (mmHg/l)

Group	Baseline	End of Phase I	End of Phase II	End of Phase III	End of Phase IV
pH					
ICI	7.38 ± 0.05	7.26 ± 0.03	7.32 ± 0.06	7.39 ± 0.04	7.40 ± 0.05
Hypo resus	7.36 ± 0.06	7.24 ± 0.02	7.30 ± 0.05	7.42 ± 0.03	7.44 ± 0.04
1/4 LR	7.37 ± 0.04	7.25 ± 0.04	7.35 ± 0.06	7.30 ± 0.02	7.37 ± 0.03
Pa_{O_2}, mmHg					
ICI	97.28 ± 12.41	94.88 ± 6.42	115.61 ± 9.19	112.63 ± 6.25*	118.94 ± 8.11*#
Hypo resus	96.34 ± 13.23	95.52 ± 8.73	109.49 ± 8.15	105.51 ± 7.61	102.96 ± 9.97
1/4 LR	98.63 ± 14.84	93.41 ± 4.95	103.04 ± 9.79	106.58 ± 9.56	107.33 ± 10.56
Pa_{CO_2}, mmHg					
ICI	44.23 ± 3.98	33.34 ± 3.12	29.15 ± 1.10	28.06 ± 2.35	28.16 ± 1.02
Hypo resus	43.89 ± 8.87	34.89 ± 2.54	29.21 ± 1.58	29.99 ± 2.14	30.26 ± 1.23
1/4 LR	44.99 ± 6.33	34.38 ± 4.34	27.39 ± 1.02	28.56 ± 1.98	32.30 ± 1.87

Data represent the mean ± SD of eight observations. Two-way ANOVA analysis showed that Pa_{O_2} had significant differences among all treated groups (ICI, hypo res, and 1/4 LR group).

*$P < 0.05$, as compared with the LR group, #$P < 0.05$, as compared with the 50-mmHg group.

Hypo resus = hypotensive resuscitation; ICI = ICI 174,864; LR = lactated Ringer's solution; Pa_{O_2} = partial pressure of arterial blood oxygen; Pa_{CO_2} = partial pressure of arterial blood carbon dioxide.

ligation, and at the end of phases III and IV. ICI 174,864 had no significant effect on blood flow in the brain (table 4).

Liver Function and Kidney Function. Parameters reflecting liver function and kidney function (levels of alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, and serum creatinine) did not show significant differences among all groups at all time points (table 4).

Discussion

The current study demonstrated that the δ opioid receptor antagonist ICI 174,864 was beneficial for the treatment of rats undergoing traumatic hemorrhagic shock at an early stage (before bleeding was controlled). ICI 174,864 (0.1–5 mg/kg) with or without infusion of a small volume of fluid (1/4 volume of LR) dose-dependently increased the MAP and prolonged the survival time and survival rate as compared with LR control and ICI 174,864 with 1/2 volume of LR infusion. A total of 3 mg/kg of ICI 174,864 plus 1/4 volume of LR infusion significantly prolonged 24-h survival rate, irrespective of whether bleeding was stopped at 1, 2, or 3 h after ICI 174,864 administration. Further study showed that this treatment strategy could significantly improve hemodynamic parameters, cardiac function, DO_2, oxygen uptake, and tissue blood flow in hemorrhagic shock rats. These results suggested that ICI 174,864 with or without small volume of fluid infusion has good beneficial effect on uncontrolled hemorrhagic shock; increasing the fluid infusion will only deteriorate its beneficial effect *via* increase of bleeding. Early application of ICI 174,864 could buy time for subsequent treatment of traumatic and hemorrhagic shock. ICI 174,864 elicits its antishock effects mainly through improving hemodynamics, cardiac function, and increasing tissue blood flow.

Endogenous opioid peptides are very important neuroendocrine mediators released mainly from the pituitary gland. Our previous studies along with some others, have demonstrated that endogenous opioid peptides are involved in shock. δ and μ opioid receptors are the main receptors participating in shock.[13,14] Several studies have shown that nonspecific opioid receptor antagonist naloxone is beneficial in the treatment of types of circulatory shock, such as hemorrhagic and septic/infectious shock. But naloxone can block μ opioid receptors to decrease the pain threshold and increase the pain of patients while eliciting its antishock effects, so its application in the treatment of traumatic shock has some limitation.[15,16]

In 1992, Kiffer and Evens cloned the δ opioid receptor in mouse brains from the NG108215 cell line. The δ opioid receptor is a 40-kD molecule comprising 372 amino acids. In 1993 and 1994, Knapp cloned the δ opioid receptor from brains of humans and rats, and it had 92% homology with the δ opioid receptor from mice.[17] Further studies demonstrated that the δ opioid receptor participated in the protective function of the heart after ischemic preconditioning *via* activation of the ATP-sensitive potassium channel and protein kinase C.[18,19] Our previous studies showed that the δ opioid receptor is involved in traumatic and hemorrhagic shock and its antagonist ICI 174,864 had beneficial effect on traumatic shock (controlled traumatic hemorrhagic shock). But whether ICI 174,864 is suitable for the management of traumatic shock at an early stage, especially for uncontrolled hemorrhagic shock, is not clear. The present study showed that the δ opioid receptor antagonist ICI 174,864 can play antishock effects at early application and buy time for subsequent treatment of shock.

In our experiments, we found some interesting results. The first was that the blood loss in the entire experimental period of the "No fluid" group in experiment part I was less than in 1/4 volume of LR and 1/2 volume of LR plus ICI

Table 3. Effects of ICI 174,864 on Cardiac Function, Oxygen Delivery, and Oxygen Consumption after Uncontrolled Hemorrhagic Shock

	Baseline	Phase I	Phase II	Phase III	Phase IV
CO, ml/min					
ICI	102.38 ± 13.81	36.04 ± 14.53	45.37 ± 3.83	56.24 ± 5.27*#	61.71 ± 6.53**#
Hypo resus	100.82 ± 21.12	42.14 ± 10.63	41.21 ± 3.18	44.57 ± 2.15	49.59 ± 2.80
1/4 LR	105.81 ± 28.93	34.12 ± 7.09	48.48 ± 3.13	44.52 ± 2.65	41.08 ± 3.27
CI, ml·min^{-1}·m^{-2}					
ICI	2.50 ± 0.21	0.80 ± 0.26	1.38 ± 0.29	1.57 ± 0.13	1.89 ± 0.21*
Hypo resus	2.53 ± 0.47	0.98 ± 0.15	1.30 ± 0.20	1.43 ± 0.20	1.56 ± 0.17
1/4 LR	2.58 ± 0.53	0.75 ± 0.04	1.49 ± 0.22	1.47 ± 0.21	1.49 ± 0.29
SI, ml·stroke^{-1}·m^{-2}					
ICI	0.5443 ± 0.0313	0.1343 ± 0.0273	0.4654 ± 0.0112	0.5612 ± 0.0214	0.5810 ± 0.0514*
Hypo resus	0.5343 ± 0.1143	0.1643 ± 0.0593	0.3990 ± 0.0179	0.4231 ± 0.0214	0.4950 ± 0.0167
1/4 LR	0.5543 ± 0.1343	0.1243 ± 0.0595	0.4620 ± 0.0376	0.4032 ± 0.0214	0.3862 ± 0.0318
DO_2, ml·min^{-1}·m^{-2}					
ICI	306.99 ± 40.83	93.80 ± 20.10	170.12 ± 18.00	202.56 ± 15.65	228.99 ± 18.97
Hypo resus	376.69 ± 96.50	112.29 ± 11.13	149.10 ± 11.70	168.52 ± 22.13	198.12 ± 24.81
1/4 LR	367.67 ± 86.09	78.48 ± 3.46	154.24 ± 11.97	174.25 ± 16.56	194.22 ± 21.62
VO_2, ml·min^{-1}·m^{-2}					
ICI	160.86 ± 36.32	51.19 ± 4.27	113.31 ± 15.76	128.53 ± 14.27*	136.80 ± 15.24*
Hypo resus	163.05 ± 47.63	61.09 ± 3.57	102.81 ± 11.83	118.56 ± 16.53	123.07 ± 17.40
1/4 LR	177.26 ± 53.01	46.38 ± 13.16	90.49 ± 14.29	99.85 ± 9.65	106.60 ± 10.27

Data represent the mean ± SD of eight observations. Two-way ANOVA analysis showed that CO, CI, SI, and VO_2 had significant differences among all treated groups (ICI, hypo resus, and 1/4 LR group).

*P < 0.05, **P < 0.01, as compared with the LR group, #P < 0.05, as compared with the 50-mmHg group.

CI = cardiac index; CO = cardiac output; DO_2 = oxygen delivery; Hypo resus = hypotensive resuscitation (50 mmHg) group; ICI = ICI 174,864, delta-opioid receptor antagonist; LR = lactated Ringer's solution; SI = stroke index; VO_2 = oxygen consumption.

174,864 group. In fact, the MAP of the "No fluid" group was higher. The reason was not because of the difference of severity in hemorrhagic shock among groups, nor because of ICI 174,864 having no hemodynamic effect that limits the bleeding. It is because ICI 174,864 has some content of vasoconstrictor effect and hemostatic effect. In "No fluid" group, ICI 174,864 may prevent and reduce the bleeding from the transected spleen and splenic artery by vasoconstriction and hemostatic effects, whereas in volume expansion groups (1/4 vol of LR and 1/2 vol of LR group), although the vasoconstriction and hemostatic effect of ICI 174,864 still remains, the volume expansion and hemodilution caused by fluid infusion may increase the bleeding of transected spleen and splenic artery. In addition, fluid expansion might interfere with the coagulation and hemostatic effect, which may be another reason that caused more blood loss in 1/4 volume of LR and 1/2 volume of LR group than in no fluid group. Of course, if ICI 174,864 has hemostatic effect and fluid expansion can interfere with the coagulation and hemostatic effect, it needs further investigation for confirmation. The second interesting result we found was that ICI 174,864 in the range of 0.1–3 mg/kg dose-dependently increased the MAP in rats with or without infusion of a small volume of LR. But MAP in the group of ICI 174,864 at 5 mg/kg with 1/4 volume of LR infusion was lower than in ICI 174,864 at 3 mg/kg with 1/4 volume of LR. There may be two reasons: the first reason is that ICI 174,864 may be its own effective dose range, and more than the maximal effective dose may produce side effects, which can alleviate the beneficial effect. The second reason may be that opioid peptides have very complex effects in cardiovascular system, central nervous system, and immune system.[20–22] Different dosages of their antagonists may produce different effects, even an opposing effect. The precise reason, however, needs further investigation.

The third interesting result was that ICI 174,864 significantly increased the blood flow of liver and kidney, whereas the increase of DO_2 and cardiac index was not coincident with the increase of blood flow of liver and kidney. We carefully looked at the changes of cardiac index and DO_2, actually, the cardiac index and DO_2 were also increased after ICI 174,864, just not as obviously as the increase of liver and kidney blood flow. On the basis of this question, we read some references. Indeed, some studies showed that endogenous opioid peptides have very complex effect on cardiovascular system—even the same opioid peptide may have different effects in different blood vessels.

The mechanism that ICI 174,864 increases blood pressure and improves the hemodynamics may be *via* two pathways: one is that ICI 174,864 directly acts on peripheral δ opioid receptors in cardiovascular system and the other is that ICI 174,864 activates the sympathetic system *via*

Table 4. Effects of ICI 174,864 on Blood Flow in the Liver, Kidney, and Brain and Their Function after Hemorrhagic Shock

	Baseline	Phase I	Phase II	Phase III	Phase IV
Blood flow of liver, U/min					
ICI	283.64 ± 14.22	195.25 ± 13.98	266.37 ± 44.75**##	276.59 ± 32.15**##	268.59 ± 26.17**##
Hypo resus	282.45 ± 13.61	184.59 ± 11.73	183.11 ± 16.23	198.56 ± 15.65*	201.98 ± 22.17*
1/4 LR	281.80 ± 14.32	191.29 ± 16.51	120.18 ± 19.59	145.27 ± 14.26	165.65 ± 26.45
Blood flow of kidney, U/min					
ICI	278.50 ± 12.34	102.99 ± 11.27	162.90 ± 22.70**##	176.53 ± 16.52**##	186.34 ± 19.46**##
Hypo resus	280.63 ± 45.51	93.11 ± 7.55	95.89 ± 13.67	120.56 ± 12.32	132.42 ± 13.80
1/4 LR	280.49 ± 9.90	100.46 ± 9.87	98.13 ± 14.78	112.35 ± 10.24	126.14 ± 14.58
Blood flow of brain, U/min					
ICI	124.09 ± 8.08	26.68 ± 1.35	117.93 ± 9.56*	136.26 ± 8.56*#	119.10 ± 7.73*#
Hypo resus	123.65 ± 4.43	26.16 ± 1.04	106.52 ± 15.06	112.56 ± 5.24	98.74 ± 7.81
1/4 LR	124.63 ± 11.98	26.03 ± 3.07	88.39 ± 10.23	98.65 ± 5.65	79.45 ± 9.29
AST, U/l					
ICI	150.41 ± 18.67	88.90 ± 2.85	222.81 ± 21.44	289.57 ± 19.57	342.64 ± 21.65
Hypo resus	155.41 ± 9.28	88.37 ± 2.33	190.84 ± 21.81	266.53 ± 18.56	364.91 ± 14.57
1/4 LR	146.44 ± 10.35	88.82 ± 1.61	206.64 ± 17.54	305.27 ± 14.25	379.48 ± 10.27
ALT, U/l					
ICI	30.47 ± 5.91	33.60 ± 4.65	50.66 ± 3.40	90.26 ± 5.65*	110.55 ± 7.06
Hypo resus	33.63 ± 4.40	34.54 ± 1.87	37.63 ± 3.03	89.56 ± 4.87	104.53 ± 6.91
1/4 LR	33.71 ± 4.98	34.19 ± 1.47	42.72 ± 3.25	104.23 ± 9.35	110.73 ± 8.70
BUN, mM					
ICI	6.14 ± 1.06	8.63 ± 1.19	12.83 ± 1.02	11.98 ± 2.35	12.49 ± 1.86
Hypo resus	6.13 ± 0.84	9.23 ± 0.84	11.47 ± 1.14	10.99 ± 1.52	10.47 ± 1.64
1/4 LR	6.17 ± 0.79	8.47 ± 0.56	11.40 ± 1.00	12.65 ± 1.05	11.99 ± 1.55
Src, mol/l					
ICI	38.23 ± 5.12	74.92 ± 8.18	56.30 ± 5.23	62.53 ± 5.25	57.58 ± 5.61*
Hypo resus	36.89 ± 1.76	81.19 ± 6.79	66.28 ± 6.99	69.21 ± 4.57	55.39 ± 5.65
1/4 LR	38.82 ± 1.97	75.42 ± 7.30	58.97 ± 3.59	69.52 ± 5.81	74.80 ± 5.81

Data represent the mean ± SD of eight observations. Two-way ANOVA analysis showed that the blood flow in liver, kidney, and brain had significant differences among all treated groups (ICI, hypo res, and 1/4 LR group).

*P < 0.05, **P < 0.01, as compared with the LR group, #P < 0.05, ##P < 0.01, as compared with the 50-mmHg group.

ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = blood urea nitrogen; Hypo resus = hypotensive resuscitation (50 mmHg) group; ICI = ICI 174,864, delta-opioid receptor antagonist; LR = lactated Ringer's solution; Scr = serum creatinine.

central or peripheral mechanism. In addition, some studies showed that inhibition of opioid receptors can increase the release of noradrenaline from postganglionic noradrenergic nerve endings innervating the vasculature. Opioid peptides are costored with noradrenaline in sympathetic nerves.[23–26]

Basic and clinical research has shown that before uncontrolled hemorrhagic shock can be controlled, permissive hypotensive resuscitation is required.[27–29] Wang *et al.* found that bleeding rate and mortality was higher in rabbits undergoing 90-mmHg resuscitation, than rabbits undergoing 50- and 70-mmHg resuscitation before hemostasis while suffering uncontrolled hemorrhagic shock. We previously found that a too-low (40 mmHg) or too-high (>80 mmHg) resuscitation pressure during uncontrolled hemorrhagic shock should be avoided. The ideal target MAP for uncontrolled hemorrhagic shock in rats was 50–60 mmHg.[3,30] Hence, the resuscitation pressure of the hypotensive resuscitation group selected in the current study was 50 mmHg. But hypotension duration is not allowed for too long. Ninety minutes was the maximal tolerance limit of hypotensive resuscitation.[3] So in the current study, the blood loss and fluid requirement in hypotensive resuscitation group was higher than that in ICI 174,864 and LR control groups over 2 h.

The current study had limitations. First, this study was mainly limited to small and anesthetized animals (rats), and whether this effect of ICI 174,864 can be extrapolated to large animals and humans needs confirmation. Second, the parameters observed in the current study were hemodynamics, cardiac function, and blood flow, and whether ICI 174,864 and fluid expansion can affect the coagulation system, which influences the blood loss, is not known, this needs further investigation. Third, if ICI 174,864 decreases tissue blood flow in some circulation, then the precise mechanism of how ICI 174,864 exerts its antishock effects also needs further study.

Conclusion

The δ opioid receptor antagonist ICI 174,864 with or without a small volume of fluid infusion is suitable for the early

treatment of traumatic hemorrhagic shock. Early application of ICI 174,864 can buy time for subsequent treatment of traumatic shock. ICI 174,864 elicits its antishock effects mainly through improving hemodynamics and cardiac function, as well as increasing the tissue blood flow of animals with shock.

References

1. Bellamy RF: The causes of death in conventional land warfare: Implications for combat casualty care research. Mil Med 1984; 149:55–62
2. Cherkas D: Traumatic hemorrhagic shock: Advances in fluid management. Emerg Med Pract 2011; 13:1–19; quiz 19–20
3. Li T, Zhu Y, Hu Y, Diao YF, Liao ZF, Li P, Liu LM: Ideal resuscitation pressure and maintenance time of hypotensive resuscitation for uncontrolled hemorrhagic shock in rats. Anesthesiology 2011; 114:111–9
4. Kanoore Edul VS, Dubin A, Ince C: The microcirculation as a therapeutic target in the treatment of sepsis and shock. Semin Respir Crit Care Med 2011; 32:558–68
5. Bauer SR, Lam SW: Arginine vasopressin for the treatment of septic shock in adults. Pharmacotherapy 2010; 30:1057–71
6. Glattard E, Welters ID, Lavaux T, Muller AH, Laux A, Zhang D, Schmidt AR, Delalande F, Laventie BJ, Dirrig-Grosch S, Colin DA, Van Dorsselaer A, Aunis D, Metz-Boutigue MH, Schneider F, Goumon Y: Endogenous morphine levels are increased in sepsis: A partial implication of neutrophils. PLoS One 2010; 5:e8791
7. Lu W, Fang ZY, Chen WL: Endogenous opioid peptides and shock. Essential Problems Trauma Surg 1994; 15:196–200
8. D'Amato R, Holaday JW: Multiple opioid receptors in endotoxic shock: Evidence for δinvolvement and μ-δ interactions *in vivo*. Proc Natl Acad Sci U S A 1984; 81:2898–901
9. Liu LM, Hu DY, Pan XK, Lu RQ, Dan FJ: Subclass opioid receptors associated with the cardiovascular depression after traumatic shock and the antishock effects of its specific receptor antagonists. Shock 2005; 24:470–5
10. Ebrahimkhani MR, Moezi L, Kiani S, Merat S, Dehpour AR: Opioid receptor blockade improves mesenteric responsiveness in biliary cirrhosis. Dig Dis Sci 2008; 53:3007–11
11. Parra L, Pérez-Vizcaíno F, Alsasua A, Martín MI, Tamargo J: mu- and delta-opioid receptor-mediated contractile effects on rat aortic vascular smooth muscle. Eur J Pharmacol 1995; 277:99–05
12. Li T, Zhu Y, Fang YQ, Liu LM: Determining the optimal mean arterial pressure for post-bleeding resuscitation in traumatized rats. Anesthesiology 2012; 116:103–12
13. Kai L, Wang ZF, Hu DY, Shi YL, Liu LM: Opioid receptor antagonists modulate Ca^{2+}-activated K+ channels in mesenteric arterial smooth muscle cells of rats in hemorrhagic shock. Shock 2003; 19:85–90
14. Hu DY, Pan XK, Liu LM: The effects of mu and delta opioid receptor antagonists on traumatic hemorrhagic shock. Chin Crit Care Med 2000; 12:101–4
15. Hackshaw KV, Parker GA, Roberts JW: Naloxone in septic shock. Crit Care Med 1990; 18:47–1
16. Pradhan AA, Befort K, Nozaki C, Gavériaux-Ruff C, Kieffer BL: The delta opioid receptor: An evolving target for the treatment of brain disorders. Trends Pharmacol Sci 2011; 32:581–90
17. Knapp RJ, Malatynska E, Fang L, Li X, Babin E, Nguyen M, Santoro G, Varga EV, Hruby VJ, Roeske WR: Identification of a human delta opioid receptor: Cloning and expression. Life Sci 1994; 54:PL463–9
18. Schultz JE, Hsu AK, Gross GJ: Ischemic preconditioning in the intact rat heart is mediated by delta1- but not mu- or kappa-opioid receptors. Circulation 1998; 97:1282–9
19. Fu SP, Jiang YZ, Zhang WJ: The relationship of opioid receptor with heart protective function after ischemic preconditioning. J Jilin Coll 2003; 9:465–7
20. E1-Sharkawy TY, A1-Shireida MF, Pilcher CW: Vascular effects of some opioid receptor agonists. Can J Physiol Pharmacol 1991; 69:846–51
21. Feuerstein G, Sirén AL: The opioid system in cardiac and vascular regulation of normal and hypertensive states. Circulation 1987; 75(1 Pt 2):I125–9
22. Fang X, Tang W, Sun S, Huang L, Huang Z, Weil MH: Mechanism by which activation of delta-opioid receptor reduces the severity of postresuscitation myocardial dysfunction. Crit Care Med 2006; 34:2607–12
23. Klein RL, Chang KJ, Gasparis MS, Viveros OH, Yang WH: Are opioid peptides co-transmitters in adrenergic vesicles of sympathetic nerves? Nature 1980; 288:707–9
24. Lang RE, Brückner UB, Kempf B, Rascher W, Sturm V, Unger T, Speck G, Ganten D: Opioid peptides and blood pressure regulation. Clin Exp Hypertens A 1982; 4:249–69
25. Ensinger H, Hedler L, Szabo B, Starke K: Bremazocine causes sympatho-inhibition and hypotension in rabbits by activating peripheral kappa-receptors. J Cardiovasc Pharmacol 1986; 8:470–5
26. Illes P, Pfeiffer N, von Kügelgen I, Starke K: Presynaptic opioid receptor subtypes in the rabbit ear artery. J Pharmacol Exp Ther 1985; 232:526–33
27. Handrigan MT, Bentley TB, Oliver JD, Tabaku LS, Burge JR, Atkins JL: Choice of fluid influences outcome in prolonged hypotensive resuscitation after hemorrhage in awake rats. Shock 2005; 23:337–43
28. Wang F, Zheng SY, Chen QF: Effect of limited crystalloid resuscitation on uncontrolled hemorrhagic shock. Acta Med Bongbu Chin 2006, 31:575–8
29. Weekes AJ, Tassone HM, Babcock A, Quirke DP, Norton HJ, Jayarama K, Tayal VS: Comparison of serial qualitative and quantitative assessments of caval index and left ventricular systolic function during early fluid resuscitation of hypotensive emergency department patients. Acad Emerg Med 2011; 18:912–21
30. Li T, Lin X, Zhu Y, Li L, Liu L: Short-term, mild hypothermia can increase the beneficial effect of permissive hypotension on uncontrolled hemorrhagic shock in rats. Anesthesiology 2012; 116:1288–98